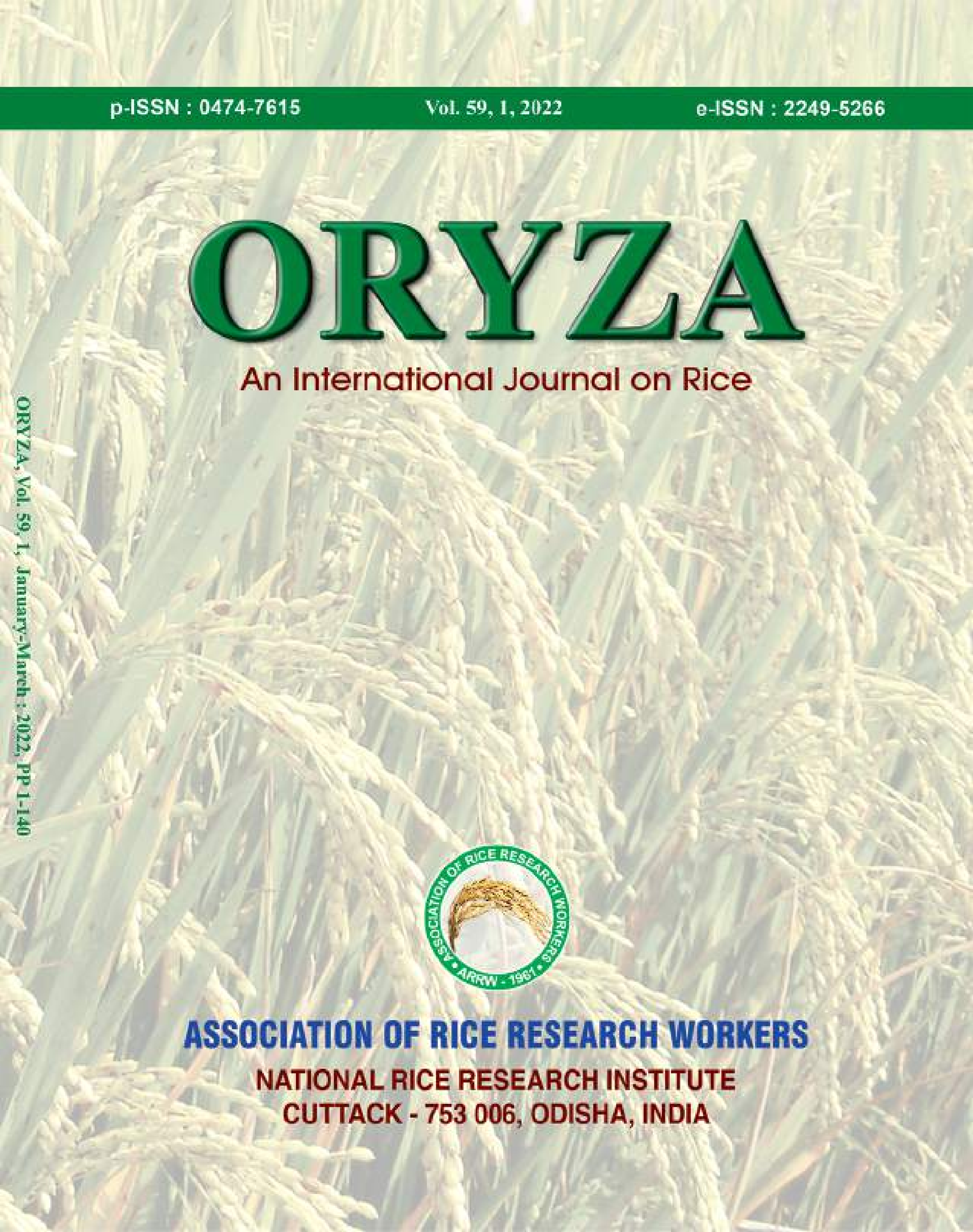

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Genetic analysis of parental lines and identification of heterotic hybrid combinations in rice (*Oryza sativa* L.)

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ABSTRACT

Thirty hybrids along with their parents (B and R lines) and standard checks viz., MTU 1001 and 27P63 were evaluated for grain yield and related traits to study combining ability and heterosis in rice. The mean performance of the hybrids for most of the characters was found higher than that of parents. The analysis of variance revealed significant differences among lines, testers and hybrids for most of the characters studied. SCA variances were found lower than GCA variances for most of the characters and average degree of dominance was far from unity indicating the predominance of additive gene action for these traits. However, for effective bearing tillers and spikelet fertility the average degree of dominance was very near to unity inferring the predominance of non-additive genetic components for these traits. The gca effects revealed that the lines, RNR 25783 and RP 5980-109-12-9-27 had significant gca effects in desired direction for yield and yield attributing traits whereas the tester, CMS 59A was a good general combiner. SCA effects showed that the hybrid, JMS 13A × RNR 19361 was found to be good specific combiner for grain yield per plant and spikelet fertility. Similarly, the hybrids, CMS 59A × SYE 503-78-34-2, CMS 46A × WGL 347 and JMS 13A × RP 5980-109-12-9-27 were identified as good specific combiner for grain yield and yield contributing characters. Based on heterotic estimates, five hybrids viz., CMS 59A × RNR 25783, CMS 59A × WGL 14, JMS 13A × RNR 25783, CMS 59A × RNR 25776, JMS 13A × RP 5980-109-12-9-27 were identified as promising with more than 5% and 20% yield advantage over hybrid and varietal checks, respectively.

Key words: *Combining ability, gene actions, heterosis, hybrid rice*

INTRODUCTION

Rice is one of the most important food crops in the world, especially in Asian countries. It is estimated that the global demand for rice will increase to 852 million tons by 2035, however, records show that annual yield growth was only around 1% in the past decade (Khush, 2013). Thus we are in need to increase rice production to meet this growing population (Kumar et al., 2014). The expected requirement of rice in India would be 130 and 168 Million Tons with cultivated area of only 42 and 40 MHa by 2030 and 2050, respectively (Gupta et al., 2020). With the limited resources along with various environmental constraints, such as pest, diseases and unfavorable farming conditions, demand for sustainable ways to increase rice production remains an enormous challenge (Gramaje et al., 2020). Theoretically, rice still has great yield potential to be tapped and there are many ways to increase yield such as molecular breeding, new plant type and hybrid rice technology. Among three, hybrid rice technology offers the most effective solution to enhance yield on suitable land for rice cultivation. The economical way to increase productivity is to develop hybrid varieties based on the fruitful experience gained in China (Galal Bakr Anis et al., 2017).

The success story of hybrid rice technology in China (Lin and Yuan, 1980) as leading producer of hybrid rice in the world (Swaminathan, 2006) and some other countries along with India has been witnessed as an important and readily adoptable genetic option to increase the rice production and offers a viable solution to meet the ever increasing food challenge in different countries (Rai, 2009; Sanghera and Wani, 2008; Virmani et al., 2003). The hybrid technology revolutionized the rice farming through boosting the yield from 35 to 40 q/ha of straight varieties of rice to the tune of 65 to 70 q/ha in rice hybrids. This helped the farmers in raising their economic status and helped in changing areas under straight varieties to rice hybrids in China (Yuan et al., 1989). Breeding strategies for the selection of hybrid combinations is based on expected level of heterosis and the specific combining ability (Satheesh kumar et al., 2016).

For generating promising hybrids, the first step is selection of desirable parents. The parental lines are considered to be the backbone of hybrid rice breeding programme since the performance of the progenies mainly depends on the genetic potential of the parents. Breeders are frequently facing the challenges while select, breed, develop, and improve superior hybrid parental lines (Gramaje, 2020). Thus parental selection should be done based on *per se* performance and gene actions. In this framework, the aim of combining ability analysis is to investigate the ability of a specific parental line to pass down genetic information to its progenies (Aly, 2013; Sprague and Tatum, 1942).

The contribution of parents in a cross and combining ability of parents in crosses can be assessed by biometrical methods through combining ability studies. Line × Tester analysis devised by Kempthorne (1957) is one of the effective mating design followed to estimate gca and sca which enables the effective screening of parental lines. Combining ability analysis is one of the powerful tool available to estimate the combining ability effects and aids in selecting the desirable parents and crosses for the exploitation of heterosis (Sarker et al., 2002). The knowledge of combining ability is useful to assess nicking ability in self-pollinated crops and at the same time elucidate the nature and magnitude of gene actions involved, provides the breeder about insight of nature and relative magnitude of fixable and non-fixable genetic variances *i.e.*, due to dominance or epistatic components (Pratap et al., 2013).

In the present investigation, studies were carried out on the combining ability and magnitude of heterosis for grain yield and important yield attributes in 30 rice hybrids.

MATERIALS AND METHODS

The experiment was conducted at Rice Research Center of Agricultural Research Institute, PJTSAU, Hyderabad, Telangana, India. During *rabi*, 2018-19, 10 restorer lines and 3 stable CMS lines (Table 1) were

Table 1. Details of experimental material used for study.

S. no.	Genotype	Pedigree	Duration group	Grain type	Source
	Lines				
1.	RNR 19361	MTU 1075 × WGL 32100	Medium	Medium Slender	RRC, ARI, Hyderabad
2.	MTU 1001	MTU 5249 × MTU 7014	Medium	Long Bold	RARS, Maruteru
3.	WGL 14	BPT 5204 x ARC 5984//BPT 3291	Medium	Medium Slender	RARS, Warangal
4.	WGL 823	NLR 34449 × BPT 5204	Medium	Medium Slender	RARS, Warangal
5.	SYE 503-78-34-2	Daya x SYE 63-2003	Medium	Short Slender	IIRR, Hyderabad
6.	RNR 25783	Badrakali × IR 79597-56-1-2-1	Mid Early	Long Bold	RRC, ARI, Hyderabad
7.	WGL 347	NLR-145 x Kavya	Medium	Medium Slender	RARS, Warangal
8.	WGL 32100	Divya x BPT 5204	Medium	Medium Slender	RARS, Warangal
9.	RNR 25776	Badrakali × IR 79597-56-1-2-1	Mid Early	Long Bold	RRC, ARI, Hyderabad
10.	RP 5980-109-12-9-27	DRR 17B x Improved Samba Mahsuri	Mid Early	Medium Slender	IIRR, Hyderabad
	Testers				
1.	CMS 46A	IR80559A	Early	Long Slender	IRRI, Philippines
2.	CMS 59A	IR79156A	Mid Early	Long Slender	IRRI, Philippines
3.	JMS 13A	JGL1870A	Medium	Medium Slender	RARS, Jagital

sown in different dates according to the flowering duration differences among the parents for synchronized flowering and effective hybrid seed production. These parents were then transplanted in main field in line × tester design for generating 30 hybrid combinations. The standard package of practices was followed *i.e.*, 30 × 15 cm spacing for male rows and 15 × 15 cm for female rows with male : female ratio of 2:6.

At boot leaf stage, barrier sheets were erected between different restorer combinations to avoid cross contamination. Leaf clipping and GA3 application were done to facilitate more pollen movement to CMS lines and better stigma exertion, respectively. Methods for adjusting the synchronization of flowering *viz*., spraying of urea (2%) for delaying and SSP (1%) for advancing the flowering were adopted by observing the panicle initiation stage of different parents (Viraktamath and Ramesha, 1996).

Vigorous roughing of pollen shedders in female lines was done to obtain pure hybrid seed. Supplementary pollination was done by shaking the pollen parent with the help of stick thrice a day at peak flowering stage from 9.30 AM to 12.30 PM to facilitate more seed setting on female lines. The hybrid seed was harvested at maturity. These 30 hybrids along with their 13 parents and two checks (27P63 and MTU 1001) were sown in nursery beds during *kharif*, 2019 and 25 days old seedlings were transplanted to main field at a spacing of 20 × 15 cm in Randomized Complete Block Design with two replications. The plot size for each treatment was 5.67 m^2 and recommended agronomic package was followed to raise good crop.

The observations were recorded on five randomly selected plants for different traits *viz*., plant height (cm), effective bearing tillers, panicle length (cm), spikelet fertility (%) and number of grains per panicle. However, days to 50% flowering and grain yield (kg/plot) was recorded on a whole plot basis (plot size 5.67 m^2), whereas, test weight (g) were recorded on a random sample taken in each plot.

The character means of each replication was subjected for combining ability analysis and the test of significance of different genotypes were done as per the procedure given by Kempthorne (1957) The estimation of heterosis over the better parent, standard variety and standard hybrid was done as per the method proposed by Fonseca and Patterson, 1968. Relative importance of *gca* and *sca* was estimated by the predictability ratio $2\sigma^2g/2\sigma^2g + \sigma^2s$ (Baker, 1978) for fixed effect model where, σ^2g and σ^2s is additive and non-additive component of variance, respectively. Average degree of dominance was estimated by the formula $(\sigma^2s/2\sigma^2g)^{1/2}$. Computer software Windostat version 9.1 has been used for the statistical analysis of data.

RESULTS AND DISCUSSION

The *per se* performance of the hybrids for most of the traits was found higher than that of parents. Among the thirty hybrids, none of the hybrids was found superior for all the traits studied. The hybrids CMS 59A × RNR 25783, CMS 59A × WGL 14, JMS 13A × RNR 25783,

Table 2. Analysis of variance for different characters in rice.

Source of variation	Degrees of	Days to 50 % Freedom	Plant height flowering	Effective bearing	Panicle length tillers	Test weight	No. of grains per panicle	Spikelet fertility	Grain yield per plot (%)
Replications	1	2.27	57.79*	10.88	16.26**	4.92*	5536.04*	87.60*	0.25
Treatments	42	47.95**	65.56**	10.75**	7.49**	22.76**	4366.65**	104.80**	2.39**
Parents	12	43.03**	94.12**	8.53**	9.92**	45.49**	5923.69**	32.52	0.28*
Lines	9	31.56**	89.49**	10.38**	9.94**	57.99**	5096.49**	7.69	0.32*
Testers	2	73.16**	9.44	2.78	9.14**	11.94**	12244.50**	17.13	0.21
Line × Tester	1	86.00**	305.18**	3.42	11.23**	0.06	726.93	286.71**	0.01
Parents × hybrids	1	238.95**	402.71**	241.29**	0.15	67.96**	19214.34**	719.84**	59.53**
Hybrids	29	43.40**	42.13**	3.72	6.74**	11.80**	3210.64**	113.51**	1.29**
Error	29	4.02	12.97	2.85	1.05	0.98	1256.35	19.43	0.13
Total		25.71	39.49	6.85	4.41	11.79	2843.55	62.42	1.25

*, ** Significant at 0.05, 0.01 probability level, respectively.

CMS 59A × RNR 25776, JMS 13A × RP 5980-109-12-9-27, CMS 59A × RP 5980-109-12-9-27, JMS 13A × RNR 19361, CMS 46A × WGL 347 and CMS 59A × WGL 347 had recorded better *per se* performance for most of the yield attributing traits. In general, eight hybrids were superior and one hybrid is on par with hybrid check 27P63 for grain yield per plant.

The results of ANOVA revealed that the parents were highly significant for all the traits except for spikelet fertility, whereas the hybrids differ significantly for all the traits except for effective bearing tillers and parent *vs* hybrids were found highly significant except for panicle length (Table 2). Mean sum of squares for crosses was partitioned into lines, testers and line × tester components. In the case of lines, significant variances were observed for all the traits except spikelet fertility. In testers, significant variances were observed for the traits *viz*., days to 50 percent flowering, panicle length, test weight and number of grains per panicle. In lines × testers, significant variances were observed for four traits *viz*., days to 50% flowering, plant height, panicle length and spikelet fertility. Parents × hybrids showed significant variance for all the characters except panicle length, indicating the superiority of hybrids and the presence of heterosis for almost all the traits studied. These results emphasized the importance of combining ability studies in the material and there is a good scope for identifying promising parents and hybrid combinations for improving yield through its components.

In the present investigation, the predictability ratios were far from unity for the traits *viz*., effective bearing tillers (0.54) and spikelet fertility (0.53) indicating the predominance of non-additive gene action, while days to 50 per cent flowering (0.87), plant height (0.80), panicle length (0.82), test weight (0.96) number of grains per panicle (1.00) and grain yield per plot (0.71) exhibited additive gene action (Table 3). GCA variances were higher than SCA variances for days to 50 % flowering, plant height, panicle length, test weight, number of grains per panicle and grain yield per plot, which indicated the predominance of additive gene action for these traits. Moreover, the average degree of dominance values were much lower than unity for these traits also indicated the additive gene effects for such traits. Similar to the present findings, certain workers reported the predominance of additive components in rice for days to fifty per cent flowering (Parimala et al., 2018), plant height (Ramesh et al., 2017; Virender et al., 2020a; Ganapati et al, 2020) panicle length (Rukmini Devi et al., 2018; Hamdi et al., 2021), number of grains per panicle (Ganapati et al., 2020), test weight and grain yield per plant (Kumar et al., 2007), 100-grain weight and aroma (Aarti Sharma and Jaiswal, 2021). In contrary Singh et al. (2020) reported predominance of non-additive gene action for majority of the traits in rice except for test weight.

Among lines, the gca effect was significant and positive for RNR 25783 (0.86) and RP 5980-109-12-9-27 (0.59) for grain yield per plot whereas in testers CMS 59A (0.50) exhibited significant and positive *gca* effect for grain yield per plot. The *gca* effects revealed that among the lines, RNR 25783 had significant *gca* effects in the desired direction for important traits *viz*.,

Table 3. Estimates of general and specific combining ability variances and proportionate gene action in rice.

Character	Source of variation			Predictability ratio ($2\sigma^2$gca/ $2\sigma^2$gca + σ^2sca)	Average degree of dominance $(s^2s/2s^2g)^{1/2}$	Nature of gene action
	σ^2gca	σ^2sca	σ^2gca/ σ^2sca			
Days to 50% flowering	12.46	3.73	3.33	0.87	0.39	Additive
Plant height (cm)	5.69	2.93	1.94	0.80	0.51	Additive
Effective bearing tillers	0.10	0.17	0.61	0.54	0.92	Non-additive
Panicle length (cm)	1.23	0.53	2.29	0.82	0.46	Additive
Test weight (g)	5.58	0.47	11.74	0.96	0.21	Additive
Number of grains per panicle	1425.43	6.20	229.81	1.00	0.05	Additive
Spikelet fertility (%)	15.79	28.53	0.55	0.53	0.95	Non-additive
Grain yield per plot (g)	0.41	0.33	1.23	0.71	0.63	Additive

σ^2: variances; gca: general combining ability; sca: specific combining ability.

Table 4. Estimates of general combining ability effects in lines and testers for yield and yield contributing characters in rice.

Parent	Days to 50 % flowering	Plant height	Effective bearing tillers	Panicle length	Test weight	No. of grains per panicle	Spikelet fertility %	Grain yield per plot
Lines								
RNR 19361	5.22**	1.39	-2.04**	-0.73	-2.00**	20.63	-8.91**	-0.08
MTU 1001	-4.78**	-3.24*	0.39	-1.29**	2.67**	-4.03	6.02**	-0.30
WGL 14	5.05**	-1.91	1.33	-1.24**	-1.33**	12.30	-0.63	0.31
WGL 823	0.22	-5.78**	-0.27	-0.16	-0.88*	6.30	-0.30	-0.62**
SYE 503-78-34-2	-0.78	1.42	-0.61	1.53**	-1.13**	-40.20**	-10.90**	-0.92**
RNR 25783	1.38	6.39**	0.58	1.41**	1.29**	-22.03	2.67	0.86**
WGL 347	-7.12**	-0.84	0.13	-1.29**	-0.74	6.80	-1.48	0.07
WGL 32100	-1.95*	-1.94	-0.24	-2.03**	-1.61**	51.13**	4.40*	0.15
RNR 25776	-1.45	6.69**	-0.17	3.46**	3.77**	-24.53	5.02**	-0.06
RP 5980-109-12-9-27	4.22**	-2.18	0.91	0.34	-0.04	-6.37	4.10*	0.59**
Testers								
CMS 46A	-0.03	-0.36	-0.18	0.20	1.34**	-8.05	-0.37	-0.16
CMS 59A	-1.83**	0.33	0.35	0.02	0.44	-24.30**	-1.20	0.50**
JMS 13A	1.87**	0.02	-0.18	-0.21	-1.79**	32.35**	1.57	-0.34**

*, ** Significant at 0.05, 0.01 probability level, respectively.

grain yield per plot, panicle length and test weight, whereas in the line RP 5980-109-12-9-27 desirable gca for grain yield per plot and spikelet fertility (Table 4). Among the testers, CMS 59A was good general combiner for the traits *viz*., grain yield per plot and days to fifty per cent flowering. However, the lines *viz*., SYE 503-78-34-2 (-0.92) and WGL 823 (-0.62) were identified as poor combiners with respect to grain yield per plot. Similarly, among testers, JMS 13A was found to be poor combiner with significant negative gca effect (-0.34) for grain yield per plot.

The lines *viz*., RNR 25776, MTU 1001, RNR 25783 and the tester, CMS 46A were found to have significant positive *gca* effects for test weight, indicates that these lines are good combiners for development of coarse grain hybrids, whereas the parents, RNR 19361, JMS 13A, WGL 32100, WGL 14, SYE 503-78-34-2 and WGL 823 were found to be best combiners for deriving fine grain hybrids. Similarly, the *gca* effects for days to 50 percent flowering indicate that, the parents *viz*., WGL 347, MTU 1001, WGL 32100 and CMS 59A were identified as good for development of early hybrids whereas, the parents, RNR 19361, WGL 14, RP 5980-109-12-9-27 and JMS 13A were found to be desirable for developing late hybrids. In the present study, the effectiveness of choice of parents based on mean performance alone was not appropriate for predicting the combining ability of the parents, hence it was clearly observed in some cases that the lines and testers with good mean performance have not been good general combiners and vice versa, thus the association between mean performance and GCA effects was evident.

The *sca* effects revealed that among thirty hybrids, JMS 13A × RNR 19361 (0.91) recorded highest significant positive effect for grain yield per plot followed by CMS 59A × SYE 503-78-34-2 (0.59), CMS 46A × WGL 347 (0.55) and JMS 13A × RP 5980-109-12-9-27 (0.53) and were considered as desirable (Table 5). However, the hybrids *viz*., CMS 59A × RNR 19361, CMS 46A × RNR 25783, JMS 13A × RNR 25776 and CMS 46A × SYE 503-78-34-2 were identified as poor hybrids with significant negative sca effects.

Two hybrids exhibited significant and negative sca effects for days to flowering *viz*., CMS 59A × WGL 32100 (-4.50) and JMS 13A × MTU 1001 (-3.37) and considered to be highly desirable for earliness. Whereas, two hybrids *viz*., JMS 13A × RNR 19361 (12.23) and JMS 13A × SYE 503-78-34-2 (6.73) exhibited significant positive sca effect for spikelet fertility, an important trait for hybrids. The hybrid, JMS 13A × RP 5980-109-12-9-27 (1.56) recorded significant

Table 5. Estimates of specific combining ability effects in crosses for yield and yield contributing characters in rice.

S. no.	Hybrids	Days to 50 % flowering	Plant height	Effective bearing tillers	Panicle length	Test weight	No of grains per panicle	Spikelet fertility %	Grain yield per plot
1	CMS 46A × RNR 19361	0.03	1.59	1.11	0.32	0.02	-25.28	0.13	0.27
2	CMS 59A × RNR 19361	-1.67	-0.10	0.58	0.65	-0.61	-2.03	-12.33**	-1.18**
3	JMS 13A × RNR 19361	1.63	-1.49	-1.69	-0.97	0.60	27.32	12.20**	0.91**
4	CMS 46A × MTU 1001	1.03	0.62	-1.02	-0.41	-0.50	13.38	1.40	-0.22
5	CMS 59A × MTU 1001	2.33	1.43	0.45	0.87	-0.52	-20.87	-2.22	0.17
6	JMS 13A × MTU 1001	-3.37*	-2.06	0.58	-0.45	1.01	7.48	0.82	0.05
7	CMS 46A × WGL 14	-0.30	-0.01	1.64	0.09	-0.28	23.05	6.35	0.11
8	CMS 59A × WGL 14	1.00	4.30	-0.89	0.87	-0.54	19.80	4.14	0.41
9	JMS 13A × WGL 14	-0.70	-4.29	-0.76	-0.95	0.82	-42.85	-10.49**	-0.52
10	CMS 46A × WGL 823	-0.97	-2.34	0.64	0.40	-1.51*	-4.45	-1.73	0.04
11	CMS 59A × WGL 823	2.83	1.37	-0.99	-1.02	0.69	0.30	4.15	0.33
12	JMS 13A × WGL 823	-1.87	0.98	0.34	0.61	0.83	4.15	-2.42	-0.37
13	CMS 46A × SYE 503-78-34-2	-1.47	-2.24	-0.32	-0.58	0.98	-14.95	-1.28	-0.70*
14	CMS 59A × SYE 503-78-34-2	1.83	0.07	1.45	0.65	-0.34	-27.20	-5.45	0.59*
15	JMS 13A × SYE 503-78-34-2	-0.37	2.18	-1.12	-0.07	-0.64	42.15	6.73*	0.11
16	CMS 46A × RNR 25783	-2.13	1.89	-0.21	-0.56	0.39	-12.12	-0.75	-0.83**
17	CMS 59A × RNR 25783	0.67	-1.10	0.36	-0.08	0.51	18.63	3.59	0.30
18	JMS 13A × RNR 25783	1.47	-0.79	-0.16	0.65	-0.90	-6.52	-2.84	0.53
19	CMS 46A × WGL 347	1.37	2.52	-0.46	1.39	-0.17	-6.95	4.05	0.55*
20	CMS 59A × WGL 347	-0.83	-2.07	0.21	-0.98	-0.52	25.30	-1.27	-0.13
21	JMS 13A × WGL 347	-0.53	-0.46	0.24	-0.40	0.69	-18.35	-2.79	-0.42
22	CMS 46A × WGL 32100	3.70*	4.32	0.81	0.92	-0.41	28.22	-2.73	0.45
23	CMS 59A × WGL 32100	-4.50**	-2.87	0.38	-1.50*	0.02	-30.53	2.10	-0.39
24	JMS 13A × WGL 32100	0.80	-1.46	-1.19	0.58	0.39	2.32	0.63	-0.06
25	CMS 46A × RNR 25776	-2.30	-2.91	-1.16	-0.11	0.92	-4.12	-3.00	0.39
26	CMS 59A × RNR 25776	-0.50	0.90	-0.49	0.67	1.04	17.63	5.14	0.38
27	JMS 13A × RNR 25776	2.80	2.01	1.64	-0.55	-1.96**	-13.52	-2.14	-0.77**
28	CMS 46A × RP 5980-109-12-9-27	1.03	-3.44	-1.04	-1.45	0.56	3.22	-2.43	-0.05
29	CMS 59A × RP 5980-109-12-9-27	-1.17	-1.93	-1.07	-0.12	0.26	-1.03	2.15	-0.49
30	JMS 13A × RP 5980-109-12-9-27	0.13	5.38*	2.11	1.56*	-0.83	-2.18	0.28	0.53*

*, ** Significant at 0.05, 0.01 probability level, respectively.

positive sca effects for panicle length.

The results clearly revealed that none of the hybrids had significant sca effects in desired direction for all the traits studied. These results are in consonance with the findings of Hamdi et. al., (2021). However, JMS 13A × RNR 19361 was found to be good specific combiner for traits *viz*., grain yield per plot and spikelet fertility. Similarly, the hybrid, JMS 13A × RP 5980-109-12-9-27 was found to be good specific combiner for grain yield per plot, plant height and panicle length. The line, WGL 32100 has produced hybrids which have contrasting *sca* effects for days to 50 percent flowering with different testers which clearly indicates that the testers CMS 46A and CMS 59A have very strong positive and negative effects, respectively resulting in development of late and early hybrids.

The relationship among *sca* effects with the *gca* effects of respective parents suggested that the crosses exhibiting significant positive *sca* effects for grain yield had parents with contrasting *gca* effects which could be due to complex interaction of positive and negative alleles of the yield controlling genes. Concurrently, majority of the crosses with significant negative *sca* effects have both the parents with negative *gca* effects and emphasizes the cumulative effect of additive × additive interactions.

Significant variation in the expression of heterobeltiosis and standard heterosis for yield and yield

Table 6. Heterobeltiosis and standard heterosis of hybrids for yield and yield contributing traits in rice.

Hybrids	Days to 50 % flowering			Plant height (cm)			Effective bearing tillers			Panicle length (cm)		
	HB	SVC	SHC	HB	SVC	SHC	HB	SVC	SHC	HB	SVC	SHC
CMS 46A × RNR 19361	3.79	-1.35	2.82	-1.48	6.27	5.04	-31.8**	-4.10	2.63	-3.23	6.44	8.62
CMS 59A × RNR 19361	0.47	-4.50*	-0.47	-2.46	5.21	3.99	-36.24**	-4.10	2.63	-13.46**	7.11	9.30
JMS 13A × RNR 19361	7.11**	1.80	6.10**	-4.14	3.40	2.21	-44.37**	-27.05	-21.93	-10.10*	-1.11	0.91
CMS 46A × MTU 1001	-4.29*	-9.46**	-5.63**	-10.14	0.32	-0.84	-29.41**	-1.64	5.26	-10.65*	0.67	2.72
CMS 59A × MTU 1001	-4.76*	-9.91**	-6.10**	-8.71	1.91	0.74	-23.71*	14.75	22.81	-14.72**	5.56	7.71
JMS 13A × MTU 1001	-6.67**	-11.71**	-7.98**	-12.33	-2.13	-3.26	-15.00	11.48	19.30	-12.43**	-1.33	0.68
CMS 46A × WGL 14	6.34**	-1.80	2.35	-13.43	1.06	-0.11	-8.24	27.87	36.84*	-6.26	3.11	5.22
CMS 59A × WGL 14	5.85**	-2.25	1.88	-8.88	6.38	5.15	-25.89**	11.48	19.30	-14.54**	5.78	7.94
JMS 13A × WGL 14	7.80**	-0.45	3.76	-16.98	-3.08	-4.20	-19.51	8.20	15.79	-8.42	-3.33	-1.36
CMS 46A × WGL 823	-0.48	-6.76**	-2.82	-4.92	-5.53	-6.62	-23.53*	6.56	14.04	-0.61	9.33*	11.56*
CMS 59A × WGL 823	1.44	-4.95*	-0.94	-3.01	-0.85	-2.00	-35.15**	-2.46	4.39	-17.41**	2.22	4.31
JMS 13A × WGL 823	0.48	-5.86**	-1.88	-5.32	-1.59	-2.73	-20.63	4.10	11.40	2.74	8.44	10.66*
CMS 46A × SYE 503-78-34-2	-3.77	-8.11**	-4.23*	-12.15	2.23	1.05	-46.08**	-4.10	2.63	2.22	12.44*	14.74**
CMS 59A × SYE 503-78-34-2	-2.36	-6.76**	-2.82	-9.41	5.42	4.20	-35.48**	14.75	22.81	-5.39	17.11**	19.50**
JMS 13A × SYE 503-78-34-2	-0.94	-5.41**	-1.41	-7.76	7.33	6.09	-49.77**	-10.66	-4.39	6.95	12.89*	15.19**
CMS 46A × RNR 25783	9.52**	-6.76**	-2.82	-1.13	11.90**	10.61**	-23.53*	6.56	14.04	1.82	12.00**	14.29**
CMS 59A × RNR 25783	2.45	-5.86**	-1.88	-3.29	9.46*	8.19*	-23.16*	15.57	23.68	-8.44*	13.33**	15.65**
JMS 13A × RNR 25783	9.55**	-1.80	2.35	-3.29	9.46*	8.19*	-18.44	6.97	14.47	9.47*	15.56**	17.91**
CMS 46A × WGL 347	-4.83*	-11.26**	-7.51**	-4.50	4.89	3.68	-27.65**	0.82	7.89	-1.21	8.67	10.88*
CMS 59A × WGL 347	-8.70**	-14.86**	-11.27**	-8.27	0.74	-0.42	-26.43**	10.66	18.42	-21.36**	-2.67	-0.68
JMS 13A × WGL 347	-4.83*	-11.26**	-7.51**	-7.02	2.13	0.95	-22.39*	6.56	14.04	-6.32	-1.11	0.91
CMS 46A × WGL 32100	9.28**	-4.50*	-0.47	-1.34	5.63	4.41	-22.35*	8.20	15.79	-6.06	3.33	5.44
CMS 59A × WGL 32100	-5.88**	-13.51**	-9.86**	-7.79	-1.28	-2.42	-27.52**	9.02	16.67	-25.85**	-8.22	-6.35
JMS 13A × WGL 32100	5.53*	-5.41**	-1.41	-6.70	-0.11	-1.26	-33.73**	-8.20	-1.75	-5.26	0.00	2.04
CMS 46A × RNR 25776	2.55	-9.46**	-5.63**	-12.08	7.12	5.88	-33.53**	-7.38	-0.88	-5.94	23.11**	25.62**
CMS 59A × RNR 25776	-1.47	-9.46**	-5.63**	-8.16	11.90**	10.61**	-31.88**	2.46	9.65	-3.90	25.78**	28.34**
JMS 13A × RNR 25776	8.04**	-3.15	0.94	-7.46	12.75**	11.45**	-12.96	15.57	23.68	-8.83*	19.33**	21.77**
CMS 46A × RP 5980-109-12-9-27	8.96**	-1.35	2.82	-5.28	-2.87	-3.99	-26.47*	2.46	9.65	-6.06	3.33	5.44
CMS 59A × RP 5980-109-12-9-27	3.43	-4.95*	-0.94	-3.01	-0.53	-1.68	-29.16**	6.56	14.04	-12.39**	8.44	10.66*
JMS 13A × RP 5980-109-12-9-27	9.95**	-0.45	3.76	2.86	6.91	5.67	-7.94	28.28	37.28*	8.84*	14.89**	17.23**

*, ** Significant at 0.05, 0.01 probability level, respectively

Table 6. *Continued...*

Hybrids	Test weight (g)			No of grains per panicle			Spikelet fertility %			Grain yield per plot (g)		
	HB	SVC	SHC	HB	SVC	SHC	HB	SVC	SHC	HB	SVC	SHC
CMS 46A × RNR 19361	1.74	-19.61	22.06**	-5.91	16.22	-24.16	-18.14**	-17.79**	-14.16**	44.97**	6.60	-6.56
CMS 59A × RNR 19361	-11.20*	-26.06	12.27	-2.84	20.00	-21.69	-32.34*8	-32.05**	-29.04**	31.61**	-7.22	-18.67**
JMS 13A × RNR 19361	9.41	-30.39	5.68	12.41	66.49**	8.64	-3.20	-2.79	1.51	41.91**	14.97*	0.78
CMS 46A × MTU 1001	-8.96*	-2.03	48.75**	45.86	23.78	-19.22	-3.43	-0.43	3.97	27.29**	-6.06	-17.66**
CMS 59A × MTU 1001	-12.58**	-5.92	42.84**	42.23	-3.51	-37.04**	-8.06	-5.20	-1.01	52.78**	12.75	-1.17
JMS 13A × MTU 1001	-15.33**	-8.88	38.34**	-3.83	42.43*	-7.05	-2.03	1.02	5.48	18.04*	-4.37	-16.17**
CMS 46A × WGL 14	3.75	-18.02	24.47**	28.14	37.84	-10.05	-4.20	-2.25	2.07	43.25**	10.70	-2.97
CMS 59A × WGL 14	-7.42	-22.91	17.05*	18.34	27.30	-16.93	-7.41	-5.52	-1.34	65.40**	27.81**	12.03*
JMS 13A × WGL 14	15.36*	-26.61	11.43	-16.24	24.05	-19.05	-19.85**	-18.22**	-14.61**	18.92*	-3.65	-15.55*
CMS 46A × WGL 823	-0.46	-21.34	19.43**	17.20	19.73	-21.87	-9.73	-10.56	-6.60	26.42**	-7.04	-18.52**
CMS 59A × WGL 823	1.09	-15.82	27.81**	11.11	13.51	-25.93*	-4.27	-5.14	-0.95	58.41**	9.98	-3.59
JMS 13A × WGL 823	18.38**	-24.68	14.35*	-1.28	46.22*	-4.59	-8.38	-9.22	-5.20	1.87	-17.47*	-27.66**
CMS 46A × SYE 503-78-34-2	11.59*	-11.82	33.88**	-29.25	-11.08	-41.98**	-18.37**	-21.44**	-17.96**	1.09	-25.67**	-34.84**
CMS 59A × SYE 503-78-34-2	-5.38	-21.21	19.62**	-41.51*	-26.49	-52.03**	-23.94*8	-26.80**	-23.56**	55.85**	9.18	-4.30
JMS 13A × SYE 503-78-34-2	7.01	-31.92	3.37	-4.38	41.62*	-7.58	-7.29	-10.77*	-6.83	5.83	-14.26*	-24.84**
CMS 46A × RNR 25783	2.65	-4.10	45.60**	1.37	0.27	-34.57**	-7.61	-6.32	-2.18	19.61	3.83	-8.98
CMS 59A × RNR 25783	-0.86	-7.38	40.62**	9.29	8.11	-29.45*	-3.91	-2.57	1.73	56.37**	35.74**	18.98**
JMS 13A × RNR 25783	-17.36**	-22.80	17.21*	-15.51	25.14	-18.34	-7.77	-6.48	-2.35	43.74	24.78**	9.38
CMS 46A × WGL 347	7.52	-15.04	29.00**	-9.30	18.65	-22.57	-7.46	-5.63	-1.45	37.51**	14.35*	0.23
CMS 59A × WGL 347	-4.32	-20.33	20.97**	-2.69	27.30	-16.93	-13.93**	-12.22*	-8.34	37.30**	14.17*	0.08
JMS 13A × WGL 347	17.60*	-24.66	14.39*	-9.31	34.32	-12.35	-12.61*	-10.88*	-6.94	12.97	-6.06	-17.66**
CMS 46A × WGL 32100	1.55	-19.75	21.84**	7.36	61.62**	5.47	-6.29	-6.59	-2.46	54.91**	13.90*	-0.16
CMS 59A × WGL 32100	-5.99	-21.72	18.85**	-19.57	21.08	-20.99	-1.99	-2.30	2.01	51.15**	10.87	-2.81
JMS 13A × WGL 32100	10.64	-29.61	6.87	12.57	69.46**	10.58	-0.59	-0.91	3.47	25.74**	1.87	-10.70
CMS 46A × RNR 25776	-7.34*	8.65	64.96**	21.66	3.24	-32.63*	-9.09	-6.22	-2.07	48.48**	9.18	-4.30
CMS 59A × RNR 25776	-10.17**	5.33	59.92**	51.15	6.22	-30.69*	-1.51	1.61	6.10	69.63**	20.94**	6.02
JMS 13A × RNR 25776	-29.04**	-16.79	26.33**	-18.98	20.00	-21.69	-6.18	-3.22	1.06	5.50	-14.53*	-25.08**
CMS 46A × RP 5980-109-12-9-27	15.18**	-8.99	38.18**	16.71	17.03	-23.63	-6.59	-6.59	-2.46	53.70**	13.01	-0.94
CMS 59A × RP 5980-109-12-9-27	3.23	-14.04	30.51**	5.66	5.95	-30.86*	-2.57	-2.57	1.73	68.55**	17.02*	2.58
JMS 13A × RP 5980-109-12-9-27	0.47	-28.09	9.18	-8.21	35.95	-11.29	-1.61	-1.61	2.74	48.40**	20.23**	5.39

*, ** Significant at 0.05, 0.01 probability level, respectively; HB = Heterobeltiosis; SHC = Standard heterosis over hybrid (27 P 63); SVC = Standard heterosis over variety (MTU 1001).

Table 7. Performance of promising hybrids identified along with per se performance, gca, sca and heterosis for grain yield per plot (kg) in rice.

Hybrid	Mean	*gca* of line	*gca* of tester	*sca* of hybrid	Standard heterosis (%)	
					Over MTU 1001 variety	Over 27P63 hybrid
CMS 59A × RNR 25783	7.62	0.86**	0.50**	0.30	35.74**	18.98**
CMS 59A × WGL 14	7.17	0.31	0.50**	0.41	27.81**	12.03*
JMS 13A × RNR 25783	7.00	0.86**	-0.34**	0.53	24.78**	9.38
CMS 59A × RNR 25776	6.78	-0.06	0.50**	0.38	20.94**	6.02
JMS 13A × RP 5980-109-12-9-27	6.75	0.59**	-0.34**	0.53*	20.23**	5.39

* Significant at 0.05 level, **Significant at 0.01 level

components was observed for all cross combinations (Table 6). Heterobeltiosis over better parent ranged from 1.09 to 69.63 % for grain yield per plot. Twenty-three hybrids showed significant positive heterobeltiosis for this trait. Highest significant positive heterobeltiosis was recorded by CMS 59A × RNR 25776 followed by CMS 59A × RP 5980-109-12-9-27, CMS 59A × WGL 14, CMS 59A × WGL 823, CMS 59A × RNR 25783, CMS 59A × SYE 503-78-34-2, CMS 46A × WGL 32100, CMS 46A × RP 5980-109-12-9-27, CMS 59A × MTU 1001 and CMS 59A × WGL 32100.

For standard heterosis over variety (MTU 1001), ten hybrids recorded positive significant heterosis. Highest significant positive heterosis was recorded by CMS 59A × RNR 25783 followed by CMS 59A × WGL 14, JMS 13A × RNR 25783, CMS 59A × RNR 25776, JMS 13A × RP 5980-109-12-9-27, CMS 59A × RP 5980-109-12-9-27, JMS 13A × RNR 19361, CMS 46A × WGL 347, CMS 59A × WGL 347 and CMS 46A × WGL 32100.

Two hybrids *viz*., CMS 59A × RNR 25783 and CMS 59A × WGL 14 recorded positive significant heterosis over the standard hybrid check (27P63) and these were identified as potential hybrids for most of the traits studied based on their per se performance and heterosis estimates.

For test weight , 24 hybrids recorded significant positive standard heterosis over hybrid check (27P63), whereas 28 hybrids have shown negative heterosis over varietal check (MTU 1001) which clearly mention that the majority of the test hybrids have intermediate test weight as compared to hybrid and varietal checks. Further, none of the test hybrids have recorded significant positive standard heterosis over hybrid check for number of grains per panicle and spikelet fertility. These findings are consistent for grain yield with those of Saravanan et al. (2008), Kumar et al. (2012), Singh et al. (2013), Sharma et al. (2013), Pratap et al. (2013), Bhati et al. (2015), Satheesh kumar et al. (2016), Yogita et al. (2016), Galal Bakr Anis et al. (2017) Manjunath et al. (2020), Virender et al. (2020b) and Babu Ramesh and Sreelakshmi (2020).

Based on *per se* performance and heterotic estimates, five hybrids *viz*., CMS 59A × RNR 25783, CMS 59A × WGL 14, JMS 13A × RNR 25783, CMS 59A × RNR 25776, JMS 13A × RP 5980-109-12-9-27 were identified as superior with more than 5% yield advantage over hybrid check, 27P63 and 20% over varietal check, MTU 1001 (Table 7). It was also evident that all these five superior crosses involve at least one parent with significant positive gca effect for grain yield per plot. Nevertheless, among them only one hybrid had significant positive sca effect.

CONCLUSION

The present investigation of evaluating 30 cross combinations along with their parents and checks revealed that significant variation was observed among lines, testers and hybrids. The different cross combinations were found superior for various traits but none of them was found superior for all the traits. This study concluded that among the lines, RNR 25783, RP 5980-109-12-9-27 and the tester, CMS 59A exhibited significant and positive gca effect for grain yield per plot and was identified as best combiners for grain yield. SCA variances were lower than GCA variances for most of the characters and concurrently the predictability ratios were near to unity for days to 50 per cent flowering, plant height, panicle length, test weight number of grains per panicle and grain yield per plot inferring the predominance of additive gene action in governing these traits. However, effective bearing tillers and spikelet fertility were found to be controlled by non-additive genetic components. Five heterotic hybrids *viz*., CMS 59A × RNR 25783, CMS 59A × WGL 14, JMS 13A × RNR 25783, CMS 59A × RNR 25776, JMS 13A × RP 5980-109-12-9-27 were identified as promising with more than 5% and 20% yield advantage over hybrid and varietal checks, respectively. These hybrids could be evaluated in multi-environments to identify most stable heterotic hybrids for commercial release.

REFERENCES

Sharma A and Jaiswal HK (2021). A study of gene action for yield and quality traits of Basmati rice (*Oryza sativa* L.) following Hayman's graphical approach. Oryza 58(1): 8-14

Aly RSH (2013). Relationship between combining ability of grain yield and yield components for some newly yellow maize inbred lines via line×tester analysis. Alexandria Journal of Agriculture Research 58(2): 115-124

Babu Ramesh P and Sreelakshmi Ch (2020). Study on heterosis for yield and quality traits in rice (*Oryza sativa* L). Oryza 57(3): 190-198

Baker RJ (1978). Issue in diallel analysis. Crop Science 18: 533-35

Bhati PK, Singh SK, Singh R, Sharma A and Dhurai SY (2015). Estimation of heterosis for yield and yield related traits in rice (*Oryza sativa* L). SABRAO Journal of Breeding and Genetics 47 (4): 467-474

Fonseca S and Patterson FL (1968). Hybrid vigor in a seven-parent diallel cross in common winter wheat (*Triticum aestivum* L). Crop Science 8: 85

Galal Bakr Anis, Ahmed Ibrahem El-Sherif, Hytham Freeg and Elsaed Farok Arafat (2017). Evaluation of some hybrid combinations for exploitation of two-line system heterotic in rice (*Oryza sativa* L). International Journal of Bio-Science, Agriculture and Technology 8(1): 1-8

Ganapati RK, Rasul MG, Sarker U, Singha A, Faruquee M (2020). Gene action of yield and yield contributing traits of submergence tolerant rice (*Oryza sativa* L.) in Bangladesh. Bulletin of the National Research Centre 44: 8

Gramaje LV, Caguiat JD, Enriuez JOS, Cruz QDD, Millas RA, Carampatana JE and Tabanao DAA (2020). Heterosis and combining ability analysis in CMS hybrid rice. Euphytica pp. 216-14 DOI: https://doi.org/10.1007/s10681-019-2542-y

Gupta HS, Dwivedi JL, Sridhar R, Samaresh Kundu, Birthal PS, Subba Rao LV and Singh TVK (2020). Quinquennial Review Team report of ICAR-Indian Institute of Rice Research (2012-2020) and All India Co-ordinated Rice Improvement Project

Hamdi F El-Mowafi, Muneera DF AlKahtani, Rizk M Abdallah, Amr M Reda, Kotb A Attia, Mahmoud A El-Hity, Hend E El-Dabaawy, Latifa Al Husnain, Talal K Al-Ateeq and Mohamed A EL-Esawi (2021). Combining Ability and Gene Action for Yield Characteristics in Novel Aromatic Cytoplasmic Male Sterile Hybrid Rice under Water-Stress Conditions. Agriculture 11(3): 226

Kempthorne O (1957). An introduction genetic statistic. John Wiley and Sons, New York

Kumar Chetan MR, Devaraju and Prasad SR (2012). Characterization of rice hybrids and their parental lines based on morphological traits. Oryza 49(4): 302-304

Kumar S, Dwivedi SK, Singh SS, Jha SK, Lekshmy S, Elanchezhian R, Singh ON and Bhatt BP (2014). Identification of drought tolerant rice genotypes by analyzing drought tolerance indices and morpho-physiological traits. SABRAO Journal of Breeding and Genetics 46 (2): 217-230

Khush G (2013). Strategies for increasing the yield potential of cereals: case of rice as an example. Plant Breeding 132: 433-436

Lin SC and Yuan LP (1980). Hybrid rice breeding in China, In: Innovative approaches to rice breeding. Manila (Philippines). International Rice Research Institute: 35-51

Manjunath K, Chandra Mohan Y, Gouri Shanker V, Balram M and Krishna L (2020). Combining ability and heterosis studies for selecting elite parents and hybrids in rice (*Oryza sativa* L.). The Bioscan 14(2): 101-108

Parimala K, Bhadru D and Surendar Raju CH (2018). Combining ability and heterosis for grain yield and its components in hybrid rice (*Oryza sativa* L.). Electronic Journal of Plant Breeding 9 (1): 244-255

Pratap N, Raj Shekhar, PK Singh and SK Soni (2013). Combining ability, gene action and heterosis using

CMS lines in hybrid Rice (*Oryza sativa* L.). The Bioscan 8(4): 1521-1528

Rai M 2009. Hybrid culture in sustainable agriculture In: Proceedings of the 9th Agricultural Science Congress, held at SKUAST-K, Shalimar, Srinagar, 22-24th June pp. 3-17

Ramesh C, Damodar Raju Ch, Surendar Raju Ch and Rama Gopal Varma (2017). Combining ability and gene action in hybrid rice (*Oryza sativa* L.). International Journal of Pure and Applied Bioscience 6(1): 497-510

Rukmini Devi K, Venkanna V, Satish Chandra B and Hari Y (2018). Gene Action and Combining Ability for Yield and Quality Traits in Rice (*Oryza sativa* L.) using Diallel Analysis. International Journal of Current Microbiology and Applied Science 7(1): 2834-2843

Sanghera GS and Wani SH (2008). Innovative approaches to enhance genetic potential of rice (*Oryza sativa* L.) for higher productivity under temperate conditions of Kashmir. The Journal of Plant Science Research 24: 99-113

Sanjeev Kumar, Singh HB and Sharma JK (2007). Combining ability analysis for grain yield andother associated traits in rice. Oryza 44 (2):108- 114

Saravanan KT, Sabesan and Kumar ST (2008). Heterosis for yield and yield components in rice (*Oryza sativa* L). Advances in Plant Science 21(1): 119-121

Sarker U, Biswas PS, Prasad B and Khaleque MMA (2002). Heterosis and genetic analysis in rice hybrid. Pakistan Journal of Biological Science 5(1): 1-5

Satheesh kumar P, Saravanan K and Sabesan T (2016). Selection of superior genotypes in rice (*Oryza sativa* L.) through combining ability analysis. International Journal of Agricultural Sciences 12(1): 15-21

Sharma SK, Singh SK, Nandan R, Amita Sharma, Ravinder Kumar, Kumar V and Singh MK (2013). Estimation of heterosis and inbreeding depression for yield and yield related traits in rice (*Oryza sativa* L). Molecular Plant Breeding 29(4): 238-246

Singh SK, Vikash Sahu, Amita Sharma and Pradeep Bhati Kumar (2013). Heterosis for yield and yield components in rice (*Oryza sativa* L). Bioinfolet 10(2B): 752-761

Sprague GA and Tatum LA (1942). General vs specific combining ability in single crosses of corn. Journal of the American Society of Agronomy 34: 923-932

Swaminathan MS (2006). An evergreen revolution. Crop Science 46: 2293-2303

Vineeta Singh, Dwivedi DK, Na Khan, Verma RL, Manish Kumar, Debarchana Jena, Diptibala Rout, Pandurang Arsode, Sanghamitra Samantaray (2020). Combining ability analysis for yield and contributing traits in short duration rice (*Oryza sativa* L.). Oryza 57(4): 271-276

Viraktamath RC and MS Ramesha (1996). Synchronization, Prediction and Adjustment Flowering. In: Hybrid Rice Technology (AM IIyas, BC Viraktamath, MS Ramaesha and CHM Kumar, eds). Directorate of Rice Research, Hyderabad pp.77-83

Virender Jeet Singh T, Damodar RajuCH, Chandra Mohan Y, Jagadeeshwar R, Balram M and Krishna L (2020a). Combining ability and heterosis studies for selecting elite parents and hybrids in rice (*Oryza sativa* L.). Journal of Rice Research 13(1): 18-32

Virender Jeet Singh T, Damodar Raju CH, Chandra Mohan Y, Jagadeeshwar R, Balram M and Krishna L (2020b). Combining ability and heterosis over locations for yield and yield components in hybrid rice (*Oryza sativa* L.) The Journal of Research PJTSAU 48 (3&4): 1-10

Virmani SS, Mao CX and Hardy B (2003). Hybrid rice for food security, poverty alleviation, and environmental protection. In: Proceedings of the 4th International Symposium on Hybrid Rice, held at Hanoi, Vietnam, 14-17th May pp. 407

Yogita S, Sahu P, Sharma D and Kujur N (2016). Heterosis and combining ability analysis for grain yield and its component traits for the development of red rice (*Oryza sativa* L.) hybrids. The Ecoscan Special issue IX : 475-485

Yuan LP, Virmani SS and Mao CX (1989). Hybrid rice - achievements and future outlook. In: Progress in irrigated rice research. IRRI, Manila, Philippines, pp. 219-235

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Study of gene action for yield and quality traits in rice (*Oryza sativa* L.)

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ABSTRACT

The present experiment was conducted to study the gene action, heterosis, inbreeding depression and heritability for different yield and quality traits in the cross WGL 48684 x IR 36. Analysis of heterosis revealed that most of the traits show duplicate type of epistasis which is non- fixable and cannot be exploited through the general breeding procedures of self pollinated crops. Additive gene effect and complementary epistasis plays a lead role for plant height, number of ear bearing tillers per plant, kernel breadth and amylase content and they can easily be exploited for developing fixed lines. Significant inbreeding depression was found for ear bearing tillers per plant, SPAD Chlorophyll Meter Reading (SCMR), harvest index, grain yield, head rice recovery and kernel elongation ratio. Therefore, few cycles of recurrent selection followed by pedigree method would be effective and useful to utilize all types of gene effects by maintaining considerable heterozygosity through mating of selected plants in early segregating generations.

Key words: *Rice, heritability, gene action, inbreeding depression, heterosis*

INTRODUCTION

The success of any breeding programme lies in thorough understanding of the genetic architecture of the genotypes and the nature of the gene action. Yield is a complex and polygenic trait which is a final product affected by the large number of its component traits (Renukadevi and Subbalakshmi, 2006). The genetic improvement primarily depends on the effectiveness of selection among progenies that differ in genetic value. The additive and dominant effects and their interactions are known as gene actions and are reported to be associated with breeding value. Generation mean analysis is used to estimate the component variance which provides information about the predominant type of gene action for the different characters (Hayman, 1958; Jinks & Jones, 1958).

Generation mean analysis provides information about the type of inter-allelic interaction besides genetic components of variance. Generation mean analysis is a tool for designing the most appropriate breeding approaches to develop crop varieties with desired traits and is commonly used in studies on the inheritance of quantitative traits (Uzokwe et al., 2017). This type of analysis provides information on the relative importance of the average effects of the genes *viz*., additive effects (d), dominance effects (h) and effects due to non-allelic gene interactions *viz*., additive x additive (i), additive x dominance (j) and dominance x dominance (l) (Subbulakshmi et al., 2016; Uzokwe et al., 2017). The objective of this study was to understand the gene action of yield and its attributing characters in rice through generation mean analysis.

MATERIALS AND METHOD

The study was carried at department of genetics and plant breeding, Agricultural Research Station, Nellore, ANGRAU, Andhra Pradesh during the four consecutive *rabi* seasons of 2014, 2015, 2016 and 2017 respectively. The crossing was accomplished in 2014 between the blast susceptible rice variety WGL 48684 as a recurrent parent and blast resistant variety IR 36 as a donor parent. In 2017 growing season, F1 seeds (WGL 48684 x IR 36) were planted. The crosses were accomplished with both the parents to obtain BC1 and BC2 generations, therefore, the six populations P1, P2, F1, F2, BC1 and BC2 were planted in 2017 *rabi* season. The experiment was planned in a Randomized block design with three replications. 75 plants of each P1and P2; 50 plants of F1, 100 plants of BC1 and BC2 and 200 plants of F2 population per replication were taken at random for subsequent measurements of the following yield traits *viz*., days to 50% flowering, days to maturity, plant height, panicle length, number of ear bearing tillers per plant, number of filled grains per panicle, number of unfilled grains per panicle, test weight, harvest index, SPAD Chlorophyll Meter Reading (SCMR) and grain yield; and quality traits *viz*., kernel length, kernel breadth, kernel L/B ratio, hulling%, Milling%, head rice recovery, water uptake, volume expansion ratio, kernel elongation ratio, gel consistency, alkali spreading value and amylose content.

Statistical analysis

The analysis of variance (ANOVA) was carried out through Statistics10. To confirm the data adequacy, Mather's (1949) scaling test (A, B, C, and D) was performed and confirmation of additive-dominance model as reported by Singh and Chaudhary (2012).

$A = P1 + F1 - 2BC1 = \frac{1}{2}([i]-[j]+[l])$

$B = P2 + F1 - 2BC2 = \frac{1}{2}([i]+[i]+[l])$

$C = P1 + P2 + 2F1 - 4F2 = 2(i) + (l)$

$D = 2F2 - BC1 - BC2$

Estimates of various gene effects, allelic interaction, and their test of significance were computed by a six parameter model of Hayman (1958) and Jinks and Jones (1958) by the following equations:

m = Mean = F2

d = Additive effect = BC1-BC2

h = Dominance effect = $2BC1 + 2BC2 + F1 - 4F2 - (1/2)P1 - 1/2\ P2$

i = Additive x Additive genetic interaction = $2BC1 + 2BC2 - 4F2$

j = Additive Dominance genetic interaction = $2BC1 - P1 - 2BC2 + P2$

l = Dominance genetic interaction = $P1 + P2 + 2F1 + 4F2 - 4BC1 - 4BC2$

Blast reaction

The observation on disease reaction was recorded when the susceptible check was severely infected by blast. Individual plants in each generation *viz*., parents (P1, P2), F1, F2, BC1 and BC2 populations were scored based on leaf blast severity following standard Evaluation system (SES, IRRI, 1996) on 0-9 scale. Similarly, the scoring was repeated 10-15 days after the first observation to avoid the escapes. Evaluation of blast resistance in all the generations was done for resistance and susceptible reactions based on the disease reaction scale where >3 was considered as susceptible. The maximum scores of each plant from two observations were considered for categorizing resistance and susceptible reaction. Chi-square test was employed to test goodness of fit of observed and expected frequencies in the segregating generations.

RESULTS AND DISCUSSION

Mean analysis of different generations

The traits *viz*., number of ear bearing tillers per plant, number of filled grains per panicle, SPAD Chlorophyll Meter Reading (SCMR), grain yield, harvest index, hulling%, head rice recovery and volume expansion ratio were showing dominance over the parents. Whereas days to 50% flowering, days to maturity, plant height, panicle length, test weight, kernel length, kernel breadth, kernel L/B ratio, milling%, water uptake, kernel elongation ratio, gel consistency, alkali spreading value and amylose content exhibited lesser values than both the parents. Similar results were already reported by Rao et al. (2017) and Ganapathi et al. (2020) in rice.

Estimation of heterosis and inbreeding depression

For the progenies of WGL 48684 x IR 36, the mid parent heterosis and better parent heterosis and inbreeding depression were calculated for 23 traits and the results were presented in Table 1. Significant positive heterosis (MP and BP) was observed for the traits *viz*., number of ear bearing tillers per plant, test weight, SPAD Chlorophyll Meter Reading (SCMR), harvest index. But in case of ear bearing tillers the component h was not significant. Significant positive heterosis but negative h was observed with the trait harvest index. But ear bearing tillers is influenced by additive x additive and dominance x dominance significant interactions. For harvest index even though dominant, dominant x

Table 1. Estimates of heterosis as deviation from mid-parent (MP) and better parent (BP) and inbreeding depression (%) for yield and contributing traits.

S. no.	Character	Mid Parent heterosis	Better Parent heterosis	Inbreeding depression
1	Days to 50% flowering	-3.80	-4.84	0.71
2	Days to maturity	-3.23*	-4.00*	-2.94
3	Plant height	-4.09	-5.54	-30.64
4	Panicle length	-7.69	-10.28	-11.08
5	Number of ear bearing tiller s per plant	43.45**	40.00**	39.51
6	Number of filled grains per panicle	0.03	-9.92	-5.84
7	Number of unfilled grains per panicle	12.81	-14.23	-48.84
8	Test weight (g)	10.25*	4.27	-10.50
9	SPAD Chlorophyll Meter Reading (SCMR)	20.84**	17.31**	23.14
10	Grain yield	-24.45**	-34.57**	30.89
11	Harvest index	63.96**	63.71**	6.82
12	Kernal length	-6.25*	-13.04**	4.76
13	Kernal breadth	-7.4	-12.95*	6.26
14	Kernal L/B ratio	3.07*	1.19	-4.49
15	Hulling %	-4.78	-5.52	1.21
16	Mlling%	1.33	0.00	2.14
17	Head rice recovery	2.48	1.98	16.21
18	Water uptake	0.25	-6.65	-20.12
19	Volume expansion ratio	4.29	2.82	-37.17
20	Kernal elongation ratio	-3.36	-4.73	12.54
21	Gel consistency	-26.07*	-37.1**	10.57
22	Alkali spreading value	-45.95**	-50.0**	1.19
23	Amylose content	-21.61**	-22.97**	-24.33

dominant interactions were negative additive x additive interactions plays a significant role in controlling the trait.

Regarding plant height, number of ear bearing tillers per plant, kernel breadth and amylose content additive gene effect and complementary epistasis have been recorded having reinforcing effect and can easily be exploited for developing fixed lines. Significant inbreeding depression was found for ear bearing tillers per plant, SPAD Chlorophyll Meter Reading (SCMR), harvest index, grain yield, head rice recovery and kernel elongation ratio.

Analysis of heterosis also revealed that most of the traits show duplicate type of epistasis. This is a non fixable and which cannot be exploited through the general breeding procedures of self pollinated crops. (Bhadra and Dey, 1985). Even more, some traits were not similar to the expected heterosis inadequacy of the model given by Mather and Jinks (1971). In the present study positive better parent heterosis was observed for number of ear bearing tillers per plant, SPAD Chlorophyll Meter Reading (SCMR), harvest index which was in agreement with the earlier findings of Rukminidevi et al. (2014) for ear bearing tillers per plant, Audilakshmi and Raghavareddy (2012) for harvest index and Bhati et al. (2015) in rice.

The scaling tests

The results of the scaling tests for the cross WGL 48684 x IR 36 was depicted in the Table 2. Estimated significant deviation from zero indicated the presence of non allelic interactions for all the traits studied.

For plant height all A, B, C and D scale tests were found to be significantly positive whereas these 4 tests were significantly negative for the traits ear bearing tillers per plant and kernel elongation ratio. The scaling tests A, B and C were positive and D was found to be negative for panicle length, number of unfilled grains per panicle, grain yield and kernel length while it was reverse in case of gel consistency and alkali spreading value. For the traits days to 50% flowering, days to maturity and milling%, A and B tests were positive and C, D tests were negative. This condition is vice-versa in case of harvest index, kernel L/B ratio, number of filled grains per panicle, water uptake and volume expansion ratio. Only A test was positive and

Table 2. Scale estimation of different traits in rice.

Sl. no.	Character	Scale effects ± standard error			
		A	B	C	D
1	Days to 50% flowering	7.40**	5.30**	-22.27**	-17.48**
2	Days to maturity	8.80**	10.40**	-5.67*	-12.43**
3	Plant height	18.35**	28.95**	80.50**	16.60**
4	Panicle length	6.19**	5.83**	4.89*	-3.57**
5	Number of ear bearing tiller s per plant	-8.90**	-3.85**	-18.88**	-3.07**
6	Number of filled grains per panicle	-95.80**	-10.75	136.50**	121.53**
7	Number of unfilled grains per panicle	46.10**	34.05**	26.02**	-27.07**
8	Test weight (g)	10.15**	-2.91**	-2.76**	-5.00**
9	SPAD Chlorophyll Meter Reading (SCMR)	1.24	-4.05**	-35.27**	-16.23**
10	Grain yield	-23.52**	-17.48**	-26.18**	7.41*
11	Harvest index	-28.65**	-27.20**	4.69	30.27**
12	Kernal length	-0.70**	-1.78**	-1.91**	0.28
13	Kernal breadth	-0.15	-0.10	-0.44*	-0.09
14	Kernal L/B ratio	-0.11	-0.74**	0.13	0.49*
15	Hulling %	-0.60	3.15**	0.95	-0.80
16	Mlling%	6.05**	11.30**	-11.60**	-14.47**
17	Head rice recovery	8.73**	-16.70**	-35.95**	-13.99**
18	Water uptake	-52.25**	-144.75**	114.08**	155.54**
19	Volume expansion ratio	-0.60**	-0.32	5.81**	3.36**
20	Kernal elongation ratio	-0.39**	-0.32**	-0.86**	-0.08
21	Gel consistency	-47.15**	-22.10**	-43.84**	12.71**
22	Alkali spreading value	-2.68**	-3.13**	-4.31**	0.75*
23	Amylose content	3.57**	3.30**	8.08**	0.60

the rest of the test were negative in case of test weight, SPAD Chlorophyll Meter Reading (SCMR) and head rice recovery.

Gene action

The results of gene action along with mean effects were given in the Table 3 indicates the highly significant mean effects for all the traits studied. All the characters in the present study showed significant scaling tests. Hence, six parameter model (additive, dominance and interactions) was identified as best fit models to study the estimates of different genetic components.

The estimates of d (additive component) were positively and highly significant for days to 50% flowering, days to maturity, plant height, number of filled grains per panicle, number of ill filled grains per panicle and alkali spreading value. Singh and Patel (2020) also recoded similar results for days to maturity, days to 50% flowering, plant height and filled grains per panicle in rice. But water uptake, gel consistency was negatively significant.

The estimates h (dominance component) were significantly positive for the traits *viz*., days to maturity, days to 50% flowering, panicle length, number of unfilled grains per panicle, test weight, SPAD Chlorophyll Meter Reading (SCMR), hulling%, milling% and head rice recovery. Whereas it was negatively significant for the traits *viz*., number of filled grains per panicle, grain yield, harvest index, water uptake, gel consistency and volume expansion ratio. These results are in agreement with the earlier findings of Lingaiah et al (2020) for days to 50% flowering, panicle length, grain yield and test weight in rice.

The estimates of i (additive x additive)were positively and highly significant for ear bearing tillers per plant, number of filled grains per panicle, grain yield, harvest index, kernel length, kernel L/B ratio, water uptake, kernel elongation ratio, volume expansion ratio, gel consistency and alkali spreading value. It was highly negatively significant for days to 50% flowering, days to maturity, panicle length, number of unfilled grains per panicle, test weight, SPAD Chlorophyll Meter Reading (SCMR), milling%, head rice recovery and

Table 3. Estimation of component of generation means for different traits in rice.

S. no.	Character	m	d	h	i	j	l	Epistasis
1	Days to 50% flowering	61.53**	6.45**	72.73**	-47.67**	34.97**	1.05	D
2	Days to maturity	103.48**	7.30**	59.13**	-44.07**	24.87**	-0.80	D
3	Plant height	107.70**	1.00**	-22.45	-14.10	-33.20**	-5.30	C
4	Panicle length	13.66**	-0.60*	24.54**	-19.17**	7.14**	0.18	D
5	Number of ear bearing tiller s per plant	7.04**	0.32	4.14	6.62*	6.13**	-2.52	C
6	Number of filled grains per panicle	392.45**	16.90**	-547.16**	349.60**	-243.05**	-42.52	D
7	Number of unfilled grains per panicle	-33.73**	6.50**	184.92**	-134.28**	54.13**	6.03	D
8	Test weight (g)	8.13**	-2.80**	22.97**	-17.25**	10.01**	6.53	D
9	SPAD Chlorophyll Meter Reading (SCMR)	10.76**	-2.47**	66.51**	-29.65**	32.46**	2.64	D
10	Grain yield	47.19**	0.01	-52.56**	55.81**	-14.82*	-3.02	D
11	Harvest index	115.53**	-1.65*	-165.51**	116.38**	-60.53**	-0.73	D
12	Kernal length	6.98**	-0.50**	-3.97**	3.03**	-0.56	0.54	D
13	Kernal breadth	1.69**	-0.03	0.15	0.06	0.19	-0.02	C
14	Kernal L/B ratio	4.42**	-0.20**	-3.05**	1.84**	-0.98*	0.32	D
15	Hulling %	72.52**	-1.38**	8.08*	-4.15	1.60	-1.88	D
16	Mlling%	38.30**	-0.35	72.20**	-46.30**	28.95**	-2.63	D
17	Head rice recovery	29.49**	-0.23	48.98**	-20.02**	27.99**	12.72	D
18	Water uptake	539.58**	-10.50**	-844.05**	508.08**	-311.08**	46.25	D
19	Volume expansion ratio	10.24**	0.04	-14.21**	7.65**	-6.73**	-0.14	D
20	Kernal elongation ratio	1.41**	-0.02	-0.45*	0.55**	0.15	-0.03	D
21	Gel consistency	78.16**	-9.25**	-133.72**	94.66**	-25.41**	-12.52	D
22	Alkali spreading value	1.72*	0.28**	-1.27	4.04**	2.70**	0.17	D
23	Amylose content	25.39**	-0.43	-0.73	-5.67*	-1.20	0.13	C

amylose content. The estimates of j were non-significant for most of the traits under study. Ganapathi et al. (2020) for days to maturity, number of filled grains per panicle and grain yield, Solanke et al. (2019) for test weight and Subbulakshmi et al. (2016) for amylose content recorded similar results in rice.

The estimates l (dominance x dominance) showed positively significant for days to maturity, days to 50% flowering, panicle length, number of ear bearing tillers per plant, number of unfilled grains per panicle, test weight, milling%, SPAD Chlorophyll Meter Reading (SCMR), head rice recovery and alkali spreading value. whereas it was negatively significant for plant height, number of filled grains per panicle, grain yield, head rice recovery, water uptake and gel consistency. These results are in consonance with the earlier results of Subbulakshmi et al. (2016) for number of ear bearing tillers per plant, test weight and panicle length.

The estimates of components of genetic variance indicated that duplicate epistatic (D, 15:1) interaction (h and l having opposite sign) was predominant for most of the traits except plant height, number of ear bearing tillers per plant, kernel breadth and amylose content which showed complementary (c; 9:7) (h and l having same sign) epistasis. Chamundeswari (2010) for days to 50% flowering, days to maturity, panicle length, kernel L/B ratio, milling recovery, alkali spreading value; Yadav et al. (2013) for number of filled grains per panicle, Roy and Senapathi (2011) for harvest index, Verma et al. (2006) for grain yield per plant, Jhansi and Satyanarayana (2015) for kernel length, volume expansion ratio, gel consistency; Gnanamalar and Vivekanandan (2013) for hulling recovery and Yadav et al. (2013) for milling recovery obtain similar results for the traits in rice.

Heritability and genetic advance

The results of heritability and genetic advance were depicted in the Table 4. The heritability values ranges from 35 to 98.63.Broadscence heritability was relatively high for all the traits (>60) except panicle length, SPAD Chlorophyll Meter Reading (SCMR), harvest index, kernel elongation ratio, gel consistency (<60) and the estimates are in the same line with those for different types of genetic variance. These results indicated that these traits could be improved by the traditional breeding methods and selection could be effective in early segregating generations. This was proved by entire

Table 4. Estimates of broad (h2b) and narrow (h2n) sense heritability and genetic advance for yield-contributing traits.

S. no.	Character	GCV	PCV	h2	GAM
1	Days to 50% flowering	5.55	6.27	78.27	10.11
2	Days to maturity	4.26	4.60	85.65	8.12
3	Plant height (cm)	6.55	7.02	87.12	12.60
4	Ear bearing tillers per plant	12.73	15.57	66.84	21.44
5	Panicle length (cm)	5.83	9.83	35.19	7.13
6	Filled grains per panicle	12.28	13.54	82.25	22.94
7	Un filled grains per panicle	20.36	25.75	62.53	33.17
8	SPAD Chlorophyll Meter Reading (SCMR)	3.79	5.65	45.01	5.24
9	Harvest index (%)	6.22	8.39	54.90	9.49
10	Test weight (g)	17.47	17.68	97.60	35.55
11	Grain yield (g/p)	16.26	17.50	86.32	31.13
12	Kernal length (mm)	6.59	6.63	98.81	13.48
13	Kernal width (mm)	4.60	4.86	89.45	8.96
14	Kernal L/B ratio	6.41	6.63	93.65	12.78
15	Hulling %	1.85	2.49	54.86	2.82
16	Milling %	2.91	3.24	80.39	5.37
17	Head rice recovery (%)	8.20	8.29	97.76	16.69
18	Water uptake (ml)	22.61	22.76	98.63	46.25
19	Volume expansion ratio	31.66	32.11	97.17	64.28
20	Kernal elongation ratio	8.96	11.66	59.04	14.19
21	Gel consistency (mm)	21.00	28.59	53.93	31.76
22	Alkali Spreading value	40.19	41.29	94.77	80.60
23	Amylose content	4.42	4.99	78.48	8.07

values of the predicted genetic advances were low in most of the cases. Similar results were already reported by Kumar et al. (2013) for head rice recovery, Kumar et al (2014) for harvest index and hulling recovery %, Kishore et al. (2015) for test weight, Veerabadhiran et al. (2009) for volume expansion ratio in rice. Considerable genetic advances were found (>20) for the traits *viz*., number of ear bearing tillers per plant, number of filled grains per panicle, number of unfilled grains per panicle, test weight, grain yield, water uptake, volume expansion ratio, gel consistency and alkali spreading value respectively. High heritability coupled with high genetic advance for these traits indicates the control of additive gene action and selection may be effective for these traits.

Blast reaction

The cross WGL 48684 x IR 36 exhibited resistant reaction (<3 score) to blast in all the plants studied in F1 generation indicating dominant gene conferring blast resistance. (Table 5). In F2 generation the plants were segregated in 3:1 ratio for resistance to susceptibility. The resistant trait in F2 population was evaluated by testing the single gene model wherein it was observed 25% of the F2 population showed susceptibility while 75% as resistant. From data analysis of chi square goodness of fit these two F2 crosses were segregated in a 3:1 ratio (X2= 0.74, df:1, at P value: 0.50-0.25 for BPT 5204 x IR 36; X2= 2.02, df:1, at P value: 0.25-

Table 5. Mode of inheritance of blast resistance in various segregating generations in the cross WGL 48684 x IR 36 of rice.

Generation	Total plants studied	Observed frequencies		Expected frequencies		Ratio	Chi square	P value
		Resistant	Susceptible	Resistant	Susceptible	R:S		
P1	46	8	38	-	-			
P2	52	46	6	-	-			
F1	51	44	7	-	-			
F2	197	156	41	144	48	3:1	2.02	0.25-0.1
BC1	45	26	19	23	22	1:1	0.52	0.5-0.25
BC2	63	49	14	45	15	3:1	0.41	0.50

0.10 for WGL 48684 x IR 36) of resistant to suceptibility. This result may suggest that there may be a single dominant gene governing the resistance in the parent IR 36. The complement of the resistant genes could be due to the donar genes from IR 36 that carried highly resistant genes. Studies from previous research also indicated that resistant gene expression is dependent on the donar cultivar genetic back ground, specificity of different isolates and effectiveness of specific host on specific strains that governed by single gene (Sharma et al. 2012).

From the overall results, it is clear that considerable amount of genetic variability was available for yield and its component traits in the cross. The magnitude of the different genetic component was unequal for different traits. However, the displaying significant additive effects can be used in future genetic improvement programs by capitalizing this variance. Significant additive effect along with complementary epistasis was recorded for number of ear bearing tillers per plant, plant height, kernel breadth and amylose content indicated the possibility of exploiting the combined variability through the evolution of the parents. It was evident from the results that the duplicate type of epistasis was common, except for some characters limiting the pace of progress through selection. Therefore, few cycles of recurrent selection followed by pedigree method would be effective and useful to utilize all types of gene effects by maintaining considerable heterozygosity through mating of selected plants in early segregating generations.

REFERENCES

Adilakshmi D and Raghavareddy P (2012). Combining ability analysis for yield components and physiological traits in rice. International Journal of Plant Sciences 7(2): 295-300.

AG Singh and PB Patel (2020). Generation mean analysis for yield and its components in rice (*Oryza sativa* L.). The Pharma Innovation Journal 9(10): 473-477

Bhadra SK and Dey A (1985). Genetic analysis of some yield contributing characters in rice (*Oryza sativa* L.). Bangladesh Journal of Agriculture 10(1):1-8.

Bhati PK, Singh SK, Singh R, Sharma A and Dhurai SY (2015). Estimation of heterosis for yield and yield related traits in rice (*Oryza sativa* L.). SABRAO Journal of Breeding and Genetics 47(4): 467-474

Chamundeswari N (2010). Genetic studies on quality traits in rice (*Oryza sativa* L.). Ph.D.Thesis submitted to Acharya N.G Ranga Agricultural University, Hyderabad

Ganapati RK, Golam Rasul Md, Sarker U, Singha A and Faruquee M (2020). Gene action of yield and yield contributing traits of submergence tolerant rice (*Oryza sativa* L.) in Bangladesh Bulletin of the National Research Centre https://doi.org/10.1186/s42269-019-0261-0

Gnanamalar RP and Vivekanandan P (2013). Genetic architecture of grain quality characters in rice (*Oryza sativa* L.). European Journal of Experimental Biology 3(2): 275-279

Hayman BI (1958). The separation of epistatic from additive and dominance variation in generation means. Heredity 12: 371-390

Jhansi RP and Satyanarayana PV (2015). Studies on combining ability analysis of traits related to grain number and grain weight for yield enhancement in rice (*Oryza sativa* L.). International Journal of Tropical Agriculture 33 (2): 723-728

Jinks J and Jones L (1958). Estimation of the components of heterosis. Genetics 43: 223-234

Kishore NS, Srinivas T, Nagabhushanam U, Pallavi M and Sameera SK (2015). Genetic variability, correlation and path analysis for yield and yield components in promising rice (*Oryza sativa* L.) genotypes. SAARC Journal of Agriculture 13(1): 99-108

Kumar A, Rangare NR and Vidyakar V (2013). Study of genetic variability of Indian and exotic rice germplasm in Allahabad agroclimate. The Bioscan 8(4): 1445-1451

Kumar VG, Koutu K, Singh SK, Mishra DK, Singh PK and Sohgaura N (2014). Genetic analysis of inter sub-specific derived mapping population (rils) for various yield and quality attributing traits in rice. International Science Journals 1(3): 1-7

Mather K, Jinks JL (1971). Biomedical genetics. Cornell University Press

Lingaiah N, Ch Surender Raju , Radhika K, Sarla N, Venkanna V, Vishnu Vardhan Reddy D (2020). Estimation of gene effects through generation mean analysis in elite rice (*Oryza sativa* L.) crosses. Journal of Experimental Biology and Agricultural Sciences. 8(1): 15 - 20

Rao M, Grithlahre S, Bisen P, Loitongbam B, Dar MH, Zaidi

NW, Singh US, Singh PK (2017). GMA for grain yield and its component traits in submergence rice. SABRAO Journal of Breeding and Genetics 49(4): 327-335

Renukadevi P, Subbalakshmi B (2006). Correlations and path co efficient analysis in chickpea. Legume Research. 29: 201-204

Roy SK and Senapati BK (2011). Estimation of genetic components for grain yield and quality traits in rice. Oryza 48(1): 22-30.

Rukminidevi K, Parimala K and Cheralu C (2014). Heterosis for yield and quality traits in rice (*Oryza sativa* L.). The Journal of Research ANGRAU 42(1): 1-11

Sharma TR, Rai AK, Gupta SK, Vijayan J, Devanna BN and Ray S (2012). Rice blast management through host-plant resistance: retrospect and prospects. Agricultural Research 1: 37-52

Singh RK and Chaudhary BD (2012). Biometrical methods in quantitative genetic analysis. Kalyani Publishers, New Delhi

Solanke AC, Patel PB and Patel PK (2019). Generation mean analysis for yield and it's contributing traits in aromatic rice (*Oryza sativa* L.). The Pharma Innovation Journal 8(8): 05-09

Subbulakshmi K, Shunmugavalli N, Muthuswamy A 2016. Generation mean analysis for yield and quality traits in F2 and F3 generation of rice (*Oryza sativa* L.). Electronic Journal of Plant Breeding 7: 491-495

Uzokwe VNE, Asafo Adjei B, Fawole I, Abaidoo R, Odeh IOA, Ojo DK, Dashiell K, Nteranya Sanginga N. (2017). Generation mean analysis of phosphorus use efficiency in freely nodulating soybean crosses grown in low phosphorus soil. Plant Breeding 136: 139-146

Veerabadhiran P, Umadevi M and Pushpam R (2009). Genetic variability, heritability and genetic advance of grain quality in hybrid rice. Madras Agricultural Journal 96(1-6): 95-99

Verma OP, Singh RV, Dwivedi JL and Prakash N (2006). Genetic analysis of yield and its components in rice. Oryza 43(1): 55-57

Yadav HN, Prasad R, Singh RP and Agarwal RK (2013). Detection of epistasis and gene effects for quality and yield traits in rice. Oryza 50(2): 115-119

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Study of genetic parameters, correlation and path analysis for yield and quality characters in fine scented rice genotypes

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ABSTRACT

During kharif 2020, the Section of Genetics and Plant Breeding at S.G. College of Agriculture and Research Station, Jagdalpur, Bastar (C.G.), examined 41 fine scented rice genotypes for 21 yield, yield contributing, and quality parameters. For all of the characters investigated, ANOVA demonstrated that there was considerable variation among genotypes. PCV levels were discovered to be slightly greater than GCV values, indicating that the environment has an impact on character expression. Plant height, test weight, head rice recovery, grain length, grain width, grain L:B, kernel length, kernel width, kernel L:B, alkali spreading value, gel consistency, and amylose content all had strong heritability and GAM, showing additive gene action. Grain yield plot^{-1} was significantly correlated with panicle length, total number of filled grains per panicle^{-1}, harvest index, test weight, grain width, kernel width, amylose content, alkali spreading value, kernel elongation ratio, number of effective tillers plant^{-1}, and days to 50% flowering. Amylose content was found to have a positive relationship with alkali spreading value and kernel elongation ratio, but a negative relationship with gel consistency.At the genetic level, path coefficient analysis revealed that traits such as harvest index, number of effective tillers plant^{-1}, grain width, number of tillers square meter^{-1}, days to 50% blooming, and kernel width had true associations with grain yield plot^{-1}. At the phenotypic level, panicle length, test weight, number of effective tillers plant^{-1}, kernel width, days to 50% flowering, harvest index, and kernel elongation ratio all showed a genuine relationship with grain yield plot^{-1}. As a result, selecting these parameters will boost grain yield in the long run.

Key words: *ANOVA, GCV, PCV, correlation, path coefficient analysis*

INTRODUCTION

Rice is the second most grown crop in the world after maize, and it is a staple diet for approximately 3.5 billion people. Carbohydrates, proteins, minerals, and energy are abundant. For billions of people around the world, it is their primary source of carbohydrates (Babu et al., 2012). As a result, rice is the only crop capable of combating hunger and poverty in developing and densely populated areas. As a result, it is known as the "Grain of Life," as it links nations and civilizations.Rice production covers roughly 162.71 million hectares globally, with 499.18 million tonnes produced in 2019. (Anonymous, 2019). Globally, the number of individuals who consume rice is increasing every day, resulting in an increase in rice demand. By 2050, the population could rise from 7.6 billion to 9.8 billion people (Anonymous, 2017) as a result, with a growing population and limited natural resources, we must boost quality output and yield has become an important goal in many breeding programmes.Quality, though, is just as vital as quantity. According to a study, quality is the second most important breeding goal in 11 of the world's major rice-growing countries (Juliano and Villared, 1993). Nowadays, the trend has shifted, with plant breeders focusing largely on adding quality characteristics such as superior grains, excellent cooking quality, lengthwise elongation, and pleasant aroma, which command a premium price in both domestic and foreign markets (Bhattacharjee et al., 2002). People nowadays demand scent in rice, and they are willing to pay a greater price for it (Bradbury et al., 2005). Rice's most valuable feature is the presence of scent (Cruz and Khush, 2000). Aromatic varieties, on the other hand, have some disadvantages (Berner and Hoff, 1986). Rice grain quality is a polygenic feature that is impacted by the environment, crop management, and crop interactions. Milling, appearance, eating, cooking, physiochemical, and nutritional quality are all factors that influence grain quality. The physicochemical and culinary properties of milled rice grains play a big role in the cooking and eating quality (Bhattacharjee et al., 2002). Because it reflects the starch content of rice grains, the amylose content is widely acknowledged as an important indicator of eating quality (Juliano et al., 1993). Within various types of rice, there is a lot of difference in quality and total diversity. However, there are few reports on the genetics of rice quality features (Rani et al., 2008). Researchers from all over the world were astounded by the range of quality attributes and wanted to learn more about them in order to produce new elite varieties with higher yields and higher quality. The purpose of this study is to evaluate genetic parameters, correlation studies and path associations studies in genotypes that may be utilised to improve genetics.

MATERIALS AND METHODS

The Section of Genetics and Plant Breeding at S.G.C.A.R.S attempted to examine the genetics of 21 yield and quality variables in 41 fine scented rice genotypes using four checks. During *kharif* 2020, at the College of Agriculture and Research Station, Kumhrawand, Jagdalpur, Bastar, Chhattisgarh. With a net plot size of 1.6 m × 4.8 m = 7.68 m^2, the experiment was conducted in Randomized Block Design with three replications with spacing of 20 cm within rows and 15 cm between plants. Some of the characters' observations were based on plots, while others were based on random plants per plot. On a plot basis, factors such as days to 50% flowering, panicles per metre square, tillers per square metre, grains yield per plot, harvest index, and test weight were calculated. Plant height, effective tiller count, panicle length, and total number of full grains per panicle^{-1} were calculated by randomly picking five plants from each plot. Kernel length, kernel width, grain length, and grain width were measured with vernier callipers by sampling 10 completely grown grains from each replication and calculating the mean, and grain and kernel L:B ratio was calculated using Shoba et al. (2006) DUS descriptors. Pokhrel et al. (2020) described a method for calculating the elongation ratio. The amount of head rice recovered was determined by milling 10 completely developed grains in a hand-held dehuller and counting the number of whole kernels produced after milling. The Gel consistency (GC) was determined using the Cagampang et al. (1973) technique. Alkali Spreading Value calculated using Little et al. (1958) technique . Spectrophotometrical approach was used to determine the amylose content in each rice sample using Juliano's modified method (1971). The analysis of variance was calculated using Panse and Sukhatme's (1985) approach, Burton's (1952) equations for the genotypic and phenotypic coefficients of variation, and Johnson et al. heritability and genetic advance (1955). According to Al-Jibouri et al. (1958) the correlation coefficient was calculated. The path coefficient analysis was done using the Wright (1921) and Dewey and Lutetium (1959) recommended process. R-studio software was used for the statistical analysis.

RESULTS AND DISCUSSIONS

Analysis of variance revealed that presence of variation in between the population for characters under study. High heritability (broad sense) was observed in days to 50% flowering (100), amylose content (99.9), alkali spreading value (97.4), grain length (96.7), kernel length (96.6), grain L:B (95.3), kernel L:B (94.6), grain breadth (92.9), gel consistency (92.4), kernel breadth (90.7), plant height (90.3), test weight (80.8) and head rice recovery (75.8). High heritability for days to 50% flowering and test weight are conformity to similar findings by Sivasubramanian and Madhavamenon, (1973). Moderate heritability was seen in kernel elongation ratio (56.8), panicle length (50.7), grain yield plot^{-1} (49.6), total number of filled grains per panicle^{-1} (48.7), number of tillers square meter^{-1} (35.5) and number of panicles square meter^{-1} (32). Low heritability was seen in number of effective tillers panicle^{-1} and harvest index. These reports were similar to previous findings of Babu et al. (2012), Ekka et al. (2015) and Sahu et al. (2017).

Genetic advance as percentage of mean was high in characters like amylose content (80.09), alkali spreading value (75.43), gel consistency (65.92), test weight (44.36), kernel L:B (42.38), grain L:B (40.46), kernel length (34.81), plant height (32.82), grain length (31.06), head rice recovery (31), total number of filled grains per panicle^{-1} (28.68), grain breadth (24.64) and kernel breadth (22.23). Moderate values are seen in grain yield plot^{-1} (17.71), days to 50% flowering (13.74), number of tillers square meter^{-1}(13), number of panicles square meter^{-1} (12.70), panicle length (11.12) and number of effective tillers panicle^{-1} (11.05). Low values were observed in harvest index (9.97) and kernel elongation ratio (8.57).

High heritability coupled with high genetic advance as percent of mean was reported for characters like plant height, test weight, head rice recovery, grain length, grain breadth, grain L:B, kernel length, kernel breadth, kernel L:B, alkali spreading value, gel consistency and amylose content indicating additive gene action. These findings were similar to previous findings of Limbani et al. (2017) and Sahu et al. (2017). High heritability coupled with moderate genetic advance as percent of mean was reported in days to 50% flowering showing both additive and non additive gene action. These finding was in agreement with previous finding of Prajapati et al. (2011).

Phenotypic Coefficient of Variation (PCV) values found to be slightly higher than Genotypic Coefficient of Variation (GCV) values showing the influence of environment on character expression. PCV is the sum of Genotypic and Environmental variances, so as more influence of environment on character expression, than higher the value of PCV. Highest PCV and GCV values were observed for characters like amylose content, alkali spreading value, gel consistency, test weight, kernel L:B and grain L:B. Dhanawni et al. (2013) reported similar results for alkali spreading value, gel consistency. Moderate PCV and GCV values were seen in head rice recovery, kernel length, plant height, grain length, grain breadth, grain yield plot^{-1}, kernel breadth, number of panicles square meter^{-1} and number of tillers square meter^{-1}. Low values of GCV and PCV was seen in characters like kernel elongation ratio and days to 50% flowering.Previous finding of Shinhaet al. (2004) is in agreement with days to 50% flowering. All genetic parameters like heritability, Genetic advance as percent of mean, GCV and PCV were presented below in (Table 1).

Grain yield plot^{-1} showed high significant positive association with panicle length, number of

Table 1. Values of mean, range, heritability broad sense [h^2], genetic advance (GA), genetic advance as percentage of mean (GAM), genetic coefficient of variation (GCV), phenotypic coefficient of variation (PCV) in present study.

Sl. no.	Characters	Mean ($\bar{X}$)	Range	h^2[b](%)	GA	GAM	GCV	PCV
1.	Plant height (cm)	112.12	78.40	90.30	36.81	32.83	16.77	112.12
2.	Panicle length (cm)	22.79	15.60	50.70	2.54	11.12	7.59	22.79
3.	Number of effective tillers per plant	8.13	8.40	29.20	0.90	11.06	9.94	8.13
4.	Total number of filled grains per panicle	115.24	187.00	48.70	33.02	28.65	19.94	115.24
5.	Number of tillers per square meter	246.41	241.00	35.50	32.20	13.07	10.64	246.41
6.	Number of panicles per square meter	236.41	259.00	32.00	30.03	12.70	10.91	236.41
7.	Days to 50% flowering	107.00	28.00	100.00	14.70	13.74	6.67	107.00
8.	Harvest index (%)	48.62	55.10	28.30	4.85	9.98	9.11	48.62
9.	Test weight (gm)	20.18	22.94	80.80	8.95	44.37	23.97	20.18
10.	Head rice recovery (%)	60.41	60.00	75.80	18.73	31.00	17.29	60.41
11.	Grain length (mm)	8.01	4.70	96.70	2.49	31.06	15.33	8.01
12.	Grain breadth (mm)	2.32	1.40	92.90	0.57	24.64	12.41	2.32
13.	Grain L:B (mm)	3.51	2.84	95.30	1.42	40.46	20.12	3.51
14.	Kernel length	5.66	3.40	96.60	1.97	34.81	17.20	5.66
15.	Kernel breadth	2.08	1.30	90.70	0.46	22.23	11.33	2.08
16.	Kernel L:B	2.77	2.40	94.60	1.17	42.38	21.16	2.77
17.	Alkali spreading value	3.60	6.00	97.40	2.72	75.43	37.10	3.60
18.	Gel consistency (mm)	48.63	86.00	92.40	32.06	65.93	33.30	48.63
19.	Amylose content	17.95	22.70	99.90	14.37	80.09	38.88	17.95
20.	Kernel elongation ratio	1.17	0.40	56.80	0.10	8.58	5.53	1.17
21.	Grain yield (kg)	2.84	2.50	49.60	0.50	17.72	12.22	2.84

Table 2. Genotypic and Phenotypic correlation of 21 yield and quality characters.

Characters		PH	PL	NETP	TNFGP	NTSM	NPSM	DTF	HI	TW	HR
PH	G	1 **									
	P	1 **									
PL	G	-0.206 NS	1 **								
	P	-0.103 NS	1 **								
NETP	G	-0.178 NS	0.544 **	1 **							
	P	-0.055 NS	0.278 **	1 **							
TNFGP	G	0.241 NS	0.159 NS	-0.035 NS	1 **						
	P	0.218 *	0.116 NS	0.038 NS	1 **						
NTSM	G	0.266 NS	0.337 *	-0.007 NS	0.546 **	1 **					
	P	0.180 *	0.166 NS	0.197 *	0.229 *	1 **					
NPSM	G	0.196 NS	0.384 *	0.055 NS	0.638 **	0.960 **	1 **				
	P	0.134 NS	0.169 NS	0.206 *	0.260 **	0.960 **	1 **				
DTF	G	0.528 **	0.100 NS	-0.065 NS	0.627 **	0.610 **	0.608 **	1 **			
	P	0.501 **	0.071 NS	-0.035 NS	0.437 **	0.364 **	0.344 **	1 **			
HI	G	0.200 NS	0.358 *	0.289 NS	0.242 NS	0.135 NS	0.219 NS	0.100 NS	1 **		
	P	0.092 NS	0.094 NS	0.064 NS	0.177 NS	-0.097 NS	-0.073 NS	0.053 NS	1 **		
TW	G	-0.306 NS	-0.113 NS	0.108 NS	0.007 NS	0.081 NS	0.127 NS	-0.149 NS	0.019 NS	1 **	
	P	-0.247 **	-0.068 NS	0.057 NS	0.008 NS	0.066 NS	0.101 NS	-0.134 NS	0.032 NS	1 **	
HR	G	0.124 NS	0.356 *	0.02 NS	0.475 **	0.388 *	0.430 **	0.481 **	-0.107 NS	-0.199 NS	1 **
	P	0.111 NS	0.193 *	-0.002 NS	0.350 **	0.174 NS	0.171 NS	0.419 **	-0.114 NS	-0.128 NS	1 **
GL	G	-0.351 *	-0.333 *	0.039 NS	-0.422 **	-0.391 *	-0.362 *	-0.561 **	0.110 NS	0.761 **	-0.579 **
	P	-0.325 **	-0.213 *	0.029 NS	-0.278 **	-0.233 **	-0.207 *	-0.552 **	0.048 NS	0.666 **	-0.487 **
GB	G	0.083 NS	0.283 NS	0.180 NS	0.433 **	0.560 **	0.551 **	0.320 *	-0.103 NS	0.497 **	0.349 *
	P	0.058 NS	0.199 *	0.072 NS	0.292 **	0.283 **	0.269 **	0.309 **	-0.098 NS	0.408 **	0.297 **
GLB	G	-0.313 *	-0.402 **	-0.055 NS	-0.593 **	-0.635 **	-0.610 **	-0.623 **	0.138 NS	0.217 NS	-0.645 **
	P	-0.280 **	-0.273 **	-0.016 NS	-0.393 **	-0.348 **	-0.322 **	-0.609 **	0.090 NS	0.200 *	-0.543 **
KL	G	-0.293 NS	-0.231 NS	0.018 NS	-0.385 *	-0.363 *	-0.316 *	-0.474 **	0.145 NS	0.756 **	-0.506 **
	P	-0.265 **	-0.163 NS	0.030 NS	-0.272 **	-0.190 *	-0.156 NS	-0.466 **	0.061 NS	0.686 **	-0.433 **
KB	G	0.223 NS	0.283 NS	0.129 NS	0.470 **	0.510 **	0.472 **	0.454 **	-0.079 NS	0.346 *	0.403 **
	P	0.223 *	0.216 *	0.087 NS	0.342 **	0.326 **	0.291 **	0.432 **	-0.006 NS	0.302 **	0.336 **
KLB	G	-0.341 *	-0.304 NS	-0.028 NS	-0.538 **	-0.529 **	-0.478 **	-0.621 **	0.148 NS	0.378 *	-0.599 **
	P	-0.320 **	-0.231 *	-0.014 NS	-0.396 **	-0.314 **	-0.272 **	-0.604 **	0.051 NS	0.345 **	-0.506 **
ASV	G	0.062 NS	0.059 NS	0.061 NS	0.447 **	0.285 NS	0.285 NS	0.136 NS	0.220 NS	0.180 NS	0.155 NS
	P	0.048 NS	0.045 NS	0.025 NS	0.307 **	0.187 *	0.178 *	0.135 NS	0.103 NS	0.156 NS	0.148 NS
GC	G	0.144 NS	0.215 NS	0.044 NS	0.032 NS	-0.314 *	-0.299 NS	-0.021 NS	0.416 **	-0.054 NS	-0.106 NS
	P	0.140 NS	0.133 NS	0.013 NS	0.057 NS	-0.161 NS	-0.138 NS	-0.020 NS	0.190 *	-0.032 NS	-0.060 NS
AC	G	-0.250 NS	0.235 NS	0.304 NS	0.376 *	0.539 **	0.528 **	0.281 NS	-0.318 *	0.171 NS	0.066 NS
	P	-0.237 **	0.166 NS	0.163 NS	0.261 **	0.321 **	0.298 **	0.281 **	-0.168 NS	0.154 NS	0.057 NS
KER	G	0.150 NS	-0.003 NS	-0.027 NS	0.629 **	0.459 **	0.455 **	0.374 *	0.090 NS	-0.217 NS	0.213 NS
	P	0.047 NS	0.019 NS	-0.062 NS	0.373 **	0.156 NS	0.161 NS	0.282 **	0.138 NS	-0.193 *	0.115 NS
GY	G	-0.271 NS	0.515 **	0.475 **	0.424 **	0.458 **	0.502 **	0.313 *	0.329 *	0.421 **	0.238 NS
	P	-0.186 *	0.237 **	0.227 *	0.238 **	0.155 NS	0.170 NS	0.220 *	0.583 **	0.298 **	0.085 NS

Continued......

(Continued…)

Characters		GL	GB	GLB	KL	KB	KLB	ASV	GC	AC	KER	GY
GL	G	1 **										
	P	1 **										
GB	G	-0.089 NS	1 **									
	P	-0.086 NS	1 **									
GLB	G	0.776 **	-0.684 **	1 **								
	P	0.769 **	-0.690 **	1 **								
KL	G	0.967 **	-0.079 NS	0.737 **	1 **							
	P	0.934 **	-0.086 NS	0.713 **	1 **							
KB	G	-0.231 NS	0.965 **	-0.754 **	-0.203 NS	1 **						
	P	-0.207 *	0.874 **	-0.692 **	-0.188 *	1 **						
KLB	G	0.860 **	-0.554 **	0.962 **	0.862 **	-0.660 **	1 **					
	P	0.816 **	-0.525 **	0.914 **	0.852 **	-0.662 **	1 **					
ASV	G	0.014 NS	0.323 *	-0.192 NS	-0.063 NS	0.341 *	-0.222 NS	1 **				
	P	0.016 NS	0.302 **	-0.179 *	-0.059 NS	0.318 **	-0.209 *	1 **				
GC	G	0.072 NS	-0.072 NS	0.094 NS	0.099 NS	-0.126 NS	0.139 NS	-0.187 NS	1 **			
	P	0.069 NS	-0.082 NS	0.101 NS	0.099 NS	-0.124 NS	0.137 NS	-0.179 *	1 **			
AC	G	-0.102 NS	0.340 *	-0.260 NS	-0.123 NS	0.320 *	-0.248 NS	0.221 NS	-0.512 **	1 **		
	P	-0.100 NS	0.327 **	-0.254 **	-0.120 NS	0.305 **	-0.241 **	0.218 *	-0.492 **	1 **		
KER	G	-0.467 **	0.014 NS	-0.331 *	-0.526 **	0.070 NS	-0.434 **	0.203 NS	0.058 NS	0.342 *	1 **	
	P	-0.344 **	0.037 NS	-0.258 **	-0.473 **	0.071 NS	-0.401 **	0.153 NS	0.015 NS	0.257 **	1 **	
GY	G	0.038 NS	0.435 **	-0.229 NS	0.087 NS	0.386 *	-0.122 NS	0.288 NS	-0.025 NS	0.416 **	0.290 NS	1 **
	P	0.024 NS	0.258 **	-0.137 NS	0.048 NS	0.297 **	-0.110 NS	0.188 *	-0.039 NS	0.293 **	0.225 *	1 **

Keywords: PH=Plant height; PL=Panicle length; NETP=Number of effective tillers per plant; TNFGP=Total number of filled grains per panicles; NTSM=Number of tillers per square meter; NPSM=Number of panicles per square meter; DTF=Days to 50% flowering; HI=Harvest index; TW=Test weight; HR=Head rice recovery; GL=Grain length; GB=Grain breadth; GLB=Grain length breadth ratio; KL=kernel length; KB=kernel breadth; KLB=Kernel length breadth ratio; ASV=Alkali spreading value; GC=Gel consistency; AC=Amylose content; KER=Kernel elongation ratio; GY=Grain yield per plot.*Significant at 5% and ** significant at 1%.

effective tillers plant^{-1}, total number of filled grains panicle^{-1}, number of tillers square meter^{-1}, number of panicles square meter^{-1}, test weight, grain breadth and amylose content. Characters like kernel breadth, days to 50% flowering and harvest index showed positive significant genotypic association with grain yield plot^{-1}. Characters like panicle length, total number of filled grains per panicle^{-1}, harvest index, test weight, grain breadth, kernel breadth and amylose content showed high significant positive phenotypic association with grain yield plot^{-1}. Positive significant phenotypic association was found between grain yield plot^{-1} with respect to characters like alkali spreading value, kernel elongation ratio, number of effective tillers plant^{-1} and days to 50% flowering. Plant height found to be phenotypical negative significant with grain yield per plot. This is due to the occurrence of lodging of plant before grain filling due to unfavourable weather conditions and weak straw. The findings from the grain yield plot^{-1} were in agreement with previous findings of Ekka et al. (2011), Selvaraj et al. (2011), Yadav et al (2010) and Veni et al. (2013) for days to 50% flowering, panicle length, total number of filled grains panicle^{-1}, number of effective tillers plant^{-1}, test weight, head rice recovery and harvest index. Plant height found to be non significant negative genotypic association with grain yield plot^{-1} was in agreement with findings of Zahid et al. (2006).

The kernel length found to be significant and negative with kernel breadth were similar with findings of Shivani et al. (2007). Grain length and kernel length showed high positive significant association with grain L:B and kernel L:B ratio were similar with findings of Mia et al. (2010). Amylose content showed positive association with alkali spreading value, kernel elongation ratio and negative association with gel consistency and gelatinization temperature. Kernel elongation ratio is a

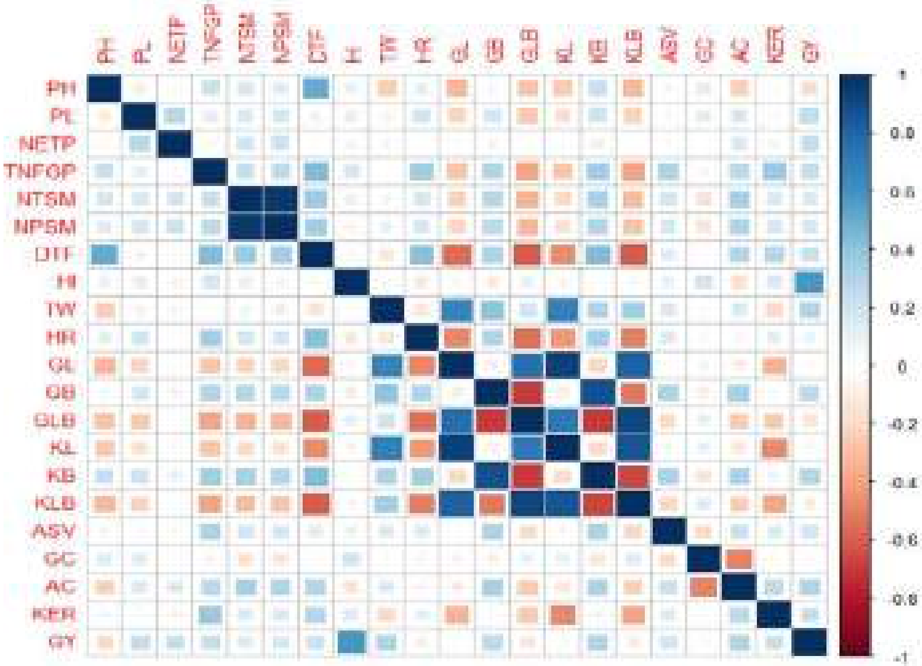

Fig.1. General Correlation matrix showing positive and negative relationship between different characters; Blue and Red shows positive and negative relationship Keywords: PH = Plant height; PL = Panicle length; NETP = Number of effective tillers per plant; TNFGP = Total number of filled grains per panicles; NTSM = Number of tillers per square meter; NPSM = Number of panicles per square meter; DTF = Days to 50% flowering; HI = Harvest index; TW = Test weight; HR = Head rice recovery; GL = Grain length; GB = Grain breadth; GLB = Grain length breadth ratio; KL = kernel length; KB = kernel breadth; KLB = Kernel length breadth ratio; ASV = Alkali spreading value; GC = Gel consistency; AC = Amylose content; KER = Kernel elongation ratio; GY = Grain yield per plot.

Table 3. Genotypic and phenotypic path coefficient analysis of 21 characters direct effect on grain yield were shown in bold letters diagonally.

Characters		PH	PL	NETP	TNFGP	NTSM	NPSM	DTF	HI	TW	HR
PH	G	-2.063	0.343	-0.231	-0.172	0.427	-0.125	0.385	0.028	0.687	-0.025
	P	-0.55	-0.011	-0.01	-0.014	0.036	-0.037	0.32	0.036	-0.052	-0.008
PL	G	0.426	-1.66	0.705	-0.114	0.541	-0.244	0.074	0.05	0.254	-0.071
	P	0.057	0.104	0.048	-0.008	0.034	-0.047	0.046	0.037	-0.014	-0.013
NETP	G	0.368	-0.903	1.297	0.026	-0.012	-0.035	-0.048	0.041	-0.242	-0.005
	P	0.031	0.029	0.172	-0.003	0.04	-0.057	-0.023	0.025	0.012	0.000
TNFGP	G	-0.498	-0.265	-0.046	-0.712	0.876	-0.406	0.457	0.034	-0.017	-0.095
	P	-0.12	0.012	0.007	-0.066	0.046	-0.072	0.279	0.069	0.002	-0.024
NTSM	G	-0.55	-0.56	-0.01	-0.389	1.603	-0.61	0.445	0.019	-0.183	-0.078
	P	-0.099	0.017	0.034	-0.015	0.202	-0.264	0.232	-0.038	0.014	-0.012
NPSM	G	-0.406	-0.638	0.072	-0.455	1.54	-0.635	0.444	0.031	-0.286	-0.086
	P	-0.074	0.018	0.036	-0.017	0.194	-0.275	0.219	-0.029	0.021	-0.012
DTF	G	-1.09	-0.167	-0.085	-0.447	0.979	-0.387	0.729	0.014	0.336	-0.096
	P	-0.276	0.007	-0.006	-0.029	0.073	-0.095	0.638	0.021	-0.028	-0.029
HI	G	-0.415	-0.595	0.375	-0.172	0.218	-0.139	0.073	0.141	-0.044	0.021
	P	-0.051	0.01	0.011	-0.012	-0.02	0.02	0.034	0.39	0.007	0.008
TW	G	0.633	0.188	0.14	-0.006	0.131	-0.081	-0.109	0.003	-2.24	0.04
	P	0.136	-0.007	0.01	-0.001	0.013	-0.028	-0.086	0.013	0.21	0.009
HR	G	-0.257	-0.591	0.034	-0.339	0.623	-0.273	0.351	-0.015	0.446	-0.2
	P	-0.061	0.02	0.022	-0.023	0.035	-0.047	0.267	-0.044	-0.027	-0.069
GL	G	0.725	0.554	0.052	0.301	-0.628	0.23	-0.409	0.016	-1.705	0.116
	P	0.179	-0.022	0.005	0.018	-0.047	0.057	-0.352	0.019	0.14	0.034
GB	G	-0.171	-0.471	0.234	-0.309	0.899	-0.35	0.234	-0.015	-1.115	-0.07
	P	-0.032	0.021	0.012	-0.019	0.057	-0.074	0.197	-0.038	0.086	-0.021
GLB	G	0.647	0.668	-0.072	0.422	-1.018	0.388	-0.455	0.019	-0.487	0.129
	P	0.154	-0.028	-0.003	0.026	-0.07	0.089	-0.388	0.035	0.042	0.038
KL	G	0.606	0.384	0.024	0.274	-0.582	0.201	-0.346	0.02	-1.695	0.101
	P	0.146	-0.017	0.005	0.018	-0.038	0.043	-0.297	0.024	0.144	0.03
KB	G	-0.461	-0.47	0.167	-0.335	0.818	-0.3	0.331	-0.011	-0.776	-0.081
	P	-0.123	0.023	0.015	-0.023	0.066	-0.08	0.275	-0.002	0.063	-0.023
KLB	G	0.705	0.505	-0.037	0.383	-0.849	0.304	-0.453	0.021	-0.848	0.12
	P	0.176	-0.024	-0.002	0.026	-0.063	0.075	-0.386	0.02	0.073	0.035
ASV	G	-0.128	-0.098	0.08	-0.318	0.457	-0.181	0.1	0.031	-0.404	-0.031
	P	-0.027	0.005	0.004	-0.02	0.038	-0.049	0.086	0.04	0.033	-0.01
GC	G	-0.298	-0.358	0.057	-0.023	-0.505	0.19	-0.016	0.059	0.122	0.021
	P	-0.077	0.014	0.002	-0.004	-0.033	0.038	-0.013	0.074	-0.007	0.004
AC	G	0.516	-0.39	0.395	-0.268	0.865	-0.336	0.205	-0.045	-0.384	-0.013
	P	0.13	0.017	0.028	-0.017	0.065	-0.082	0.18	-0.066	0.032	-0.004
KER	G	-0.311	0.006	-0.036	-0.448	0.736	-0.289	0.273	0.013	0.488	-0.043
	P	-0.026	0.002	-0.011	-0.025	0.031	-0.044	0.18	0.054	-0.04	-0.008

Continued.......

Characters		GL	GB	GLB	KL	KB	KLB	ASV	GC	AC	KER	Cor~GY
PH	G	1.451	0.034	0.533	-1.439	0.367	-0.935	0.044	0.026	0.213	0.18	-0.271 NS
	P	-0.767	-0.148	1.072	1.728	1.197	-3.057	-0.001	-0.025	0.067	0.035	-0.186 *
PL	G	1.379	0.117	0.683	-1.135	0.465	-0.833	0.042	0.039	-0.2	-0.004	0.515 **
	P	-0.502	-0.507	1.043	1.063	1.159	-2.203	-0.001	-0.024	-0.047	0.014	0.237 **
NETP	G	-0.164	0.075	0.095	0.091	0.212	-0.078	0.044	0.008	-0.259	-0.033	0.475 **
	P	0.071	-0.181	0.063	-0.197	0.477	-0.137	-0.001	-0.002	-0.047	-0.045	0.227 *
TNFGP	G	1.742	0.179	1.008	-1.89	0.771	-1.474	0.321	0.006	-0.32	0.753	0.424 **
	P	-0.656	-0.743	1.502	1.771	1.831	-3.779	-0.008	-0.01	-0.074	0.272	0.238 **
NTSM	G	1.617	0.232	1.079	-1.781	0.837	-1.45	0.204	-0.057	-0.459	0.549	0.458 **
	P	-0.549	-0.721	1.331	1.237	1.746	-3.005	-0.005	0.029	-0.091	0.114	0.155 NS
NPSM	G	1.497	0.228	1.038	-1.552	0.775	-1.309	0.205	-0.054	-0.45	0.544	0.502 **
	P	-0.488	-0.683	1.231	1.02	1.561	-2.603	-0.005	0.025	-0.085	0.117	0.170 NS
DTF	G	2.32	0.133	1.06	-2.329	0.745	-1.703	0.098	-0.004	-0.24	0.448	0.313 *
	P	-1.301	-0.785	2.326	3.037	2.314	-5.772	-0.004	0.004	-0.08	0.206	0.220 *
HI	G	-0.458	-0.043	-0.235	0.713	-0.131	0.407	0.158	0.076	0.271	0.108	0.329 *
	P	0.115	0.249	-0.347	-0.398	-0.031	0.487	-0.003	-0.034	0.048	0.101	0.583 **
TW	G	-3.143	0.206	-0.369	3.711	0.568	1.036	0.129	-0.01	-0.146	-0.26	0.421 **
	P	1.57	-1.039	-0.766	-4.467	1.617	3.298	-0.004	0.006	-0.044	-0.14	0.298 **
HR	G	2.39	0.144	1.097	-2.482	0.661	-1.643	0.111	-0.019	-0.056	0.255	0.238 NS
	P	-1.147	-0.755	2.076	2.822	1.801	-4.836	-0.004	0.011	-0.016	0.083	0.085 NS
GL	G	-4.129	-0.037	-1.32	4.746	-0.379	2.355	0.011	0.013	0.087	-0.559	0.038 NS
	P	2.355	0.218	-2.939	-6.083	-1.109	7.788	0.000	-0.012	0.029	-0.251	0.024 NS
GB	G	0.368	0.413	1.164	-0.388	1.584	-1.518	0.232	-0.013	-0.289	0.017	0.435 **
	P	-0.202	-2.525	2.63	0.549	4.703	-5.028	-0.008	0.015	-0.093	0.028	0.258 **
GLB	G	-3.208	-0.283	-1.699	3.616	-1.237	2.635	-0.138	0.017	0.222	-0.396	-0.229 NS
	P	1.812	1.749	-3.824	-4.64	-3.71	8.712	0.005	-0.018	0.072	-0.188	-0.137 NS
KL	G	-3.996	-0.033	-1.253	4.904	-0.333	2.362	-0.045	0.018	0.105	-0.629	0.087 NS
	P	2.2	0.214	-2.723	-6.505	-1.005	8.134	0.002	-0.018	0.034	-0.343	0.048 NS
KB	G	0.954	0.399	1.281	-0.995	1.64	-1.808	0.245	-0.023	-0.272	0.084	0.386 *
	P	-0.488	-2.231	2.646	1.222	5.337	-6.336	-0.008	0.022	-0.087	0.034	0.297 **
KLB	G	-3.552	-0.229	-1.635	4.231	-1.083	2.738	-0.159	0.025	0.211	-0.52	-0.122 NS
	P	1.922	1.338	-3.487	-5.548	-3.556	9.537	0.006	-0.025	0.069	-0.296	-0.110 NS
ASV	G	-0.061	0.134	0.328	-0.309	0.56	-0.608	0.717	-0.034	-0.188	0.243	0.288 NS
	P	0.039	-0.771	0.686	0.383	1.694	-1.999	-0.027	0.032	-0.062	0.113	0.188 *
GC	G	-0.3	-0.03	-0.16	0.487	-0.208	0.383	-0.135	0.182	0.436	0.07	-0.025 NS
	P	0.163	0.211	-0.386	-0.65	-0.668	1.314	0.005	-0.179	0.14	0.012	-0.039 NS
AC	G	0.422	0.141	0.442	-0.604	0.525	-0.679	0.159	-0.093	-0.851	0.409	0.416 **
	P	-0.236	-0.832	0.971	0.787	1.635	-2.301	-0.006	0.088	-0.284	0.188	0.293 **
KER	G	1.93	0.006	0.563	-2.581	0.115	-1.191	0.146	0.011	-0.291	1.196	0.290 NS
	P	-0.811	-0.099	0.982	3.055	0.252	-3.866	-0.004	-0.003	-0.073	0.69	0.225 *

Keywords: PH=Plant height; PL=Panicle length; NETP=Number of effective tillers plant-1; TNFGP=Total number of filled grains panicles-1; NTSM=Number of tillers square meter-1; NPSM=Number of panicles square meter-1; DTF=Days to 50% flowering; HI=Harvest index; TW=Test weight; HR=Head rice recovery; GL=Grain length; GB=Grain breadth; GLB=Grain length breadth ratio; KL=kernel length; KB=kernel breadth; KLB=Kernel length breadth ratio; ASV=Alkali spreading value; GC=Gel consistency; AC=Amylose content; KER=Kernel elongation ratio; Cor~GY= Correlation of respective characters with grain yield.*Significant at 5% level ** Significant at 1% level complex trait which is related with physicochemical and genetic factors like type of genotypes, aging temperature, time, water uptake, amylose content and gelatinization temperature. As the kernel elongation ratio is positively correlated with amylose content, this shows the high amount of starch and in turn it is positively correlated with kernel breadth, this in turn positively correlated with grain yield. These findings were similar to previous findings of Wang et al. (2005) and Golam and Prodhan (2013). The genotypic and phenotypic correlation was shown in (Table 2). General correlation was presented in (Fig. 1).

Genotypic path coefficient analysis revealed characters like kernel length (4.904), kernel length breadth ratio (2.738), kernel breadth (1.640), number of tillers square meter^{-1} (1.603), number of effective tillers plant^{-1} (1.297), kernel elongation ratio (1.196), days to 50% flowering (0.729), alkali spreading value (0.717), grain breadth (0.413), gel consistency (0.182) and harvest index (0.141) showed positive direct effect of which no. of effective tillers plant^{-1}, harvest index, number of tillers square meter^{-1}, grain breadth, days to 50% flowering, kernel breadth showed true relationship with grain yield plot-1 at genotypic level. Plant height, total number of filled grains panicle^{-1}, panicle length, number of panicles square meter^{-1}, test weight, grain length, head rice recovery, grain length breadth ratio, amylose content showed direct negative effect with grain yield plot^{-1}. Kernel length breadth ratio (9.537), kernel breadth (5.337), grain length (2.355), kernel elongation ratio (0.690), days to 50% flowering (0.638), harvest index (0.390), test weight (0.210), number of tillers square meter^{-1} (0.202), number of effective tillers plant^{-1} (0.172) and panicle length (0.104) showed positive direct effect of which panicle length, number of effective tillers plant^{-1}, days to 50% flowering, harvest index, test weight, kernel breadth and kernel elongation ratio showed true relationship with grain yield plot^{-1} at phenotypic level.

Traits like plant height, head rice recovery, total number of filled grains panicle^{-1}, grain breadth, number of panicles square meter^{-1}, grain length breadth ratio, kernel length, alkali spreading value, gel consistency and amylose content recorded direct negative effect with grain yield plot^{-1}. Therefore, selection of the characters which have direct effect with true relationship results in increasing the grain yield.These findings found to be satisfactory with previous results of Nandeshwar et al. (2010), Yadav et al. (2010), Selvaraj et al. (2011), Yadav et al. (2011) and Osman et al. (2012) for panicle length. Nandeshwar et al. (2010), Yadav et al. (2010), Osman et al. (2012), Allam et al. (2015) and Devi et al. (2017) for test weight. Yadav et al. (2010) and Yadav et al. (2011) for harvest index. Immanuel et al. (2011), Khare et al. (2014) and Ekka et al. (2015) for days to 50% flowering. Veniet al. (2013), Hossain et al. (2015) and Gunasekaran et al. (2017) for number of effective tillers plant^{-1}. Allam et al. (2015) for kernel elongation ratio and Veniet al. (2013) for kernel breadth. The genotypic and phenotypic path coefficient analysis is shown in (Table 3).

CONCULSION

High heritability (broad sense) was mostly observed in quality characters. High heritability coupled with high genetic advance as percentage of mean was reported for characters like plant height, test weight, head rice recovery, grain length, grain breadth, grain L:B, kernel length, kernel breadth, kernel L:B, alkali spreading value, gel consistency and amylose content indicating additive gene action. PCV values found to be slightly higher than GCV values showing the influence of environment on character expression. Characters like panicle length, total number of filled grains per panicle^{-1}, harvest index, test weight, grain breadth, kernel breadth and amylose content showed high significant positive phenotypic association with grain yield plot^{-1}. Amylose content showed positive association with alkali spreading value, kernel elongation ratio and negative association with gel consistency and gelatinization temperature. Characters like number of effective tillers plant^{-1}, grain breadth, number of tillers square meter^{-1}, harvest index, days to 50% flowering, kernel breadth showed true relationship with grain yield plot^{-1} at genotypic level. Panicle length, number of effective tillers plant^{-1}, days to 50% flowering, test weight, harvest index, kernel breadth and kernel elongation ratio showed true relationship with grain yield plot^{-1} at phenotypic level so selection of these traits will increase the grain yield.

REFERENCES

Al-Jibouri HR, Miller PA and Robinson HF (1958). Genotypic and environmental variance and covariance in an upland cotton crop of inter specific origin. Agron.

J. 50: 633-637

Allam CR, Jaiswal HK and Qamar A (2015). Character association and path analysis studies of yield and quality parameters in basmati rice (*Oryza sativa* L.). The Bioscan 9(4): 1733-1737

Anonymous (2017). the world population prospects: The 2017 Revision, published by UN Department of Economics and Social Affairs

Anonymous (2019). World: Rice Area, Yield and Production, U.S.Department of Agriculture

Babu VR, Shreya K, Dangi KS, Usharani G and Nagesh P (2012). Genetic variability studies for qualitative and quantitative traits in popular rice (*Oryza sativa* L.) hybrids of India. Int. J. Sci. Res. 2(6): 1-5

Berner DK and Hoff BJ (1986). Inheritance of Scent in American Long Grain Rice 1. Crop Sci. 26(5): 876-878

Bhattacharjee P, Singhal RS and Kulkarni PR (2002). Basmati rice: a review. Inter. J. of Food Sci. and Tech. 37(1): 1-12

Bradbury LM, Henry RJ, Jin Q, Reinke RF and Waters DL (2005). A perfect marker for fragrance genotyping in rice. Mol. Breed. 16(4): 279-283

Burton GW (1952). Quantitative inheritance in grasses. Proc. 6th Intr. Grassland Cong. 1: 227-283

Cagampang GC, Perez CM and Juliano BO (1973). A gel consistency test for eating quality of rice. J. Sci. Food Agric. 24(1): 1589-1594

Cruz ND and Khush GS (2000). Rice grain quality evaluation procedures. Aromatic rices 3: 15-28

Devi KR, Chandra BS, Lingaiah N, Hari Y and Venkanna V (2017). Analysis of variability, correlation and path coefficient studies for yield and quality traits in rice (*Oryza sativa* L.). Agri. Sci. Digest 37(1): 1-9

Dewey DR and Lu HK(1959). A correlation and path coefficient analysis of components of created wheat grass seed production. Agron. J. 51(3): 515- 518

Dhanwani RK, Sarawgi AK, Solanki A and Tiwari JK (2013). Genetic variability analysis for various yield attributing and quality traits in rice (*Oryza sativa* L.). The Bioscan 8(4):1403-1407

Ekka RE, Sarawgi AK and Kanwar RR (2011). Correlation and path analysis in traditional rice accessions of Chhattisgarh. J. Rice Res. 4(1 & 2): 11- 17

Ekka RE, Sarawgi AK and Kanwar RR (2015). Genetic variability and inter-relationship analysis for various yield attributing and quality traits in traditional germplasm of rice (*Oryza sativa* L.). Plant Archives 15(2): 637-645

Golam F and Prodhan ZH (2013). Kernel elongation in rice. Journal of the Science of Food and Agriculture 93(3): 449-456

Gunasekara K, Sivakami R, Sabariappan R, Ponnaiah G, Nachimuthu VV and Pandian BA (2017). Assessment of genetic variability, correlation and path coefficient analysis for morphological and quality traits in rice (*Oryza sativa* L.). Agric. Sci. Digest 37(4): 251-256

Hossain S, Haque M and Rahman J (2015). Genetic variability, correlation and path coefficient analysis of morphological traits in some extinct local Aman rice (*Oryza sativa* L). J. Rice Res. 3: 158

Immanuel SC, Pothiraj N, Thiyagarajan K, Bharathi M and Rabindran R (2011). Genetic parameters of variability, correlation and path coefficient studies for grain yield and other yield attributes among rice blast disease resistant genotypes of rice (*Oryza sativa* L.). African J. of Biotech. 10(17): 3322-3334

Johnson HW, Robinson HF and Comstock RE (1955). Estimation of genetic variability and environmental variability in soybean. Agron. J. 47: 314-318

Juliano BO (1971), A simplified assay for milled rice amylose. Cereal Sci. Today 16(1): 334-338

Juliano BO and Villareal CP (1993). Grain quality evaluation of world rices. IRR1, Philippines pp. 205

Juliano BO, Perez CM and Cuevas-Perez F (1993). Screening for stable high head rice yields in rough rice. Cereal Chern. J. 70(1): 650-655

Khare R, Singh AK, EramS and Singh PK (2014). Genetic variability, association and diversity analysis in upland Rice (*Oryza sativa* L). SAARC J. of Agri. 12(2): 40-51

Limbani PL, Gangani MK and Pandya MM (2017). Genetic variability, heritability and genetic advance in rice (*Oryza sativa* L.). Int. J. Pure App. Bio Sci. 5(6): 1364-1371

Little RR, Hilder GB and Dawson EH (1958). Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chem. 35: 111-126

Mia MF, Begum SN, Islam MM, Manidas AC and Halder J (2010). Identification and differentiation of aromatic rice genotypes using SSR markers. Int. J. Bio. Res.

2(9): 7-12

Nandeshwar BC, Pal S, Senapati BK and De DK (2010). Genetic variability and character association among biometrical traits in F_2 generation of some Rice crosses. Electron. J. Plant Breed. 1(4): 758-763

Osman KA, Mustafa AM, Ali F, Yonglain Z and Fazhan Q (2012). Genetic variability for yield and related attributes of upland rice genotypes in semi arid zone (Sudan). African J. of Agri. R. 7(33): 4613-4619

Panse VG and Sukhatme PV (1985). Statistical methods for agricultural workers. 2nd Edition, ICAR, New Delhi pp. 359

Pokhrel A, Dhakal A, Sharma S and Poudel A (2020). Evaluation of physicochemical and cooking characteristics of rice (*Oryza sativa* L.) landraces of lamjung and Tanahun Districts, Nepal. International Journal of Food Science 2020: 1-11

Prajapati MK, Singh CM, Babu GS, Lavanya GR and Jadhav P (2011). Genetic parameters for grain yield and its component characters in rice. Elect. J. of Plant Breed. 2(2): 235-238

Rani NS, Madliav MS, Pandey MK, Sundaram RM, Prasad GSV, Sudarshan I, Rao LV and Ravindrababau (2008). Genetics and molecular approaches for improvement of grain quality traits in rice. Indian J. Crop Sci. 3(1): 1-14

Sahu PK, Sharma D, Mondal S, Kumar V, Singh S, Baghel S, Tiwari A, Vishwakarma G and Das BK (2017). Genetic variability for grain quality traits in indigenous rice landraces of Chhattisgarh India. J. of Exp. Bio. and Agri. Sci. 5(4):439-455

Shinha SK, Tripathi AK and Bisen (2004). Study of genetic variability and correlation coefficient analysis in midland landraces of rice. Annal of Agric. Res. 25 (1): 1-3

Shivani D, Viraktamath BC and Shobha Rani N (2007). Correlation among various grain quality characteristics in rice. Oryza 44(3): 212-215

Shobha Rani N, Shobha Rao LV, Viraktamath BC and Mishra B (2006).National guidelines for the conduct of tests for distinctiveness, uniformity and stabilit y. Directorate of Rice Research pp. 6-13

Sivasubramanian S and Madhavamenon P (1973). Heritability in rice. Madras Agric. J. 60: 1777-1778

Veni BK, Lakshmi BV and Ramana JV (2013). Variability and association studies for yield components and quality parameters in rice genotypes. J. of Rice Res. 6(2): 16-23

Wang D, Zhang X, Zhu Z, Chen N, Min J, Yao Q, Yan J and Liao X (2005). Correlation analysis of rice grain quality characteristics. Zuowuxuebao 31: 1086-1091

Wright S (1921). Correlation and causation. J. Agric. Res. 20: 557-565

Yadav SK, Pandey P, Kumar B and Suresh BG (2011). Improvement in Rice (*Oryza sativa* L.). Pakistan Journal of Biological Sciences 14(9): 540-545

Yadav SK, Suresh BG, Pandey P and Kumar B (2010). Assessment of genetic variability, correlation and path association in rice (*Oryza sativa* L.). J. of bio-Sci. 18:1-8

Zahid MA, Akhter M, Sabar M, Manzoor Z and Awan T (2006). Correlation and path analysis studies of yield and economic traits in Basmati rice (*Oryza sativa* L.). Asian J. Plant Sci. 5(4): 643-645

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Phenotypic assessment of rice landraces for genetic variability and diversity studies under heat stress

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ABSTRACT

The present investigation was carried out to estimate the genotypic and phenotypic variability, heritability, genetic advance and divergence based on heat stress and yield associated traits using 48 landraces of rice grown during rabi 2016 and 2017 at ICAR-Indian Institute of Rice Research farm, Hyderabad. ANOVA revealed the existence of significant differences for all the traits under study. Phenotypic coefficient of variation (PCV) was found slightly elevated than the genotypic coefficient of variation (GCV). All the characters under study except time to maturity exhibited high heritability coupled with high genetic advance as a per cent of mean, which revealed the predominance of additive gene action in controlling these traits. Cluster analysis grouped the 48 landraces into ten distinct clusters. Cluster I consisted of one landrace, while cluster II and III had 9 landraces each. Clusters IV, V, VI, VII, VIII, IX and X had 6, 2, 3, 3, 1, 6 and 8 landraces respectively. Cluster I included one landrace Byama Jhupi which was superior for the trait number of grains per panicle. Highest single plant yield (Mugei), fertility percentage (Neta) and time of heading (Neta) were recorded by entries of cluster II and highest panicle number per plant was recorded by landrace (Chiiti Mutyalu) of cluster IX. The genotypes of cluster II, VIII and IX showed high spikelet fertility percentage. Hence the genetic resources of these clusters can be utilized in the breeding programmes for development of heat tolerant varieties. The study helped to understand the extent of genetic diversity among the genetic resources which serve as a treasure of highly useful traits which can be exploited in developing high yielding and stress tolerant varieties.

Key words: *Rice, landraces, heat tolerance, genetic variability, diversity, cluster analysis*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food crops grown worldwide. In India, rice is grown in an area of 43.79 million ha with a total production of 112.91 million tones and an average productivity of 2578 kg ha^{-1} (Anonymous, 2018). Heat stress has become a serious problem of agriculture in different regions around the world (Wahid et al., 2007; Ahmad et al., 2016; Zafar et al., 2018). Most of the rice is currently grown in regions where current temperatures are already close to optimum for rice production. Therefore, any further increase in mean temperatures or of short episodes of high temperatures during sensitive stages, may be supra optimal and reduce grain yield. Grain yields of rice declined by 10% for each 1.8°C increase in minimum temperature during the growing-season (Peng et al., 2004). The predicted 2-4 °C increment in temperature by the end of the 21st century poses a threat to rice production. The increase in temperature has been striking and can cause irreversible damage to plant growth and development (Wahid et al., 2007).

Heat tolerance studies in rice have mainly focused on the reproductive stage due to its high sensitivity and its immediate relevance to grain yield (Prasad et al., 2008; Jagadish et al., 2010b; Aghamolki et al., 2014; Das et al., 2014; Hatfield and Prueger, 2015). According to Bahuguna et al., 2015 rice grain yield and quality are negatively affected by high temperature stress at reproductive or grain-filling stages. In tropical and subtropical rice growing regions, high day temperature is a major obstacle during anthesis and grain-filling stages (Kobata and Uemuki, 2004; Fahad et al., 2016a). It was suggested that spikelet fertility at high temperature can be used as a screening tool for heat tolerance during the flowering stage (Prasad et al., 2006). Hence, heat stress poses a serious threat to sustaining rice production in the most productive regions of tropical Asia.

Under changing climate, enhancing heat tolerance through the use of diverse germplasm of landraces is vital for breeding to develop temperature tolerant rice genotypes. Landraces harbour a great deal of useful traits with genetic potential for rice improvement and landraces are also important genetic resources for resistance to pests and diseases; they provide "adaptability genes" for specific environmental conditions. Incorporation of adaptability genes from landraces could ensure optimum grain yield for the region (Vijayakumar et al., 2012). Landraces, given their past evolutionary history and adaptation to stress environments, often out-yield modern cultivars under low-input production systems (Dwivedi et al., 2016).Thus, the present investigation was undertaken to evaluate landraces to understand the extent of genetic diversity for further utilization in crop improvement programs.

MATERIAL AND METHODS

The experimental material consisted of 48 landraces of rice (Table 1) collected from different geographical locations were sown separately in raised bed nursery. These genetic resources were evaluated for heat stress during *rabi* 2016 and 2017, such that the temperature sensitive flowering and grain filling stages of the genotypes coincided with the peak summer temperatures *i.e.*, from April to May, 2016 & 2017, at ICAR - Indian Institute of Rice Research farm, ICRISAT, Patancheru, Hyderabad, India. Experimental farm is situated at 17.53°N latitude, 78.27°E longitude and altitude of 545 m above mean sea level. Twenty-five days old seedlings of each landrace were transplanted in a plot comprising 11 rows of 6 m length at spacing of 20 cm between rows and 15 cm between plants in Randomized Block Design with three replications. Recommended agronomic and plant protection measures for raising a healthy nursery and main crop were taken up during the experiment.

Observations were recorded on five randomly chosen plants of each genotype for all the 22 traits under study at different stages of growth with appropriate procedures. Analysis of variance was performed for 22 traits using SAS software, version 9.2 to estimate genetic variation for the traits. The Pearson's correlation coefficient (r) was analysed to evaluate the relationships among different variables. Cluster analysis was carried out based on standardized Euclidean distances and dendrogram was generated using Ward's (1963) minimum variance.

RESULTS AND DISCUSSION

The analysis of variance (ANOVA) showed that differences are significant ($P < 0.05$) among the 48 landraces for twenty two traits studied, indicating the presence of considerable genetic variation in the experimental material. The estimates of phenotypic coefficient of variation (PCV) were slightly higher than those of genotypic coefficient of variation (GCV) for all the traits studied in the present investigation. The extent of the environmental influence on traits is explained by the magnitude of the difference between PCV and GCV. Large differences between PCV and GCV values reflect high environmental influence on the expression of traits. In this study, slight differences indicated environmental influence and consequently high influence of genetic factors on the expression of traits. High PCV and GCV was observed for gelatinization temperature (40.67, 40.64) followed by number of grains per panicle (35.90, 33.34), while low PCV and GCV was observed for days to maturity (9.20, 9.13). Similar finding of PCV and GCV have been reported for total grains per panicle, grain yield per plant and number of effective tillers per plant, while the lowest for days to maturity and days to 50 per cent flowering in rice reported by Khare et al. (2014). Similar observations were also noted earlier by Mustafa and Elsheikh (2007), Kole et al. (2008), Syoum et al. (2012), Malimar et al., 2015; Rashid et al., 2017; Gyawali et al., 2018; Adhikari et al., 2018 in rice.

Table 1. List of landraces used in present investigation.

S. no.	Landrace	Origin	S. no.	Landrace	Origin
KR1	Ajay	Uttar Pradesh	KR25	Him-Chhortu	Himachal Pradesh
KR2	Bhramarmali	West Bengal	KR26	Kamad	Jammu & Kashmir
KR3	Sadhu Bhog	Jharkhand	KR27	Mushk Budgi	Jammu & Kashmir
KR4	Charka Dhusri	Jharkhand	KR28	Red Rice (Zag)	Jammu & Kashmir
KR5	Rajesh	Bihar	KR29	Surjeet Basmati	Haryana
KR6	Neta	Jharkhand	KR30	Karad	Himachal Pradesh
KR7	Jhulur	West Bengal	KR31	Ambemohar	Maharashtra
KR8	China Goda	Jharkhand	KR32	ChittiMutyalu	Telangana
KR9	Khajurchari	West Bengal	KR33	Heitupphou	Manipur
KR10	Byama Jhupi	West Bengal	KR34	Tolen	Manipur
KR11	Neta Shawl/ Neta Shal	West Bengal	KR35	Mugei	Orrisa
KR12	Nagrasal	West Bengal	KR36	Govardhan Badshah Bhog	Chattisgarh
KR13	Gelei Dhan	Orissa	KR37	Govardhan Vishnu Bhog	Chattisgarh
KR14	Annapurna	Uttar Pradesh	KR38	Atharav	Uttar Pradesh
KR15	N22	Uttar Pradesh	KR39	AMAR	Bihar
KR16	Dular	Uttar Pradesh	KR40	Mahadi	Maharastra
KR17	Kuruka (Kuruna)	Kerala	KR41	Sagara Mutyalu	Andhra Pradesh
KR18	Azucena	IRRI	KR42	Kakirekkalu	Andhra Pradesh
KR19	Nipponbare	IRRI	KR43	Manipur Black Rice	Manipur
KR20	Kalahitta	Bangladesh	KR44	Him-Begmi	Himachal Pradesh
KR21	Ganjarangwala	Central India	KR45	Champaisali	Orrisa
KR22	Darbariroodbar	Central India	KR46	Nun-Bovel	Jammu & Kashmir
KR23	Nerica-L-45	IRRI	KR47	KABI RAJ	West Bengal
KR24	Lemont	IRRI	KR48	Jeeraka Samba	Tamil Nadu

Heritability and genetic advance

The estimates of broad sense heritability ranged from 68.54 to 99.97 %. High estimate of heritability (above 60%) were exhibited for all the characters in present study (Table 2). High heritability values indicate that the characters under study are less influenced by environment in their expression. The plant breeder, therefore adopt simple selection method on the basis of the phenotype of the characters which ultimately improves the genetic background of these traits. Sarawgi et al., 2000; Gannamani, 2001; Sao, 2002 and Jalandhar Ram et al., 2017 reported that the experimental finds nearer to the present work. But character exhibiting high heritability may not necessarily give high genetic advance (Gandhi et al., 1964) because of involvement of non-additive gene action. Thus, selection for the characters should be based on high heritability as well as high genetic advance (Johnson et al., 1955).

The estimates of genetic advance as percent of mean provide more reliable information regarding the effectiveness of selection in improving the traits. Among the studied characters, high and moderate estimates of genetic advance as percent of mean was recorded and it varied from 18.67 to 83.64 (Table 2). Among the characters time to maturity exhibited moderate (18.67%) genetic advance as a percent of mean. Remaining all the traits exhibited high estimates of genetic advance as a per cent of mean, among them gelatinization temperature recorded highest (83.64 %) value. In this study high heritability (98.48%) coupled with moderate genetic advance as percent of mean (18.67%) was recorded in the days to maturity. Such phenomenon suggested that these traits are mostly governed by interaction of genetic and environmental components. Present experimental findings are in unison with the reports of Tiwari et al., 2019 and Bekele et al., 2013. Heritability and genetic advance were found to be high for the remaining characters, selection for these characters in early generation would be ideal as the additive gene action is controlling those traits. Hence direct selection of such characters would be effective in improving the seed yield. Current investigation exhibited the findings that are in agreement with the findings of Kumar et al., 2015; Tuhina-Khatun et al., 2015; Tripathi et al., 2016 and Jalandhar Ram et al., 2017.

Table 2. The mean performance, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), H2b Broad sense heritability (H2), genetic advance (GA) and Genetic advance as percent of mean (GAM) for all the traits are furnished.

S. no.	Character	Mean	Range		Coefficient of variation (%)		H^2b (%)	GA	GAM
			Min	Max	Phenotypic	Genotypic			
1	Leaf: Length of blade	33.81	17.62	57.91	28.28	28.21	99.53	19.60	57.98
2	Leaf: Width of blade	1.12	0.69	1.97	24.16	24.08	99.32	0.55	49.43
3	Time of heading	97.00	70.38	117.86	12.03	12.01	99.75	23.97	24.72
4	Stem: Thickness	1.08	0.29	5.37	13.91	13.90	99.90	3.08	28.64
5	Stem: Length	77.06	40.38	137.67	32.07	32.01	99.63	50.72	65.82
6	Panicle: Length of main axis	21.30	13.51	29.40	15.63	15.52	98.53	6.76	31.73
7	Panicle: Number per plant	13.20	5.63	19.87	24.26	23.13	90.89	6.00	45.42
8	Time maturity (days)	126.66	99.36	145.72	9.20	9.13	98.49	23.65	18.67
9	Grain: Weight of 1000 fully developed grains	22.37	13.67	29.16	13.54	13.39	97.86	6.11	27.30
10	Grain: Length	8.27	5.86	11.58	13.84	13.70	97.98	2.31	27.93
11	Grain: Width	2.51	1.77	3.62	18.14	18.02	98.64	0.93	36.87
12	Decorticated grain: Length	5.81	3.88	8.63	15.05	14.92	98.32	1.77	30.47
13	Decorticated grain: Width	2.10	1.49	2.81	19.14	19.02	98.73	0.82	38.93
14	Endosperm: Content of amylose	22.24	7.31	29.22	18.09	17.97	98.65	8.17	36.76
15	Gelatinization temperature through alkali spreading value	39.73	6.96	88.43	40.67	40.64	99.83	33.23	83.64
16	No. of grains/panicle	106.39	55.98	298.03	35.90	33.34	86.24	67.86	63.78
17	Fertility %	83.02	43.31	97.19	15.69	12.99	68.54	18.39	22.15
18	Single plant yield(in gms)	17.01	10.24	23.74	19.34	16.42	72.14	4.89	28.74
19	germination %	73.37	9.97	103.67	34.66	34.58	99.50	52.13	71.05
20	No of days to Germination	4.69	3.03	8.07	27.91	27.90	99.97	2.70	57.47
21	Coleoptile length	12.61	5.42	18.77	28.09	27.98	99.23	7.24	57.41
22	Radicle length (cm)	8.78	3.48	13.97	24.27	24.15	99.07	4.35	49.52

Cluster analysis

The cluster analysis based on 22 morphological data grouped the 48 landraces into ten distinct clusters at the Euclidean squared distance of 100. Cluster I consisted of one landrace, while clusters II and III had 9 landraces each. Clusters IV V, VI, VII, VIII, IX, X, XI had 6, 2, 3, 3, 1, 6, 6, 2 landraces respectively, as shown in Fig. 1. Cluster I included one landrace Byama Jhupi which was superior for the character number of grains/panicle. Highest single plant yield, fertility percentage and time of heading were recorded in entries of cluster II and highest panicle number per plant was recorded by cluster IX landrace. The genotypes of cluster II, VIII and IX showed high spikelet fertility percentage. Hence, the genotypes of the cluster I, cluster II, cluster VIII and cluster IX can be used in breeding programmes for the development of heat tolerant varieties.

The clustering pattern reflects the closeness between the clusters and the geographical adaptation of the genotypes (Ram et al., 1970). Grouping of IRRI landraces into one cluster (cluster VI) indicated genetic relatedness between these genotypes. This can be further explained by free flow of genes between these accessions. The clustering pattern showed that the landraces originating from similar geographical regions were categorized into different clusters, revealing that geographical diversity and genetic diversity were not related. Therefore, the kind of genetic diversity found among the genotypes belonging to same geographical region might be due to differences in adaptation, selection criteria, selection pressure and environmental stress. Similar studies has been reported earlier by Chakravorty and Ghosh, 2013; Rashid et al., 2013; Chandramohan et al., 2016; Guru et al., 2017; Solanki et al. 2019; Kumar et al., 2019; Sangeetha et al., 2019; Sandeep et al., 2020.

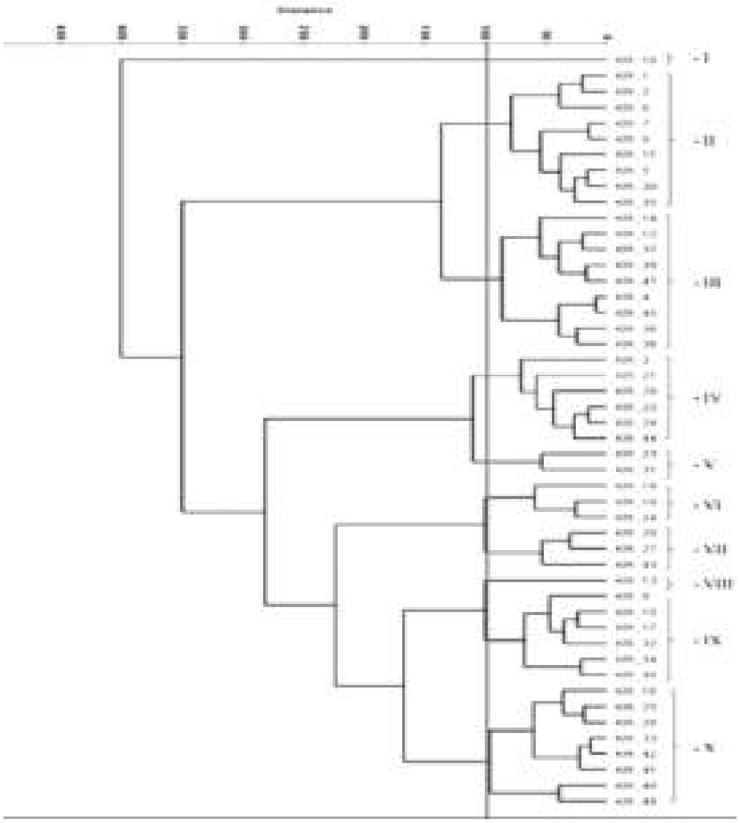

Fig. 1. Cluster analysis of 48 landraces of rice based on yield and heat tolerance associated traits.

CONCLUSION

Analysis of variance exhibited the presence of significant differences among the traditional varieties of rice for all the traits included in the study, thereby confirming the presence of genetic variation in the experimental material. Phenotypic coefficient of variation was found slightly higher than the genotypic coefficient of variation revealed less influence of environment on the characters under study. Therefore, response to direct selection may be effective in improving these traits. Selection of traits with high heritability coupled with high genetic advance would help in improving the traits under study. The landraces of rice under study were grouped into ten different clusters. Few landraces from same geographic location grouped into same cluster suggesting genetic relatedness between these genotypes. This can be further explained by free flow of genes between these accessions. The clustering pattern also showed that the landraces/traditional varieties originating from close geographical regions were categorized into different clusters, informing that geographical diversity and genetic diversity probably were not related. Therefore, the kind of genetic diversity found among the genotypes belonging to same geographical region might be due to differences in adaptation, selection criteria, selection pressure and environmental stress. Hence, utilization of these landraces/genetic resources from different clusters serve as potential donors for improvement of several agronomic traits in hybridization programmes by tailoring these useful traits in modern high yielding varieties with wide adaptability and resilience.

REFERENCES

Adhikari BN, Joshi BP, Shrestha J and Bhatta NR (2018). Genetic variability, heritability, genetic advance and correlation among yield and yield components of rice (*Oryza sativa* L.). Journal of Agriculture and Natural Resources 1(1): 149-160

Aghamolki MTK, Yusop MK, Oad FC, Zakikhani H, Jaafar HZ, Kharidah S et al. (2014). Heat stress effects on yield parameters of selected rice cultivars at reproductive growth stages. J. Food Agric. Environ. 12: 741-746

Ahmad W, Noor MA, Afzal I, Bakhtavar MA, Nawaz MM, Sun X, Zhou B, Ma W, Zhao M (2016). Improvement of sorghum crop through exogenous application of natural growth-promoting substances under a changing climate. Sustainability 8: 1330

Anonymous (2018). Agricultural statistics at a glance 2018. Directorate of Economics and Statistics, Department of Agriculture and Cooperation, Ministry of Agriculture, Government of India, New Delhi

Bahuguna RN, Jha J, Pal M, Shah D, Lawas LM, Khetarpal S et al. (2015). Physiological and biochemical characterization of NERICA-L-44: a novel source of heat tolerance at the vegetative and reproductive stages in rice. Physiol. Plant. 154: 543-559. doi: 10.1111/ppl.12299

Bekele BD, Naveen GK, Rakhi S and Shashidhar HE (2013). Genetic evaluation of recombinant inbred lines of rice (*Oryza sativa* L.) for grain zinc concentrations, yield related traits and identification of associated SSR markers. Pakistan Journal of Biological Sciences 16(23): 1714-1721 doi:10.3923/pjbs.2013.1714.1721

Chakravorty A and Ghosh PD (2013). Characterization of landraces of rice from Eastern India. Indian Journal of Plant Genetic Resources 26 (1): 62-67

Chandramohan YB, Thippeswamy SS and Padmaja D (2016). Diversity and variability analysis for yield parameters in rice genotypes.Indian Journal of Agricultural Research 50 (6)

Das S, Krishnan P, Nayak M and Ramakrishnan B (2014). High temperature stress effects on pollens of rice (*Oryza sativa* L.) genotypes. Environ. Exp. Bot. 101: 36-46 doi: 10.1016/j.envexpbot.2014.01.004

Dwivedi SL, Ceccarelli S, Blair MW, Upadhyaya HD, Are A K and Ortiz R (2016). Landrace Germplasm for Improving Yield and Abiotic Stress Adaptation. Trends in Plant Science 21(1): 31-42. doi:10.1016/j.tplants.2015.10.012

Fahad S, Hussain S, Saud S, Hassan S, Chauhan BS, Khan F (2016a). Responses of rapid viscoanalyzer profile and other rice grain qualities to exogenously applied plant growth regulators under high day and high night temperatures. PLoS One 11 (7). Available from: https://doi.org/10.1371/journal.pone.0159590.

Gandhi SM, Sanghli AK, Nathawat KS and Bhatnagar MP (1964). Genotypic variability and correlation coefficient relating to grain yield and few other quantitative characters in Indian wheat. Indian J. Genet. 24: 1-8

Gannamani N (2001). Study of heterosis and combining ability by utilizing cytoplasmic genetic male sterility and fertility restoration system in rice (*Oryza sativa* L.), M. Sc. (Ag.) Thesis, GAU, Raipur

Guru T, Padma V, Reddy VV, Rao PR, Rao DS, Ramesh T and Radhakrishna KV (2017). Genetic diversity analysis for yield attributing traits in rice genotypes. Res. on Crops.18(2): 311-315

Gyawali S, Poudel A and Poudel S (2018). Genetic variability and association analysis in different rice genotypes in mid hill of western Nepal. Acta Scientific Agriculture 2(9): 69-76. Retrieved from https://actascientific.com/ASAG/pdf/ASAG-02-0173.pdf

Hatfield JL and Prueger JH (2015). Temperature extremes: effect on plant growth and development. Weather Clim. Extrem. 10: 4-10. doi: 10.1016/j.wace.2015.08.001

Jagadish SVK, Muthurajan R, Oane R, Wheeler TR, Heuer S, Bennett J et al. (2010b). Physiological and proteomic approaches to address heat tolerance during anthesis in rice (*Oryza sativa* L.). J. Exp. Bot. 61: 143-156 doi: 10.1093/jxb/erp289

Jalandhar Ram B, G Suresh Babu, G Roopa Lavanya, K Madhu Kumar and B Spandana (2017). Genetic variability for yield attributing traits of elite rice Germplasm (*Oryza sativa* L.) Journal of Pharmacognosy and Phytochemistry 6(3): 832-834

Johnson HW, Robinson HF and Comstock RE (1955). Estimates of genetic and environmental variability in soybean. Agronomy Journal 47: 314-318

Khare R, Singh AK, Eram S and Singh PK (2014). Genetic Variability, Association and Diversity Analysis in upland rice (*Oryza sativa* L.). SAARC J. Agri. 12(2): 40-51

Kobata T, Uemuki N (2004). High temperatures during the grain-filling period do not reduce the potential grain dry matter increase of rice. Agron. J. 96: 406-414

Kole PC, Chakraborty NR and Bhat JS (2008). Analysis of variability, correlation and path coefficients in induced mutants of aromatic non-basmati rice. Trop. Agric. Res. Exten. 11: 60-64

Kumar R, Suresh Babu G, Rai SK and Sandhya (2015). Genetic variability and divergence studies in elite rice genotypes for biometrical traits. The Ecoscan 9(1-2): 209-212

Mallimar M, Surendra P, Hanamaratti NG, Jogi M, Sathisha TN and Hundekar R (2015). Genetic variability for yield and yield attributing traits in F_3 generation of rice (*Oryza sativa* L.). Research in Environment and Life Sciences 9(1): 24-28

Mustafa MA and Elsheikh MAY (2007). Variability, correlation and path coefficient analysis for yield and its components in rice. Afr. Crop Sci. J. 15: 183-189

Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, Centeno GS, Khush GS and Cassman KG (2004). Rice yield decline with higher night temperature from global warming. In E.D. Redona, A.P. Castro & G.P. Llanto, eds. Rice Integrated Crop Management: Towards a RiceCheck syatem in the Philippines, p. 46-56: Nueva Ecija, Philippines, PhilRice populations of rice for heat tolerance during anthesis. Crop Science.48 (3): 1140-1146

Prasad PVV, Boote KJ, Allen LHJr., Sheehy JE and Thomas JMG (2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crops Res. 95: 398-411 doi: 10.1016/j.fcr.2005.04.008

Prasad PVV, Staggenborg SA, Ristic Z, Ahuja LR, Reddy VR, Saseendran SA and Yu Q (2008). Impacts of drought and/or heat stress on physiological, developmental, growth, and yield processes of crop plants. Response of Crops to Limited Water: Understanding and Modeling Water Stress Effects on Plant Growth Processes. doi:10.2134/advagricsystmodel1.c11

Praveen SKC, Mahesh Naik B, Ebenezer BRR, Suji DB (2019). Studies on genetic divergence among rice genotypes (*Oryza sativa* L.) under coastal saline condition. Plant Archives 19(2): 2825-2828

Ram J and Panwar DVS (1970). Intra specific divergence in rice. Indian J. Genet. 30: 1-10

Rashid MM, Nuruzzaman M, Hassan L and Begum SN (2017). Genetic variability analysis for various yield attributing traits in rice genotypes. Journal of the Bangladesh Agricultural University 15(1): 15-19

Rashid K, Khaliq I, Farooq MO and Ahsan MZ (2013). Correlation and cluster analysis of some yield and yield related traits in rice (*Oryza sativa*). International Journal of Agricultural Science and Research (4): 25-30

Sandeep S, Sujatha M, Subbarao LV and Neeraja CN (2020). Assessment of morphometric diversity for yield and yield attributing traits in rice (*Oryza sativa* L.) for tolerance to heat stress. Current Journal of Applied Science and Technology 39(10): 29-49

Sangeetha R, Saraswathi R, Amudha K and Senthil Kumar G (2019) Genetic divergence studies in subspecies of *Oryza sativa* L. International Journal of Current Microbiology and Applied Sciences 8(7): 834-845

Sao A (2002). Studies on combining ability and heterosis in F1rice hybrids using cytoplasmic male sterile lines, M. Sc. (Ag.) Thesis, IGAU, Raipur

Sarawgi AK, Rastogi NK, Soni DK (2000). Studies on some quality parameters of indigenous rice in Madhya Pradesh. Annuals of Agricultural Research 21(2): 258-261

Seyoum M, Sentayehu A and Kassahum B (2012). Genetic variability, heritability, correlation coefficient and path analysis for yield and yield related traits in upland rice. J. Plant Sciences 7(1): 13- 20

Tiwari DN, Tripathi SR, Tripathi MP, Khatri N and Bastola BR (2019). Genetic variability and correlation coefficients of major traits in early maturing rice under rainfed lowland environments of Nepal. Hindawi Advances in Agriculture Article ID 5975901 pp. 9

Tripathi A, Kumar S, Singh MK, Kumar A and Karnwal MK (2017). Phenotypic assessment of rice (*Oryza sativa* L.) genotypes for genetic variability and varietal diversity under direct seeded condition. J ournal of Applied and Natural Science 9(1): 6 - 9

Tuhina-Khatun, Mohamed M. Hanafi, Mohd Rafii Yusop, M. Y. Wong, Faezah M. Salleh and Jannatul Ferdous (2015). Genetic variation, heritability and diversity analysis of upland rice (*Oryza sativa* L.) genotypes based on quantitative traits. BioMed Research International Article ID 290861 pp. 7

Ujjval IS, Mahesh BP, Lalji NG (2019). Assessment of genetic divergence in rice genotypes under middle Gujarat condition. International Journal of Chemical Studies. 7(3): 2825-2828

Vijayakumar, Chakrabarty SK, Singh Y, Vashisht V and Dadlani M (2012). Characterization of farmers' varieties of rice based on morphological, physicochemical properties. Oryza 49 (3): 222-226

Wahid A, Gelani S, Ashraf M and Foolad M (2007). Heat tolerance in plants: An overview. Environmental and Experimental Botany 61(3): 199-223

Ward JH (1963). Hierarchical grouping to optimize an objective function, Journal of the American Statistical Association 58: 236-244

Zafar SA, Hameed A, Nawaz MA, MA W, Noor MA, Hussain M and Mehboob-ur-Rahman (2018). Mechanisms and molecular approaches for heat tolerance in rice (*Oryza sativa* L.) under climate change scenario. Journal of Integrative Agriculture 17(4): 726-738 doi:10.1016/s2095-3119(17)61718-0

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Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers in contrasting rice (*Oryza sativa* L.) genotypes under drought stress

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ABSTRACT

The role of antioxidative enzymes as reactive oxygen species-scavengers under water-limited (WL) conditions was studied in five contrasting rice genotypes, including two checks (Sahabhagidhan as a tolerant genotype and IR 64 as a susceptible genotype). The experiment was performed in pots, and the irrigation was withdrawn five days before flowering for 15 days. For stress imposition, stress pots were maintained at 50% field capacity whereas nonstress pots were maintained at 100% field capacity. The antioxidant enzyme activity such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX), was significantly increased under the WL conditions. Genotypes Sahabhagidhan and Parijata had the highest SOD, CAT, and POX activity with the lowest production of superoxide radical (O_2^-) and hydrogen peroxide (H2O2). At the same time, IR 64 and Prasad exhibited the lowest SOD, CAT, and POX activity, suggesting a lower potential to eliminate oxidative stress. The higher scavenging capacity of free radicals in Sahabhagidhan and Parijata was supplemented by the higher level of relative water content (RWC), membrane stability index (MSI), Maximum quantum yield of PSII photochemistry (Fv/Fm), chlorophyll content, and low lipid peroxidation which resulted in higher grain yield accompanied by higher biomass partitioning towards the grain.

Key words: *Grain yield, lipid peroxidation, superoxide dismutase, catalase, hydrogen peroxide*

INTRODUCTION

Water is a limited resource but an essential factor for growing crops (Wang et al., 2012). Due to the effects of ongoing climate change, the occurance of abiotic stresses such as drought has increased in most rice-growing areas, severely affecting rice production (Liu et al., 2018). The grain yield of rice plants is associated with various aspects such as biomass production, biomass partitioning to grains, and rate of grain filling (Jeng et al., 2006). Under water-limited condition, high translocation of assimilates may help to improve grain yield and grain filling percentage (Puteh et al., 2014). It has been reported that under water limited condition, the production of reactive oxygen species (ROS) cause oxidative damage to the cell and results in poor biomass translocation (Basu et al., 2017). The production of reactive oxygen species (ROS) such as superoxide radical (O_2^-) and hydrogen peroxide (H_2O_2) can lead to oxidative damage to lipids cell membrane damage, protein degradation, and pigment bleaching (Dudziak et al., 2019; Wang et al., 2019). Under these situations, plants have a defense mechanism to neutralise the free radicals using enzymes such as superoxide dismutase (SOD), catalase (CAT), and peroxidase (POX) (Samota et al., 2017; Sahoo et al., 2019). During the flowering stage under drought stress, the activity of SOD is believed to be more than the activity of CAT and POX (Wang et al., 2019). A study on the effects of drought stress found that tolerant genotypes have a greater increase in antioxidant activity with lower lipid peroxidation (Yin et al., 2005). The production of antioxidants is essentail for metabolic activity in the cell by improving tolerance strategies against drought stress (Kamarudin et al., 2018; Li et al., 2019). The present experiment was aimed to characterize five genotypes interms of their ROS and their scavenging activity under drought stress.

MATERIALS AND METHODS

Plant materials and treatments

The performance of five rice (*Oryza sativa* L.) genotypes comprising three genotypes (Parijata, Lalat, and Prasad) selected from the vegetative stage drought screening experiment conducted during the dry season 2018 and two checks (Sahabhagidhan as a tolerant and IR 64 as a susceptible) was studied in a pot experiment conducted during dry season-2019. The experiment was laid out with three replications for each genotype at ICAR-National Rice Research Institute, Cuttack, India in two sets under well-watered (WW) and water limited (WL) conditions. Each pot was filled with a 4 kg mixture of farm yard manure (FYM) and farm soil in a 1:6 ratio and fertilized with 200 mg urea, 384 mg single superphosphate, and 160 mg murate of potash. In each pot, four seeds were sown. After 15 days of germination, thinning was done to maintain one plant per plant. Drought stress was imposed in one set of pots (three replications for each genotype) before five days of flowering by withdrawing irrigation and maintaining at 50% field capacity for 15 days, while the other set was irrigated regularly for maintaining 100% field capacity. Standard agronomic practices were followed for the management of crops. Following physiological and biochemicak prameters were estimated at the end of the stress period.

Biochemical estimations

i) Superoxide radical (O_2^-)

The production of Superoxide radical (O_2^-) was determined by the oxygenated hydroxylamine method of Wang and Luo, 1990. The reaction mixture consisting of 0.5 ml potassium phosphate buffer (pH 7.8) and 0.5 ml of enzyme extract was incubated for 30 minutes at 25 °C. One milliliter of 3-aminobenzenesulfonic acid (58 mM) and an equal volume of 7 mM of 1-naphthyl amine was added to the reaction mixture and again incubated for 20 minutes. The absorbance was taken at 530 nm, and the amount of O_2^- production was calculated from the standard curve of sodium nitrite ($NaNO_2$).

ii) Hydrogen peroxide (H_2O_2)

Hydrogen peroxide (H_2O_2) was determined by the method of Alexieva et al., 2001. Fifty milligram of fresh leaf sample macerated with liquid nitrogen using pre-chilled mortar and pestle, and further homogenized with 10 ml of 0.1% TCA (w/v). The product was centrifused for 15 minutes at 1000 rpm under 4°C. The supernatant was stored at -80°C refrigerator and used for enzyme activity assays. The reaction mixture consisted of 0.5 ml 100 mM potassium phosphate buffer, 2 ml KI (1M) (W/V), and 0.5 ml of leaf enzyme extract. The mixture was incubated for 1 hour at room temperature under dark conditions, and the absorbance was taken at 390 nm. The amount of H_2O_2 was calculated from the standard curve obtained from the known concentrations of H_2O_2.

iii) Lipid peroxidation

The amount of malondialdehyde (MDA) (nmole per g tissue) is a decomposition product and is an indicator of lipid peroxidation, which was estimated according to the protocol of Yagi, 1998. Five hundred milligrams of freshly chopped leaves were homogenized in 2 ml of 0.1% (W/V) trichloroacetic acid (TCA. The homogenate was centrifuged at 4°C for 5 minutes at 10,000 rpm, and the supernatant was used for MDA estimation. One milliliter of the reaction mixture [4% of TCA+ 0.5% of thiobarbituric acid (TBA)] was added with 0.5 ml of supernatant. Mixture was boiled for 1 hour in a water bath and reaction was terminated by placing the tubes at room temperature. Then samples were centrifuged for 10 minutes at 10,000 rpm and taken absorbance at 520 nm and 600 nm using spectrophotometer (Genesys 200).

$$\text{Amount of MDA } \left(\text{nmoles/gm tissue}\right) = \frac{\text{Abs at 532 nm - Abs at 600 nm}}{155} \text{x1000}$$

155 is the extinction coefficient of the MDA-TBA abduct at 532

iv) Antioxidant enzyme activity

Fifty milligrams of fresh leave samples were homogenized with 3 ml of extraction buffer [100 mM potassium phosphate buffer (PH 7.8), 0.1 mM EDTA, and 1% polyvinylpyrrolidone (w/v)] in an ice water bath. Tthe homogenized material was centrifuged at 13000g for 10 minutes at 4°C. The supernatant was stored at -80°C refrigerator and used for enzyme activity assays. Activity of Super oxide dismutase (SOD) was determined by measuring the photochemical reduction of nitro-blue tetrazolium chloride (NBT) following the procedure of Giannopolitis and Ries, 1977. One unit of SOD was determined as the amount of enzyme causing 50% inhibition of NBT by photo-reduction at 560 nm absorbance.

Catalase activity was measured following the method of Beers and Sizer, 1952. To 0.1 ml of enzyme extract, 0.9 ml of reaction mixture containing 0.1 ml of H2O2, 0.7 ml of 50 mM potassium phosphate buffer (PH 7.0), and 0.1 ml of EDTA was added. Rate of CAT activity was determined from the degradation of H_2O_2 per minute at 240 nm absorbance.

Peroxidase activity was assayed following the procedure of Putter, 1974. The reaction mixture contained 1.5 ml of 0.1M potassium phosphate buffer (PH 7.0), freshly prepared 1 ml 10 mM guaiacol, 0.1 ml of H_2O_2, and an enzyme extract of 0.1ml. Initial absorbance at 436 nm was noted and then increase in absorbance was recorded per minute. One unit of enzyme activity was calculated from the enzyme assay that caused the degradation of 1 μmol of H_2O_2 per minute and expressed as unit/mg protein.

Quantification of physiological observations

i) Relative water content (RWC)

Healthy, fully expanded leaves were taken at mid-day, cleaned, and cut into small pieces. Fresh weights of cut leaves were recorded and then submerged in distilled water for 8 hours. After measuring the turgid weight under room temperature, the leaves were dried at 800C till a constant weight was achieved, and the dry weight of leaves was recorded. The relative water content of the leaves was calculated by using the formula given by Bhusan et al., 2007.

RWC (%) = [(Fresh weight- Oven dry weight) / (Turgid weight- Oven dry weight)] x 100

ii) Membrane stability index (MSI)

Second leaves from the top were collected and washed. One hundred milligram of leaf sample was incubated at 30°C in a hot water bath for 4 hours. After taking first initial electrical conductivity (EC) by an EC meter (Eutech Instruments, United Kingdom), the samples were again incubated at 100°C for 15 minutes, and the second EC reading was taken. MSI was calculated according to the formula given by Sairam, 1994.

$$\text{MSI (\%)} = 1 - \frac{\left(1 - \frac{T_1}{T_2}\right)}{\left(1 - \frac{C_1}{C_2}\right)} \times 100$$

Where:

T_1 and T_2: First and second electical conductivity of stressed plant leaves

C_1 and C_2: First and second electical conductivity of control plant leaves

iii) Total chlorophyll

The total chlorophyll content of the leaf sample was determined following the protocol of Arnon, 1949. Measured amount of fresh leave samples were placed in 80% acetone and incubated for 48 hours at 4°C in dark conditions. After filtering the supernatant absorbance was measured at 645 nm and 663 nm using a spectrophotometer (UV-2600, SHIMADZU, Europe). Total chlorophyll content was calculated using following the formula :

Total chlorophyll (mg g^{-1} fw) = (20.2 (OD_{645}) + 8.02 (OD_{663}) × V/W) x 1000

Where: OD: Optical density; V: Final volume of solution (10 ml);

W: Weight of sample (25 mg)

iv) Maximum quantum yield of PSII photochemistry (Fv/Fm)

Chlorophyll fluorescence (ChlF) was measured using Imaging PAM- MAXI version (Heinz Walz, Effeltrich, Germany) and the data were analyzed with the imaging Win v2.46i software. The healthy second leaf from the top of the plant was used for chlorophyll fluorescence measurement following 30 minutes of dark adaption.

The value of Fv/Fm was measured following the protocol of Pradhan et al., 2019.

Grain yield and yield components at maturity

After the end of the experiment, the plant of each pot was harvested separately and the panicle and stem weight were recorded after drying. The value was expressed as grain yield per plant at 14% grain moisture content. The weights of stems were recorded after oven drying at 80°C until a constant dry weight was achieved. Each observation was taken in three replications from both WW and WL conditions and expressed in gram per plant. Grain filling percentage per plant was calculated from a number of completely filled grains per plant to total grains per plant and the value was expressed in percentage.

Statistical analysis

The data were subjected to ANOVA, taken from two treatments, each consisting of six replications for each genotype. Pearson's correlation coefficients of the parameters were analyzed using Microsoft Excel. The data were analyzed with XLSTAT-2014 and Crop Stat version 7.2 (IRRI, 2009) statistical software.

RESULTS AND DISCUSSION

Induction of drought stress at flowering stage on rice plants caused significant reduction ($p<0.05$) in grain yield and biomass accumulation. WL lead to significant reduction ($p<0.05$) in RWC, MSI, chlorophyll content, Fv/Fm and, significant increase ($p<0.05$) in lipid peroxidation and antioxidative enzymes activity.

Reactive oxygen species (ROS)

Excessive production of ROS i.e. O_2^- and H_2O_2 due to water loss causes oxidative damage and lipid peroxidation under WL conditions (Mittler, 2002). However, ROS at minimal level acts as a secondary messenger for stress tolerance (Sauer et al., 2001). Drought stress significantly increased ($p<0.05$) O_2^- and H_2O_2 in all the studied genotypes. The range of O_2^- and H_2O_2 varied from 0.022 μmolg^{-1} fw to 0.030 μmolg^{-1} fw (mean 0.026 μmolg^{-1} fw) and 1.74 mg g-1fw to 1.83 mg g^{-1}fw (mean 1.80 mg g^{-1}fw) respectively under WW condition (Fig. 1 A & Fig. B). Under WL condition, the range of O_2^- varied from 0.032 μmolg^{-1} fw to 0.043 μmolg^{-1} fw (mean 0.038) and H_2O_2 varied from 2.20 mg g^{-1} fw to 4.24 mg g^{-1} fw (mean 3.31 mg g^{-1}fw). In particular, Parijata (0.032 μmolg^{-1} fw) and Sahabhagidhan (0.035 μmolg^{-1} fw) had the loweer O2? whereas, Prasad (0.043 μmolg^{-1} fw) and IR 64 (0.041 μmolg^{-1} fw) recorded with the higher production of O_2^- under WL. Furthermore, Sahabhagidhan (2.20 mg g^{-1}fw) showed the lowest production of H2O2, followed by Parijata (2.56 mg g^{-1}fw) and Lalat (3.47 mg g^{-1}fw), while IR 64 (4.24 mg g^{-1}fw) and Prasad (4.08 mg g-1fw) had the highest H_2O_2 under WL condition. Higher ROS production destroys photosynthetic apparatus, inactivates Calvin cycle's enzymes, and oxidizes cellular ultrastructures that cause cell death (Guo et al., 2006, Mahmud et al., 2019). Tolerant genotypes have antioxidant defense mechanisms to scavenge ROS production and mitigate oxidative damage (Gill and Tuteja, 2010). In this study, Parijata and Sahabhagidhan possessed lower ROS production with higher antioxidant activity than IR 64 and Prasad. The results agreed with previous studies that lower ROS production increased the efficiency of plant cells and showed tolerance under WL conditions (Lu et al., 2010; Kumar et al., 2014; Dudziak et al., 2019).

Lipid peroxidation

Panda et al. (2020) reported that lipid peroxidation is associated with oxidative stress and quantification of cell membrane damage. The production of MDA is a clear marker of lipid peroxidation (Celik et al., 2017). The level of MDA content was significantly increased ($p < 0.05$) when plants were subjected to WL conditions (Scandalios, 1993; Panda, 2007). In the present study, the value of MDA was 19.26 nmol g^{-1} fw to 24.14 nmol g^{-1} fw (mean 21.20 nmol g^{-1} fw) under WW condition and 25.65 nmol g^{-1} fw to 42.88 nmol g^{-1} fw (mean 34.25 nmol g^{-1} fw) under WL condition (Fig. 1 C). In particular, the genotype Parijata (25.65 nmol g^{-1} fw) showed lower production of MDA followed by Sahabhagidhan (28.21 nmol g^{-1} fw), whereas; higher production was observed in IR 64 (42.88 nmol g^{-1} fw) and Prasad (40.68 nmol g^{-1} fw). It was observed that WL has a positive impact on lipid peroxidation. MDA had significantly negative correlation with SOD ($r = -0.896$), CAT ($r = -752$), POX ($r = -0.762$), GY ($r = -0.829$) and filled grain % ($r = -0.822$) while positive association with O_2^- ($r = 0.758$) and H_2O_2 ($r=0.790$) (Table 1). The observed lowest production of MDA in tolerant genotypes reflects their

Table 1. Correlation coefficient among different traits of studied rice genotypes under WL condition.

Parameters	RWC	Chlorop-hyll	Fv/Fm	MSI	MDA	SOD	CAT	POX	O_2^-	H_2O_2	GY
Chlorophyll	0.836**										
Fv/Fm	0.858**	0.778**									
MSI	0.862**	0.807**	0.844**								
MDA	-0.887**	-0.732**	-0.896**	-0.843**							
SOD	0.931**	0.826**	0.910**	0.840**	-0.896**						
CAT	0.812**	0.666**	0.778**	0.799**	-0.752**	0.812**					
POX	0.797**	0.667**	0.706**	0.650**	-0.762**	0.757**	0.710**				
O_2^-	-0.587**	-0.558**	-0.785**	-0.662**	0.758**	-0.737**	-0.393*	-0.475*			
H_2O_2	-0.864**	-0.834**	-0.757**	-0.732**	0.790**	-0.454*	-0.722**	-0.789**	0.586**		
GY	0.951**	0.838**	0.809**	0.851**	-0.829**	0.894**	0.870**	0.744**	-0.639**	-0.807**	
Filled grain %	0.944**	0.811**	0.811**	0.835**	-0.822**	0.878**	0.855**	0.684**	-0.559**	-0.825**	0.981**

*, ** Significant level at 0.05 and 0.01 probability level.

RWC: relative water content, Fv/Fm: photosynthetic efficiency of PSII photochemistry, MSI: membrane stability index, MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase, POX-peroxidase, O_2^- : superoxide radical, H_2O_2: hydrogen peroxide, GY: grain yield.

minimum membrane leakiness with higher membrane stability and less oxidative damage with higher antioxidant activity that improves tolerance under WL conditions (Zhang et al., 2014; Khaleghi et al., 2019).

Antioxidant defense mechanism

Plants have an antioxidant defense mechanism to protect the cells from oxidative stress by producing antioxidant enzymes under WL conditions (Chutipaijit, 2016). Increased the expression level of antioxidants such as SOD, CAT, and POX serve as effective antioxidant systems in the plant cell to combat toxic ROS hence, increased tolerance under WL (Lu et al., 2010; Sharma et al., 2012). In this study, WL significantly increased ($p < 0.05$) SOD activity in Sahabhagidhan (2.09 fold), Parijata (1.75 fold), and Lalat (1.47 fold) whereas, non-significant increment ($p > 0.1$) was observed in IR 64 (1.06 fold) and Prasad (1.09 fold) (Fig. 2 C). The value of SOD was 0.89 units mg^{-1} protein to 1.10 units mg^{-1} protein (mean 1.03 units mg^{-1} protein) under WW condition and 1.10 units mg^{-1} protein to 2.19 units mg^{-1} protein (mean 1.54 units m^{-1} protein) under WL condition. In particular, Sahabhagidhan (2.19 units mg^{-1} protein), Parijata (1.93 units mg^{-1} protein), and Lalat (1.31 units mg^{-1} protein) showed higher production of SOD than that of Prasad (1.10 units mg^{-1} protein) and IR 64 (1.17 units mg^{-1} protein). The activity of SOD was markedly increased

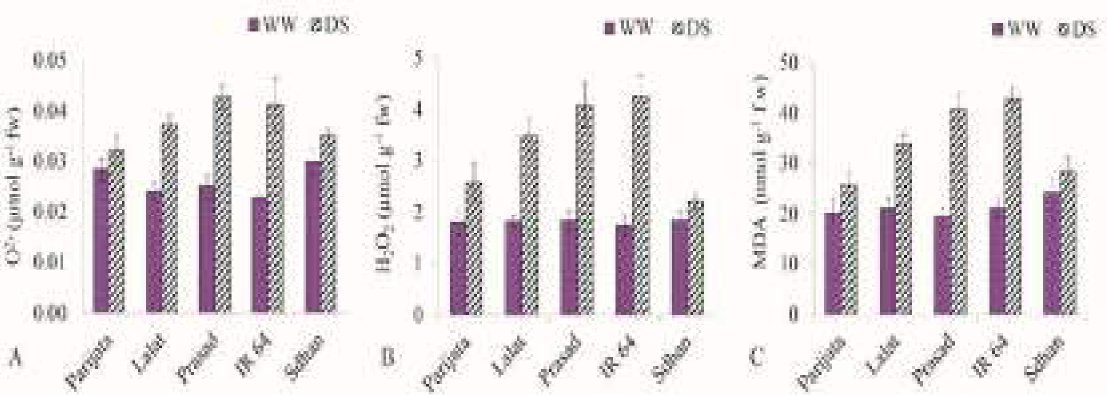

Fig. 1. Variations in superoxide radical (O_2^-) (A), hydrogen peroxide (H_2O_2) (B), malonaldehyde content (MDA) (C) of different rice genotypes under well-watered (WW) and water limited (WL) conditions. Data are expressed as means ± SE (n=3).

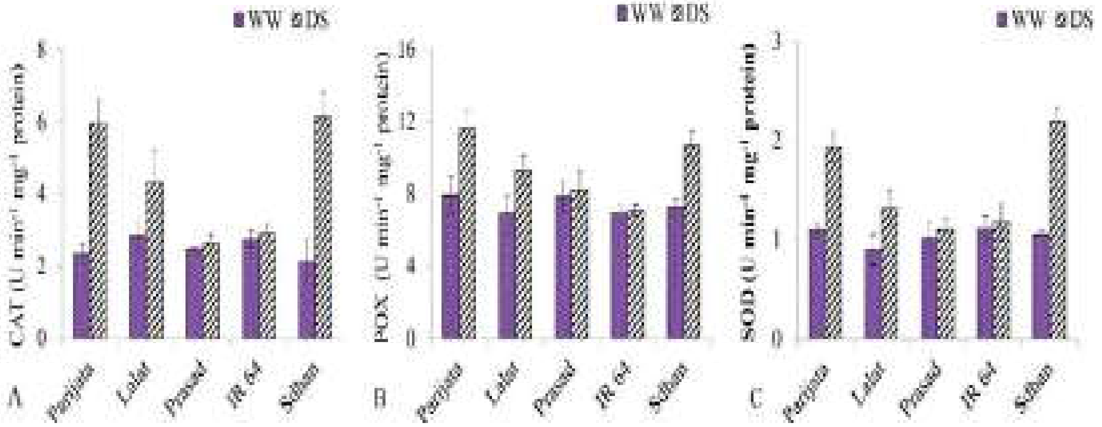

Fig. 2. Variations in catalase (CAT) (A), peroxidase (POX) (B), superoxide dismutase (SOD) (C) of different rice genotypes under well-watered (WW) and water limited (WL) conditions. Data are expressed as means ± SE (n=3).

in tolerant genotypes to mitigate the toxic effects of O_2^-. The production of SOD had significantly positive association with CAT (r = -0.812), POX (r = 0.757), GY (r = 0.894) and filled grain percentage (r = 0.878) and negative correlation with O_2^- (r=-0.737) and H_2O_2 (r = -0.454) (Table 1). The findings agreed with previous studies that higher production of SOD increase the efficiency to scavenge O_2^-, increase photosynthetic efficiency, GY, and reduce membrane leakiness under WL (Xu et al., 2011; Samota et al., 2017; Wang et al., 2019).

Under oxidative stress, the production of H_2O_2 by the activity of SOD leads to the production of CAT enzymes in plants (Mahmud et al., 2019). CAT neutralises H_2O_2 by converting it to H_2O and O_2. Hence, under WL, increasing CAT activity could protect the plant from oxidative stress and help build up stress tolerance. In the present study, CAT activity was significantly increased ($p<0.05$) in Sahabhagidhan (2.88 fold), Parijata (2.54 fold), and Lalat (1.52 fold) whereas, non-significant increment ($p > 0.1$) was observed in IR 64 (1.05 fold) and Prasad (1.06 fold) under WL compared to WW condition (Fig. 1 A). The value of CAT was 2.12 units mg^{-1} protein to 2.82 units mg$_2$ protein (mean 2.50 units mg$_2$ protein) under WW and 2.62 units mg^{-1} protein to 6.11 units mg^{-1} protein (mean 4.38 units mg^{-1} protein) under WL condition. In particular, Sahabhagidhan (6.11 units mg^{-1} protein) showed higher production of CAT followed by Parijata (5.94 units mg^{-1} protein) and Lalat (4.31 units mg^{-1} protein) than that of Prasad (2.62 units mg^{-1} protein) and IR 64 (2.89 units mg^{-1} protein). The production of CAT had significantly positive association with POX (r = 0.710), GY (r = 0.870) and filled grain % (r = 0.855) and negative correlation with O_2^- (r = -0.393) and H_2O_2 (r = -0.722) (Table 1). An increase in CAT activity strengthens antioxidant machinery, decreases oxidative damage by deactivating the H_2O_2 pathway, minimizes lipid peroxidation, and improves tolerance under WL conditions (Noctor et al., 2014; El-Esawi and Alayafi, 2019).

Similarly, POX activity was significantly increased ($p < 0.05$) in Parijata (1.47 fold), Sahabhagidhan (1.46 fold), and Lalat (1.34 fold) under WL conditions (Fig. 2 B). However, POX activity was non-significantly increased ($p > 0.1$) in IR 64 (1.02 fold) and Prasad (1.04 fold) indicating a lower potential to eliminate H_2O_2. The value of POX was 6.88 units mg-1 protein to 7.91 units mg^{-1} protein (mean 7.36 units mg^{-1} protein) under WW condition and 7.06 units mg^{-1} protein to 11.65 units mg^{-1} protein (mean 9.37 units mg^{-1} protein) under WL condition. Among the tested genotypes, Parijata (11.65 units mg^{-1} protein), Sahabhagidhan (10.63 units mg^{-1} protein) showed higher production of POX activity whereas, IR 64 (7.06 units mg^{-1} protein) and Prasad (8.21 units mg^{-1} protein) showed lower production of POX under WL condition. The production of POX had significantly positive association with GY (r = 0.744) and filled grain percentage (r = 0.684) and negative correlation with

O_2^- (r = -0.475) and H_2O_2 (r = -0.789) (Table 1). As a result, up-regulation of POX activity in tolerant genotypes could protect the cell for scavenging H_2O_2, minimizing lipid peroxidation, thereby improving drought tolerance and GY (Oskuei et al., 2013; Celik et al., 2017).

Relative water content

Relative water content (RWC) indicates tissue hydration and is a sensitive indicator of water status in plants (Silva et al., 2007). RWC decreased significantly ($p < 0.05$) under WL conditions (Fig. 3 A). Under WW conditions, RWC varied from 86.13% to 88.52%, with a mean of 87.16% and it varied from 23.67% to 71.93%, with a mean of 50.47% under WL conditions. The genotype Parijata (71.93%) and Sahabhagidhan (68.16 %) maintained the higher value of RWC while the genotype IR 64 (23.67%) and Prasad (28.28%) recorded the lowest value and Lalat (60.29%) showed intermediate value under WL conditions. Hence, the genotypes maintaining higher RWC signifies better protoplast hydration and better healthiness of tissue under WL conditions (Sikuku and Onyango, 2012; Yang et al., 2019). Dien et al., 2019 reported that minimum reduction in RWC signifies better tolerance potential under WL conditions.

Membrane stability index

The reduction in membrane stability index (MSI) is due to oxidative damage by overproduction of reactive oxygen species (ROS), leading to disruption of cell membranes (Swapna and Shylaraj, 2017). MSI decreased significantly ($p < 0.05$) under WL. Under WW condition, MSI was 82.23% to 86.96 % (mean 84.04%) and under WL conditions, it varied from 25.81% to 52.71% (mean 40.21%) (Fig. 3B). It was observed that WL has a negative impact on MSI. Among the five tested genotypes, Sahabhagidhan (52.71%) and Parijata (48.93%) were able to maintain the highest value of MSI, while the genotype IR 64 (25.81%) and Prasad (29.61%) recorded the lowest value under WL conditions. MSI had significantly negative correlation with MDA (r = -0.843), O_2^- (r = -0.662) and H_2O_2 (r = -0.732) while positively associated with antioxidant enzyme SOD (r = 0.840), CAT (r=0.799) and POX (r = 0.650) (Table 1). Hence, the genotypes that

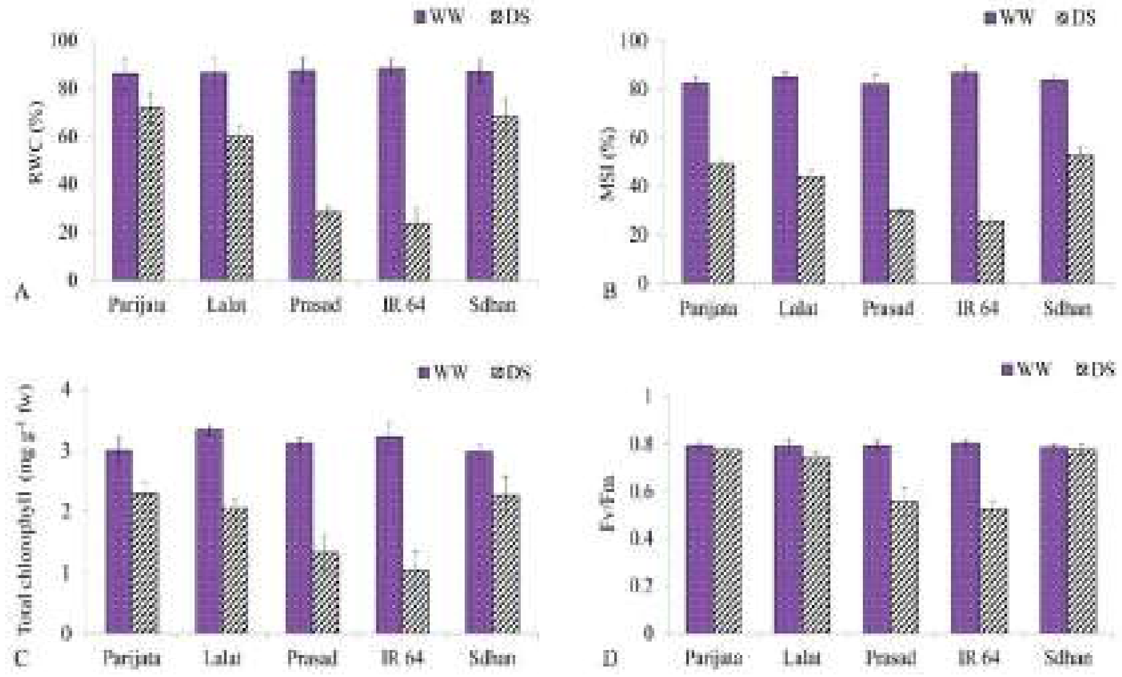

Fig. 3. Variations in relative water content (RWC) (A), membrane stability index (MSI) (B), total chlorophyll (C) and photochemical efficiency of PSII photochemistry (Fv/Fm) (D) of different rice genotypes under well-watered (WW) and water limited (WL) conditions. Data are expressed as means ± SE (n=3).

maintained higher MSI are likely to uphold good health with minimum tissue leakage, thus ensuring higher productivity (Larkunthod et al., 2018). Previously, it was reported that the ability of the genotype to retain higher MSI is one of the adaptive methods of tolerance under WL (Bangar et al., 2019).

Total chlorophyll content

Chlorophyll is the major photosynthetic pigment that significantly decreased ($p < 0.05$) under WL conditions (Moonmoon et al., 2017). The reduction of chlorophyll pigment is mainly due to chloroplast photo-oxidation, ultrastructure degradation and increment in chlorophyllase activity (Mafakheri et al., 2010; Kabiri et al., 2014). Total chlorophyll content varied from 2.97 mg g-1fw to 3.33 mg g^{-1} fw (mean 3.12 mg g^{-1}fw) under WW condition and it varied from 1.03 mg g^{-1}fw to 2.29 mg g^{-1}fw (mean 1.79 mg g-1 fw) under WL (Fig. 3 C). The genotype Parijata (2.29 mg g^{-1}fw) and Sahabhagidhan (2.26 mg g^{-1}fw) exhibited maximum value of chlorophyll content while IR 64 (1.03 mg g^{-1}fw) and Prasad (1.32 mg g^{-1}fw) had minimum chlorophyll content, and Lalat (2.05 mg g^{-1}fw) showed intermediate value under WL conditions. Photo-inhibition and ROS production (superoxide radical and hydrogen peroxide) lead to lipid peroxidation, degradation of chlorophyll pigments, and reduced chlorophyll bio-synthesis under WL conditions (Alaei, 2011; Chutia and Borah, 2012). The results showed that tolerant genotypes were able to maintain higher chlorophyll content and minimum reduction than susceptible genotypes.

Photosystem (PS) II activity

Maximum quantum efficiency of PSII photochemistry (Fv/Fm) defined the health status of PS II and was significantly reduced ($p < 0.05$) under WL condition (Praba et al., 2009; Stirbet and Govindjee, 2011; Gashi et al., 2013). Over-production of ROS that damage Rubisco activity leads to a reduction in Fv/Fm, resulting in a higher restriction in photosynthesis (Zhou et al., 2007). The value of Fv/Fm was 0.785 to 0.799, with an average of 0.791 under WW conditions (Fig. 3 D). Under WL conditions, it varied from 0.523 to 0.775 with an average of 0.674. The genotypes Sahabhagidhan (0.775) and Parijata (0.774) had higher Fv/Fm while IR 64 (0.523) and Prasad (0.554) showed lower Fv/Fm. Lalat showed intermediate value (0.741) under WL conditions. Fv/Fm had significantly negative correlatioon with MDA (r = -0.896), O_2^- (r = -0.785) and H_2O_2 (r = -0.757) and positive association with SOD (r = 0.910), CAT (r = 0.778), POX (r = 0.706), GY (r = 0.809) and filled grain percentage (r = 0.811) (Table 1). In this study, tolerant genotypes Parijata and Sahabhagidhan had higher Fv/Fm, maintained better PSII photochemical efficiency, and thylakoid membrane integrity compared to Prasad and IR64 under WL conditions.

Yield and yield attributes as affected by reproductive stage drought stress

Total above ground biomass was sharply reduced (22.23%) in all the studied genotypes under WL conditions. Total biomass was higher in IR 64 (37.26 g $plant^{-1}$) and Prasad (33.60 g $plant^{-1}$) than Lalat (31.25 g $plant^{-1}$), Parijata (28.48 g $plant^{-1}$) and Sahabhagidhan (25.67 g $plant^{-1}$) under WL condition (Fig. 4 A). Although IR 64 showed the highest biomass but had lowest grain yield (GY) per plant indicating that partitioning of biomass to grains was poor (Puteh et al., 2014). The value of GY per plant was 13.58 g to 16.82 g, with an average of 14.81 g $plant^{-1}$ under WW conditions (Fig. 4 B). Under WL conditions, it varied from 2.23 g $plant^{-1}$ to 8.11 g $plant^{-1}$ with an average of 5.39 g $plant^{-1}$. The genotype Parijata (8.11 g $plant^{-1}$) and Sahabhagidhan (7.88 g $plant^{-1}$) maintained the highest value of GY, while the genotype IR 64 (2.23 g $plant^{-1}$) and Prasad (2.77 g $plant^{-1}$) recorded the lowest value and Lalat (5.96 g $plant^{-1}$) showed intermediate value under WL conditions. Fahad et al., 2017 reported that WL severely affects biomass partitioning due to disruption in phloem loading and unloading process. The genotypes maintaining higher GY suggest efficient partitioning of assimilates to grains under WL condition. Results further showed that filled grain percentage was recorded highest in Sahabhagidhan (68.95 %) followed by Parijata (62.18%) and Lalat (45.59%) than that of IR 64 (15.38%) and Prasad (18.98%) (Fig. 4 C) leading to higher GY per plant. Filled grain percentage had significantly positive association with GY (r = 0.981) (Table 1) and highest filled grain percenttage with less biomass indicate that tolerant genotypes have more ability to efficiently utilize the incoming assimilates during the grain filling period. Here, biomass

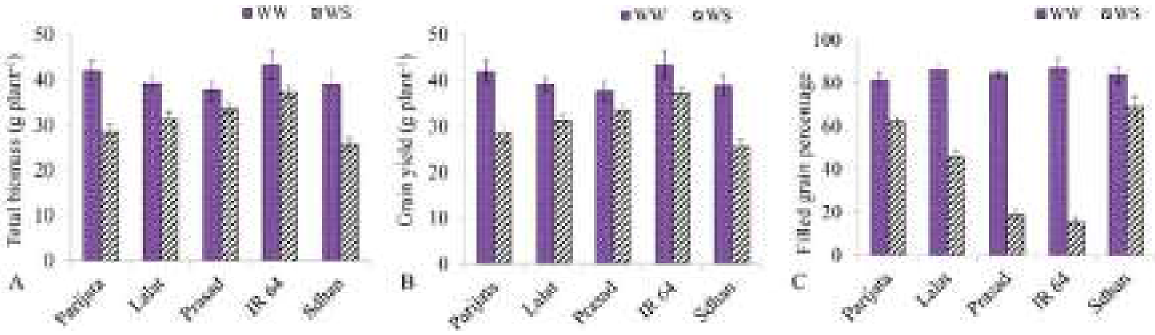

Fig. 4. Variations in total biomass per plant (A), grain yield per plant (B), and filled grain percentage (C) of different rice genotypes under well-watered (WW) and water limited (WL) conditions. Data are expressed as means ± SE (n=3).

translocation is more important than biomass production. Laza et al., 2003 reported that GY is strongly correlated with biomass partitioning and translocation rather than biomass production.

CONCLUSIONS

The differential response of the antioxidative enzymes (SOD, CAT, and POX) played an important role in protecting the plants from oxidative damage and was responsible for maintaining grain yield under drought stress conditions. Sahabhagidhan and Parijata maintained significantly higher GY per plant with higher SOD, CAT, and POX activity with lower production of O_2^- and H_2O_2. Higher GY is mainly due to efficient translocation of biomass. At the same time, IR 64 and Prasad exhibited the lowest production of antioxidant enzymes suggesting a lower potential to eliminate oxidative stress. Higher MSI, Fv/Fm, chlorophyll content, and lesser lipid peroxidation are mainly associated with higher antioxidant enzyme activity under drought stress conditions.

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REFERENCES

Alaei Y (2011). The effect of amino acids on leaf chlorophyll content in bread wheat genotypes under drought stress conditions. Middle-East Journal of Scientific Research 10: 99-101

Alexieva V, Sergiev I, Mapelli S, Karanov E (2001). The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. Plant, Cell and Environment 24: 1337-1344

Arnon DI (1949). Copper Enzymes in Isolated Chloroplasts. Polyphenoloxidase in Beta Vulgaris . Plant Physiology 24: 1-15

Bangar P, Chaudhury A, Tiwari B, Kumar S, Kumari R, Bhat KV (2019). Morphophysiological and biochemical response of mungbean [*Vigna radiata* (L.) Wilczek] varieties at different developmental stages under drought stress. Turkish Journal of Biology 43: 58-69

Basu S, Giri RK, Benazir I, Kumar S, Rajwanshi R, Dwivedi SK, Kumar G (2017). Comprehensive physiological analyses and reactive oxygen species profiling in drought tolerant rice genotypes under salinity stress. Physiology and Molecular Biology of Plants 23: 837-850

Beers RF and Sizer IW (1952). A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. The Journal of biological chemistry 195: 133-140

Bhushan D, Pandey A, Choudhary MK, Datta A, Chakraborty S, Chakraborty N (2007). Comparative proteomics analysis of differentially expressed proteins in chickpea extracellular matrix during dehydration stress. Molecular and Cellular Proteomics 6: 1868-1884

Çelik Ö, Ayan A, Atak Ç (2017). Enzymatic and non-enzymatic comparison of two different industrial tomato (*Solanum lycopersicum*) varieties against drought stress. Botanical Studies pp. 58

Chutia J and Borah SP (2012). Water stress effects on leaf growth and chlorophyll content but not the grain yield in traditional rice (*Oryza sativa* Linn.) genotypes of Assam, India II. Protein and proline status in seedlings under PEG induced water stress. American Journal of Plant Sciences 03: 971-980 DOI:

10.4236/ajps.2012.37115

Chutipaijit S (2016). Changes in physiological and antioxidant activity of Indica rice seedlings in response to mannitol-induced osmotic stress. Chilean Journal of Agricultural Research 76: 455-462

Dien DC, Mochizuki T, Yamakawa T (2019). Effect of various drought stresses and subsequent recovery on proline, total soluble sugar and starch metabolisms in rice (*Oryza sativa* L.) varieties. Plant Production Science 22: 530-545

Dudziak K, Zapalska M, Börner A, Szczerba H, Kowalczyk K, Nowak M (2019). Analysis of wheat gene expression related to the oxidative stress response and signal transduction under short-term osmotic stress. Scientific Reports 9: 1-14

El-Esawi MA, Alayafi AA (2019). Overexpression of rice Rab7 gene improves drought and heat tolerance and increases grain yield in rice (*Oryza sativa* L.). Genes pp. 10

Fahad S, Bajwa AA, Nazir U, Anjum SA, Farooq A, Zohaib A, Sadia S, Nasim W, Adkins S, Saud S, Ihsan MZ, Alharby H, Wu C, Wang D, Huang J (2017). Crop Production under drought and heat stress: plant responses and management options. Front. Plant Sci. 8: 1147

Gashi B, Babani F, Kongjika E (2013). Chlorophyll fluorescence imaging of photosynthetic activity and pigment contents of the resurrection plants Ramonda serbica and Ramonda nathaliae during dehydration and rehydration. Physiol. Mol. Biol. Plants 19(3): 333–341 doi: 10.1007/s12298-013-0175-5

Giannopolitis CN and Ries SK (1977). Superoxide dismutases: I. Occurrence in higher plants. Plant physiology 59: 309-14

Gill SS and Tuteja N (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. Plant Physiology and Biochemistry 48: 909-930

Jeng TL, Tseng TH, Wang CS, Chen CL, Sung JM (2006). Yield and grain uniformity in contrasting rice genotypes suitable for different growth environments. Field Crops Research 99(1): 59-66

Kabiri R, Nasibi F, Farahbakhsh H (2014). Effect of exogenous salicylic acid on some physiological parameters and alleviation of drought stress in Nigella sativa plant under hydroponic culture. Plant Protection Science 50: 43-51

Kamarudin ZS, Yusop MR, Muda Mohamed MT, Ismail MR, Harun AR (2018). Growth performance and antioxidant enzyme activities of advanced mutant rice genotypes under drought stress condition. Agronomy 8

Khaleghi A, Naderi R, Brunetti C, Maserti BE, Salami SA, Babalar M (2019). Morphological, physiochemical and antioxidant responses of Maclura pomifera to drought stress. Scientific Reports 9: 1-12

Kumar M, Lee SC, Kim JY, Kim SJ, Aye SS, Kim SR (2014). Over-expression of dehydrin gene, *OsDhn1*, improves drought and salt stress tolerance through scavenging of reactive oxygen species in rice (*Oryza sativa* L.). Journal of Plant Biology 57: 383-393

Laza MaR, Peng S, Akita S, Saka H (2003). Contribution of biomass partitioning and translocation to grain yield under sub-optimum growing conditions in irrigated rice. Plant Prod. Sci. 6: 28-35

Larkunthod P, Nounjan N, Siangliw JL, Toojinda T, Sanitchon J, Jongdee B, Theerakulpisut P (2018). Physiological responses under drought stress of improved drought- Tolerant rice lines and their parents. Notulae Botanicae Horti Agrobotanici Cluj-Napoca 46: 679-687

Li P, Yang H, Wang L et al. (2019). Physiological and transcriptome analyses reveal short-term responses and formation of memory under drought stress in rice. Frontiers in Genetics 10: 1-16

Liu Y, Wang G, Yu K, Li P, Xiao L, Liu G (2018). A new method to optimize root order classification based on the diameter interval of fine root. Scientific Reports 8: 1-11

Lu YY, Deng XP, Kwak SS (2010). Over expression of CuZn superoxide dismutase (CuZn SOD) and ascorbate peroxidase (APX) in transgenic sweet potato enhances tolerance and recovery from drought stress. African Journal of Biotechnology 9: 8378-8391

Mafakheri A, Siosemardeh A, Bahramnejad B, Struik PC, Sohrabi E (2010). Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. Australian Journal of Crop Science 4: 580-585

Mahmud JA, Bhuyan MHMB, Anee TI, Nahar K, Fujita M and Hasanuzzaman M (2019). Reactive oxygen species metabolism and antioxidant defense in plants under metal/metalloid stress, Plant Abiotic Stress Tolerance pp. 221-257

Mittler R (2002). Oxidative stress, antioxidants and stress tolerance. Trends in Plant Science 7, 405-410

Moonmoon S, Fakir M, Islam M. 2017. Effect of drought stress on grain dry weight, photosynthesis and chlorophyll in six rice genotypes. Sch. J. Agric. Vet. Sci. 4: 13-17

Noctor G, Mhamdi A, Foyer CH (2014). The roles of reactive oxygen metabolism in drought: Not so cut and dried. Plant Physiology 164: 1636-1648

Oskuei BK, Valizadeh M, Rostaei M (2013). Antioxidant Isoenzymes Activities in Seedling Roots of Wheat Exposed to Drought Stress. 3: 33-40

Panda SK (2007). Chromium-mediated oxidative stress and ultrastructural changes in root cells of developing rice seedlings. Journal of Plant Physiology 164: 1419-1428

Panda D, Mandal L, Barik J, Padhan B, Bisoi SS (2020). Physiological response of metal tolerance and detoxification in castor (*Ricinus communis* L.) under fly ash-amended soil. Heliyon 6: e04567

Praba ML, Cairns JE, Babu RC, Lafitte HR (2009). Identification of physiological traits underlying cultivar differences in drought tolerance in rice and wheat. Journal of Agronomy and Crop Science 195: 30-46.

Pradhan B, Chakraborty K, Prusty N, Deepa, Mukherjee AK, Chattopadhyay K, Sarkar RK (2019). Distinction and characterisation of rice genotypes tolerant to combined stresses of salinity and partial submergence, proved by a high-resolution chlorophyll fluorescence imaging system. Functional Plant Biology 46: 248-261

Puteh AB, Mondal MMA, Ismail MR, Latif MA (2014). Grain sterility in relation to dry mass production and distribution in rice (*Oryza sativa* L.), Biomed Research International pp. 1- 6

Sahoo S, Saha B, Awasthi JP, Omisun T, Borgohain P, Hussain S, Panigrahi J, Panda SK (2019). Physiological introspection into differential drought tolerance in rice cultivars of North East India. Acta Physiologiae Plantarum, 41

Sairam RK (1994). Effect of Moisture Stress on Physiological Activities of Two Contrasting Wheat Genotypes. Indian Journal of Experimental Biology 32: 584-593

Samota MK, Sasi M, Singh A (2017). Impact of Seed Priming on Proline Content and Antioxidant Enzymes to Mitigate Drought Stress in Rice Genotype. International Journal of Current Microbiology and Applied Sciences 6: 2459-2466

Sauer H, Wartenberg M, Hescheler J (2001). Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem 11(4): 173-86

Scandalios JG (1993). Oxygen stress and superoxide dismutases. Plant Physiology 101: 7-12

Sharma P, Jha AB, Dubey RS, Pessarakli M (2012). Reactive Oxygen Species, Oxidative Damage, and Antioxidative Defense Mechanism in Plants under Stressful Conditions. Journal of Botany, 1-26

Sikuku P, Onyango J (2012). Physiological and biochemical responses of five nerica rice varieties (*Oryza sativa* L.) to water deficit at vegetative and reproductive stage. Agriculture and Biology Journal of North America 3: 93-104

Silva MDA, Jifon JL, Da Silva JAG, Sharma V (2007). Use of physiological parameters as fast tools to screen for drought tolerance in sugarcane. Brazilian Journal of Plant Physiology 19: 193-201

Stirbet A, Govindjee (2011). On the relation between the Kautsky effect (chlorophyll a fluorescence induction) and Photosystem II: Basics and applications of the OJIP fluorescence transient. Journal of Photochemistry and Photobiology B: Biology 104: 236-257

Swapna S, Shylaraj KS (2017). Screening for Osmotic Stress Responses in Rice Varieties under Drought Condition. Rice Science 24: 253-263

Wang ZX, Chen L, Ai J, Qin HY, Liu YX, Xu PL, Jiao ZQ, Zhao Y, Zhang QT (2012). Photosynthesis and activity of photosystem II in response to drought stress in Amur Grape (*Vitis amurensis* Rupr.). Photosynthetica 50: 189-196

Wang X, Liu H, Yu F, Hu B, Jia Y, Sha H, Zhao H (2019). Differential activity of the antioxidant defence system and alterations in the accumulation of osmolyte and reactive oxygen species under drought stress and recovery in rice (*Oryza sativa* L.) tillering. Scientific Reports 9: 1-11

Xu L, Han L, Huang B (2011). Antioxidant enzyme activities and gene expression patterns in leaves of kentucky bluegrass in response to drought and post-drought recovery. Journal of the American Society for Horticultural Science 136: 247-255

Yagi K (1998). Simple assay for the level of total lipid

Peroxides. 108: 101-6 doi: 10.1385/0-89603-472-0:101

Yin C, Peng Y, Zang R, Zhu Y, Li C (2005). Adaptive responses of Populus kangdingensis to drought stress. Physiologia Plantarum 123: 445-451

Zhang L, Peng J, Chen TT, Zhao XH, Zhang SP, Liu SD, Dong HL, Feng L, Yu SX (2014). Effect of drought stress on lipid peroxidation and proline content in cotton roots. Journal of Animal and Plant Sciences 24: 1729-1736

Zhou Y, Lam HM, Zhang J (2007). Inhibition of photosynthesis and energy dissipation induced by water and high light stresses in rice. Journal of Experimental Botany 58:1207-1217

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Glycolate catabolic bypass pathway integration in rice could be effective in lowering photorespiratory rate with modulating starch content and grain quality

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ABSTRACT

Photorespiration, which is prevalent under higher temperature and arid conditions, significantly affects crop productivity by reducing yields up to 50% in C_3 *crops like rice under severe stress conditions. This is primarily attributed to a reduction in net photosynthetic rate (PN). Rice flag leaf photosynthesis is the primary supplier of sugar to the maturing spikelets after anthesis. This study evaluated the grain quality traits and starch content of the wild type (WT) and transgenic rice generated by introducing Escherichia coli (E. coli) glycolate catabolic pathway bypassed (GCPB) through agrobacterium mediated transformation. Leaf soluble protein, photosynthetic* CO_2 *assimilation rate, leaf non-structural carbohydrate content, grain quality traits such as hulling and milling percentages, head rice recovery, water uptake, volume expansion, alkali spreading value, gel consistency, grain breadth, grain starch content and amylose content were affected to a great extent in GCPB transgenic plants* (T_4)*. This study indicates the possible role of photorespiratory bypass mechanism in the regulation of source-sink communication, starch biosynthesis and grain quality in rice.*

Key words: *Photorespiration, photosynthesis, starch biosynthesis, grain quality, yield, rice*

INTRODUCTION

Rice (*Oryza sativa* L.) is consider as most significant staple food in Asia where it is providing nutrition to a large proportion population. As per economical perspective, it is a chief food crop with nutritional variation that could help in poverty alleviation (Otegbayo et al., 2004). At the same time, it is classified as the world's most significant human food crop (Itani et al., 2002). For C_3 plants like rice, the basis for the increased productivity at high CO_2 is thought to be higher leaf CO_2 assimilation rates (*A*). In our previous reported work, we have developed the transgenic rice having increased chloroplastic CO_2 concentrations and reduced ribulose 1,5 bisphosphate (RuBP) oxygenation by bypassing the endogenous photorespiration that eventually increases photosynthesis and grain yield (Nayak et al., 2021). These physiological and biochemical modifications may not only influence productivity but also influence the grain quality attributes of rice. Wang et al., 2011 reported that alteration in different physiological processes due to improvement in CO_2 utilization are very likely to impact the physical and chemical components of rice crops and ultimately grain quality.

Rice grain quality is determined by the various aspects such as grain physicho-chemical properties, cooking quality, eating quality and nutritional value (Juliano, 1990). The processing properties and nutritional values are very vital for the overall health perspective of the people along with commercial purposes including the economy of the rice growers. Grain quality is a very important factor for the consumer acceptance, market value and end users. Consumer's preference mainly depends on cooking processes, appearance , grain shape and size (Sharma and Khanna, 2019). Demand for superior grain quality is increasing in economically developed as well as in developing countries. Therefore, grain quality is the main breeding aspects in rice breeding programs in all rice developing countries. As unsatisfactory cooking property and poor grain character hampers the consumer acceptance and spread of modern released variety, quality improvement programme receives special emphasis in the current scenario. Rice grain quality is regulated by several environmental factors where grain starch content largely contributes to it (Sreenivasulu et al., 2015). Previous studies showed that the reduction of amylose and starch contents of rice grain depleted overall grain quality (Kumar et al., 2018). Different grain quality traits such as appearance, milling properties , cooking quality and nutritional value are mainly correlated to the starch and protein content in the endosperm (Pratap and Tyagi, 2020). Villareal and Juliano, 1978 reported that rice grain nutritional value is determined by the seed-storage protein content which can make up to 5-12%. Till now, several strategies have been developed for the upliftment of grain quality of rice. However, the impact of photorespiration suppression has not been studied in association with rice grain quality. The present study aims to evaluate the impact of elevated CO_2 and higher photosynthetic rate on the grain quality in the glycolate catabolic pathway bypassed (GCPB) transgenic rice.

MATERIALS AND METHODS

Plant material

The rice plant (Cultivar. Naveen) was transformed with glycolate catabolic pathway genes related to three subunits of glycolate dehydrogenase (GDH) to get the partial bypass pathway transgenic plants. After stable confirmations with southern hybridization and RT-qPCR, physiological, morphological, agronomical and biochemical analysis were done in the T_3 progenies. On the basis of these analysis, we confirmed that the transformed lines with reduced photorespiration showed superior plant architecture and higher grain yield (Nayak et al., 2021). The GCPB transgenic rice (T_4) was further grown in the greenhouse chamber at ICAR-National Rice Research Institute (ICAR-NRRI), Cuttack, India and their morpho-agronomical data were recorded. Additionally, the grains harvested from the wild-type control (WT; Cultivar Naveen) and GCPB lines were assessed for starch accumulation and grain quality traits.

Estimation of grain quality parameters

The grain quality parameters related to physical traits, chemical traits and cooking properties were determined as per standard procedures (Bhonsle and Sellapan, 2010; Kumar et al., 2020) with few modifications.

Physical traits

Brown rice (BR) yield, grain size and head rice recovery (HRR)

One hundred grams of rough paddy seeds were dehulled using a mini 'Satake Rice Medium' and the average whole-grain BR yield was determined. The grain size was calculated from de-husked brown rice. About six full grains per replication was calculated using rice digital meter and L/B ratio was measured. For HRR estimation, about 100 grams of grains were de-hulled and milled to 10% to get the head rice recovery percentage (HRR%) was calculated on a paddy weight basis Bhonsle and Sellapan, 2010.

Chemical traits

Grain starch content

Grain starch content of GCPB transgenic lines and WT was estimated by total starch assay kit (K-TSTA100A, Megazyme International, Ireland Limited, Bray, Ireland). About 0.1 gm of rice powder sample was added with 200 µl of 80% aqueous ethanol (v/v) and mixed by nonstop stirring on a vortex. The rice sample and ethanol solution were added to the 300 µl of thermostable α-amylase, the tubes were incubated for 6 min with stirring every 2 min intervals in water bath at 50°C. Again, amyloglucosidase (100 ml) added to the contents and incubated at 50°C for 30 min. The solution was added with 100 ml of distilled water. Additional, distilled water

(10 ml) was diluted with 1.0 ml of solution and centrifugation at 3000 rpm for 10 min. About 3.0 m of glucose oxidase-peroxidase (GOPOD) reagent was mixed with pure supernatant measuring 100 µl and incubated for 20 min at 50°C. About 3.0 ml GOPOD reagent and D-glucose standard solution (1.0 ml) were used as the glucose control. The absorbance at 510 nm of D-glucose control and the sample were calculated against the reagent blank.

Grain amylose content

According to Kumar et al the amylose content (AC) of rice grains was determined by colorimetric measurement of the amylose-iodine solution (Kumar et al., 2018). About 100 mg powdered rice was soaked with 1ml of distilled ethanol in a boiling tube. By added 9 ml of 1 N sodium hydroxide to the rice solution then incubated in a water bath for 10 min. 1 ml of 1 N acetic acid were mixed with 5ml of three aliquots followed by the addition of 2 ml iodine solution (1 g iodine and 10 g KI/500 ml distilled water). For 20 min the flasks were placed in darkness and the absorbance was calculated at 620 nm after the contents diluted to 100 ml.

Grain total protein content

The total protein content in rice grain was estimated by total nitrogen in the sample through the Kjeldhal method. About 200 mg brown rice grain was taken in the Kjeldhal flask and equal weight of salt mixture (467 gm K_2SO_4 with 28 gm $CuSO_4\,5H_2O$ and 4.7 mg metallic Selenium) was added. Thereafter, 3 ml of conc. H_2SO_4 was added and left it overnight for digestion. The completion of sample's digestion was marked by formation of clear solution. Distillation process was started after completion of digestion. For distillation, 10 ml of 4% H_3BO_3 was taken in conical flask followed by addition of 2 drops mixed indicator (Methyl blue: 0.2 g and methyl red: 0.4 g dissolved in 50 ml of 90 % ethanol). The flasks were kept under condenser as such that tip of the condenser outlet remained beneath the surface of the solution in the flask. Digested samples were transferred to the distillation apparatus with minimum three washing. Then, 10ml NaOH was added to the distillation unit. The steam from boiling water was allowed to pass. The released NH_3 was collected in the flask containing H_3BO_3 and mixed indicator. The distillation process was carried out for 5min each followed by titration with 0.01 N HCl until changing of colour. The titre value was measured for calculation of quantity of nitrogen present in the sample with blank value (titration without sample) taken as 0.3.

$$\text{Protein content}\left(\%\right) = \frac{\left(\text{Titre value x Normality of HCl x 14 x 100 x 5.95}\right)}{\left(\text{Weight of sample }\left(\text{g}\right)\text{x 1000}\right)}$$

In rice, 5.95 is used as a correction factor for total protein content.

Estimation of Glycemic Index

Glycemic Index (GI) value can be evaluated through the hydrolysis index (HI) method. Three digestive enzymes specifically amyloglucosidase, α-amylase and pepsin were used to digest starch. About 0.2 g of rice powder was heated with distilled water (2 ml) for two minutes in a tube placed in boiling water bath. About 5 ml of 0.1 M phosphate buffer (pH-6.9) were added after cooling. By added 10% o-phosphoric acid the pH of the contents was brought down to 2.5. Thereafter, reaction mixture was mixed with 200 µl of pepsin (250 mg ml^{-1}) and incubated at 37°C in a water bath shaker at 110 rpm for 60 min to hydrolyze the rice protein. Approximately 20% potassium hydroxide was added to adjust pH (6.9) followed by addition of 200 µl of α-amylase (125 mg ml^{-1}). The volume was adjusted to 40 ml with 0.1 M of phosphate buffer (pH-6.9) and incubated at 37°C with 110 rpm. Suspension were drawn about 500 α at every 30 min intervals for 3 h. About 1.5 ml of sodium acetate buffer (0.4 M, pH 4.75) and 30 α of amyloglucosidase (3300 U ml^{-1}) was added to each sample followed by incubation for 30 min at 50°C. The solution was then diluted with distilled water (10 ml). About 300 µl of duplicate aliquots were incubated with GOPOD reagent at 50°C for 20 mins and measured at 510 nm of absorbance. By using 0.2g glucose as standard carbohydrate the area under the curve (AUC) was calculated. By dividing AUC of sample by that of glucose and expressed in percentage, the HI for each rice genotypes was calculated. The predicted glycemic index (PGI) was calculated using the following formula (Kumar et al., 2020).

$$\text{PGI} = 39.71 + (0.549 \times \text{HI})$$

Cooking properties

Alkali spreading value (ASV)

Six milled rice grains of each transgenic line along with the wild type were taken in a plastic cube containing potassium hydroxide (10 mL). For twenty-three hours the dish was incubated at 27-30°C and seven-point scale was used to record the ASV value. The standard photograph was used to compare the spreading of rice grains and ranked 1, 2, 3 up to 7 (Bhonsle and Sellappan, 2010).

Gel consistency (GC)

About 100 mg of rice powder was added 0.2 ml of ethanol mixture (thymol blue (0.25%) and 2.0 ml of KOH solution (250 ml distilled water was added in 2.8 g of KOH). After that the rice solution incubated in water bath for 8 min and then the mixture kept in the ice bath for 20 mins. The spreading of gel (mm) was recorded by placing the tube horizontally on a graph paper for 1 h.

Water uptake (WU)

About 2gm of rice grains were soaked in 10 ml of distilled water for 30 min. The rice solution and control (without rice grains) was boiled at about 80°C temperature of water bath for 45 min and placed in a cold water for cooling after boiling. Then, the supernatant was transferred into a graduated cylinder and water level was noted. Water uptake was measured using the following formula (Kumar et al., 2020):

$$\text{Water uptake} = 100/2\ \text{g} \times \text{actual water absorbed.}$$

Kernel length after cooking (KLAC), volume expansion ratio (VER)

Five gram of head rice grain was added to 15 ml of distilled water in 50 ml graduated centrifuge tubes. The primarily enhanced volume was calculated as Y and kept for 10 min. Then, enhanced volume before cooking was noted (Y-15). Rice grains were cooked for 20 min on a water bath and placed on Whatman no. 1 filter paper. KLAC was calculated using graph paper. About 5 g of cooked rice were kept in 50 ml distilled water and volume an increase of cooked rice was calculated as X. Then, the volume increase was measured as X-50. VER was estimated by added 50 ml distilled water. After taking kernel length, increased volume was recorded of the cooked rice (Juliano, 1971).

RESULTS AND DISCUSSION

The quality traits were analyzed in the glycolate catabolic pathway bypassed (GCPB) transgenic rice grain collected in the T_4 generation. Previously we have established that via introducing the *E. coli* glycolate catabolic pathway in rice, photorespiration reduced by 31.5 %, enhanced the photosynthetic rate up to 30% and grain yield by 67%, respectively which ensured a positive impact of photosynthetic rate on the sink capacity. To further understand the influence of enhanced photosynthetic rate on the starch accumulation and grain quality traits, we grew the WT and GCPB transgenic lines (T_4 generation) in the greenhouse and evaluated the physical, chemical and cooking traits including the starch accumulation rate.

Physical characteristics

The de-hulling of rice is one of the crucial post-harvest procedures where a higher hulling percentage ensures an enhanced head rice recovery (HRR) rate. The hulling percentage (HP) for GCPB lines was compared with WT (Table 1). The HP of WT and GCPB lines ranged from 70-72% and 79-82% respectively. Overall, a 12.59% significant increment in HP was recorded in the GCPB lines than WT lines. An idealized HP in rice is considered to be 80% or more than that (Bisne and Sarawgi, 2008). Further, milling percentage (MP) is a measure of the coarse rice recovery during milling. At the production level, rice millers prefer varieties with

Table 1. Grain quality physical traits of WT and transgenic line (GCPB).

Parameters	WT	GCPB
KL (mm)	4.56±0.05	5.06±0.08^{ns}
KB (mm)	2.05±0.04	2.18±0.03^{ns}
L/B ratio	2.51±0.09	2.88±0.11^{ns}
HP (%)	71.50±0.71	80.50±0.71**
MP (%)	61.00±0.71	63.50±0.71**
HRR (%)	54.50±2.12	65.50±0.71**

Kernel length, KL; Kernel breadth, KB; Length to breadth ratio, L/B; Hulling percentage, HP; Milling percentage, MP; Head rice recovery, HRR. Values represent the means, SE ± (n = 6), non-significant, NS, *, ($p < 0.05$); **,($p < 0.01$).

high MP (Merca and Juliano, 1981). Our data reflect a significant increment of MP in GCPB lines than WT by 4.10%. This suggests a higher processing performance of GCPB transgenic grains indicating its quality enhancement. The HRR designates the average weight of the wholesome grains obtained after the industrial processing. This is the part of the intact grain obtained after the milling of rice. Thus, HRR is considered a significant rice grain quality trait. HRR of more than 65% is considered as a lucrative value (Singh et al., 2018). HRR of WT and GCPB lines ranged from 53-56% and 64-68% respectively. Overall, a 20.25% significant increment in HRR was recorded in the GCPB lines than WT lines (Table 1). Previously, Dipti et al., 2003 established a positive correlation between HRR with grain type, chalkiness index, cultivation process, and degree of drying. Our data suggest a non-significant increment (14.93%) of L/B ratio in transgenic lines than in WT plants. According to Bhonsle et al., (2010), the L/B ratio in high-yielding varieties is maintained between 2.10 to 3.31 and the grain size and shape is short to medium with a translucent appearance.

Chemical characteristics

Amylose content (AC) is the primary factor for the rice eating quality as it is the part of endosperm starch along with amylopectin (Eggum et al., 1993). It eventually determines the texture and color of the cooked rice (Rodríguez et al., 2020). The AC of WT and GCPB lines ranged from 20.50-20.58% and 24.78-25.04% respectively (Table 2). Overall, a 21.28% significant increment in AC was recorded in the GCPB lines than WT lines (Table 2). Utilizing preference, most consumers consider rice with intermediate AC ranging between 20-25% (Rachmat et al., 2016). The AC was correlated with volume expansion and water absorption during cooking. A similar observation was previously reported by Zhou et al., 2002. An increase in AC is reflected in a higher accumulation of starch by 29.40% in GCPB lines than in WT grains (Table 2). We hypothesize that an increment in the photosynthetic rate as a consequence of photorespiration suppression results in higher starch and amylose accumulation in GCPB grains than WT. A similar correlation between photosynthesis and grain starch accumulation was reported by Wang et al., 2011.

Table 2. Grain quality chemical traits of WT and transgenic line (GCPB).

Parameters	WT	GCPB
PC (%)	9.64±0.05	10.69±0.04^{ns}
GS (%)	57.59±0.11	74.52±0.42**
AC (%)	20.54±0.04	24.91±0.13**
GI	69.02±0.78	62.80±0.71*

Protein content, PC; Grain starch, GS; Amylose content, AC; Glycemic Index, GI. Values represent the means, SE ± (n = 6), non-significant, NS; *, ($p < 0.05$); **,($p < 0.01$).

Glycemic index (GI) is a crucial grain quality trait related with starch digestibility. GI is a measurement of the potency of the grain to maintain sugar level in the blood after absorption. Preferably a diet with low GI is considered as a part of the balanced diet that prevents obesity, diabetes, and cardiovascular diseases (Kumar et al., 2019). In our study, GI was significantly reduced in the GCPB lines than WT by 8.99% (Table 2). This suggests that a photosynthetic enhancement not only upregulates the processing but also the dietary quality related to starch digestibility of rice. Additionally, the protein content of the GCPB lines increased than WT by 10.90% which is in parallel to the previous reports (Santos et al., 2013).

Cooking characteristics

The volume expansion ratio (VER) after cooking non-significantly increased in the GCPB lines than the WT by 6.21% (Fig. 1C). Furthermore, VER was correlated with AC, water uptake (WU) and alkali spreading value (ASV). A similar observation was previously reported (Xiongsiyee et al., 2018). These observations suggest that the rice grain absorb more water can generate greater volume after cooking. Furthermore, in our study, kernel length after cooking (KLAC) non-significantly increased in the GCPB lines than in the WT lines by 8.96% (Fig. 1A). Similarly, the WU significantly decreased in the GCPB lines than in the WT lines by 9.99% (Fig. 1B). Additionally, a negative correlation (r= -0.888) between WU and AC was recorded. Similar results were previously reported by Metcalf et al., 1985. The ASV and gel consistency (GC) were calculated for WT and GCPB lines. ASV significantly decreased in GCPB lines than WT lines by 81.67% (Fig. 1D). ASV is considered as an indirect evaluation of starch properties (Chowdhury et al., 2016). There is significantly correlation between the ASV, grain protein

Table 3. Estimates of correlation coefficients of various chemical and cooking traits of grain quality parameters of GCPB transgenic lines.

	AC	GS	Protein	GI	KLAC	WU	VER	ASV	GC
AC	1								
GS	.922**	1							
Protein	.925**	.996**	1						
GI	-.860*	-.962**	-.951**	1					
KLAC	.308	.568	.554	-.689	1				
WU	-.888**	-.995**	-.985**	.974**	-.618	1			
VER	-.059	.305	.272	-.363	.796*	-.379	1		
ASV	-.787*	-.938**	-.918**	.983**	-.714	.965**	-.479	1	
GC	-.717*	-.897**	-.868*	.932**	-.816*	.930**	-.630	.959**	1

** - Correlation is significant at the 0.01 level (2-tailed). * - Correlation is significant at the 0.05 level (2-tailed).

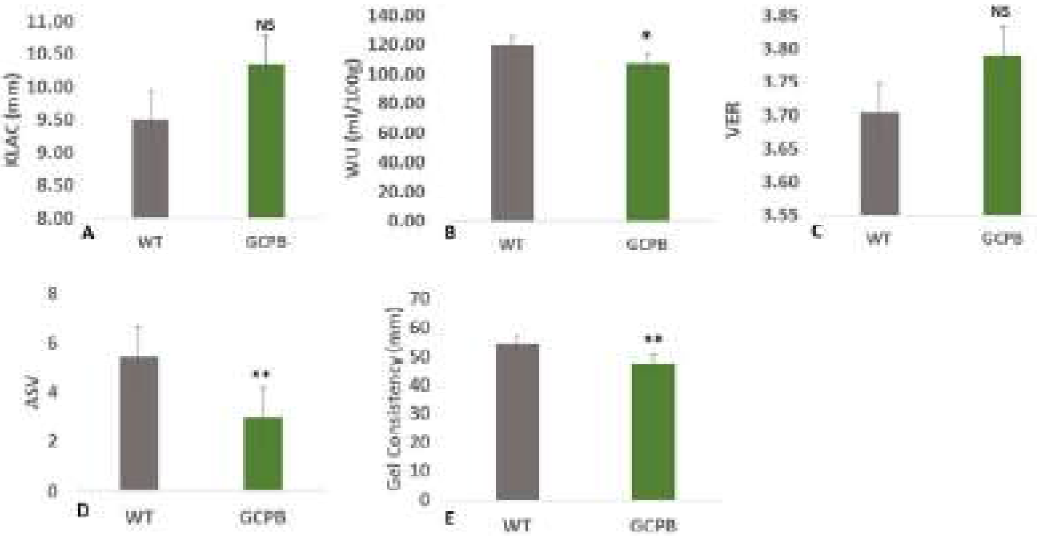

Fig. 1. Grain quality cooking parameters analysis of transgenic lines (GCPB) and WT control. (A) Kernel length after cooking, KLAC; (B) Water uptake, WU; (C) Volume expansion ratio, VER; (D) Alkali spread value, ASV, (E) Gel consistency, GC in the transgenic lines (n=6) and WT. SE(±), Non-significant, NS. *, ($p < 0.05$); **,($p < 0.01$).

and AC (Table 3). Additionally, GC is a measurement of the cold paste viscosity which is an index used to segregate rice texture of high amylose rice genotypes (Prasad et al., 2021). The GC was primarily developed to distinguish high-amylose rice with amylograph pasting viscosities (Cagampang et al., 1973). In our work, GC significantly decreased in GCPB lines than WT lines by 13.72% (Fig. 1E). Furthermore, GC was found to be correlated with AC (Table 3). A similar observation was reported by (Hu et al., 2021).

CONCLUSIONS

In the present study, physical, chemical and cooking characteristics of photorespiratory bypassed rice and its wild type control lines (WT) were evaluated for grain quality traits. Taken together, transgenic lines grain maintained superior quality than WT lines by maintaining higher HP, MP, HRR, L/B, starch content and AC. This could be ascribed to the higher photosynthetic rate in transgenic lines than the WT (30% higher) lines. Further studies are necessary to understand the relationship between photosynthetic enhancement and rice grain quality at molecular level. These studies can aid breeders to instrumentalize photosynthesis for the enhancement of grain quality in rice.

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REFERENCES

Bhonsle SJ and Sellapan K (2010). Grain Quality Evaluation of Traditionally Cultivated Rice. Recent Research in Science and Technology 2: 88-97

Bisne R and Sarawgi AK (2008). Agro-morphological and quality characterization of Badshah bhog group from aromatic rice germplasm of Chhattisgarh. Bangladesh Journal of Agricultural Research 33: 479-492

Cagampang GB et al. (1973). A gel consistency test for eating quality of rice. Journal of the Science of Food and Agriculture 24: 1589-1594

Chowdhury BD et al. (2016). Characterization and variability analysis of Rice genotypes with reference to Cooking Quality Parameters. IOSR Journal of Agriculture and Veterinary Science (IOSR-JAVS) 9: 2319-2380

Dipti sharifa et al. (2003). Grain Quality Characteristics of Some Beruin Rice Varieties of Bangladesh. Pakistan Journal of Nutrition 2: 242-245

Eggum BO et al. (1993). Starch, energy, and protein utilization by rats in milled rats of IR36-based amylose extender mutant. Cereal Chemestry71: 275-279

Hu, Y., et al. 2021. Comparison of the Grain Quality and Starch Physicochemical Properties between Japonica Rice Cultivars with Different Contents of Amylose, as Affected by Nitrogen Fertilization. Agriculture 2021, Vol. 11, Page 616 11, 616

Itani T et al. (2002). Distribution of Amylose, Nitrogen, and Minerals in Rice Kernels with Various Characters. Journal of Agricultural and Food Chemistry 50: 5326-5332

Juliano BO (1990). Rice grain quality: problems and challenges. Cereal Foods World 35: 245-253

Juliano OB (1971). A simplified assay for milled rice amylase. Cereal Sci. Today 16: 360

Kumar A et al. (2020). Status of glycemic index of paddy rice grain (*Oryza sativa* L.) on infestation by storage pest Sitotrogacerealella. Journal of Stored Products Research 89: 101697

Kumar A et al. (2019). Low light stress influences resistant starch content and Glycemic index of rice (*O. sativa* L). Starch - Stärke71: 1800216

Kumar A et al. (2018). Rice with pulses or cooking oils can be used to elicit lower glycemic response. Journal of Food Composition and Analysis 71: 1-7

Merca FE, Juliano BO (1981). Physicochemical properties of starch of intermediate-amylose and waxy rices differing in grain quality. undefined 33: 253-260

Metcalf SL and Lund DB (1985). Factors Affecting Water Uptake in Milled Rice. Journal of Food Science 50: 1676-1679

Nayak L et al. (2021). A chloroplast Glycolate catabolic pathway bypassing the endogenous photorespiratory cycle enhances photosynthesis, biomass and yield in rice (*Oryza sativa* L.). Plant Science 111103

Nayak R et al. (2017). Yield quality and economics of basmati rice as influenced by different organic nutrient management practices. Oryza 54: 44-49

Otegbayo BO et al. (2004). Effect of Parboiling on Physico-chemical Qualities of Two Local Rice Varieties in Nigeria. Journal of Food Technology in Africa 6, 130-132

Prasad T et al. (2021). Study on physicochemical properties of rice landraces for amylose, gel consistency and gelatinization temperature. Electronic Journal of Plant Breeding 12: 723-731

Prathap V and Tyagi A (2020). Correlation between expression and activity of ADP glucose pyrophosphorylase and starch synthase and their role in starch accumulation during grain filling under drought stress in rice. Plant Physiology and Biochemistry 157: 239-243

Rachmat R et al. (2016). The empirical relationship between price and quality of rice at market level in west java. Indonesian Journal of Agricultural Science 7: 27

Rodríguez JP et al. (2020). Healthy and Resilient Cereals and Pseudo-Cereals for Marginal Agriculture: Molecular Advances for Improving Nutrient Bioavailability. Frontiers in Genetics 11: 49

Santos KFD et al. (2013). Storage protein profile and amino acid content in wild rice oryzaglumaepatula. PesquisaAgropecuariaBrasileira 48: 66-72

Sharma N and Khanna R (2019). Rice Grain Quality: Current Developments and Future Prospects. Recent Advances in Grain Crops Research

Singh V et al. (2018). Pusa Basmati 1121 - a rice variety with exceptional kernel elongation and volume expansion after cooking. Rice 11: 1-10

Sreenivasulu N et al. (2015). Designing climate-resilient rice with ideal grain quality suited for high-temperature stress. Journal of Experimental Botany 66: 1737-1748

Villareal RM and Juliano BO (1978). Properties of glutelin from mature and developing rice grain. 17: 177-182

Wang Y et al. (2011). The impact of atmospheric CO_2 concentration enrichment on rice quality - A research review. Acta EcologicaSinica 31: 277-282

Xiongsiyee V et al. (2018). Variation in Grain Quality of Upland Rice from Luang Prabang Province, Lao PDR. Rice Science 25: 94-102

Zhou Z et al. (2002). Composition and functional properties of rice. International Journal of Food Science & Technology 37: 849-868

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Molecular characterization and varietal identification for multiple abiotic stress tolerance in rice (*Oryza sativa* L.)

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ABSTRACT

Coexistence of two or more abiotic stresses is common in most of the rainfed lowland and upland rice growing areas of India and worldwide. Rice production under these conditions is not sustainable. Identification and development of multiple abiotic stress tolerant rice varieties are to be addressed. Here we tried to identify multiple abiotic stress tolerant varieties from a collection of earlier identified varieties for single stress and validated the known SSR markers for stress tolerance. Twenty rice genotypes were evaluated for individual abiotic stress such as drought, salinity and temperature initially and the tolerant three genotypes in each case were further evaluated for combination of stresses various physio-morphological and biochemical parameters were recorded. Among the genotypes evaluated for combination of stresses, PTB-7 was found to be tolerant for drought and salinity, Nagina-22 was tolerant against high temperature and salinity. However, the seeds did not germinate in the presence of all three stresses simultaneously. Twenty rice varieties viz., Chomala, MO-16, PTB-35, PTB-60, PTB-39, PTB-55, PTB-30, PTB-7, CRdhan307, Apo, Vyttila-3, Vyttila-4, Vyttila-5, Vyttila-6, Vyttila-7, Vyttila-8, Vyttila-9, Vyttila-10, Nagina-22 and NL-44 were further investigated using microsatellite markers to confirm the genotypic level of tolerance to combination of abiotic stresses. Rice genotypes were screened using 30 reported simple sequence repeat (SSR) markers that are linked to drought, salinity and temperature. Molecular marker analysis of rice genotypes also confirmed that RM8904 and RM1287 were associated with salinity tolerance, RM2612, RM6100 and RM5749 were linked to high temperature tolerant trait. Population analysis also revealed that there is five subpopulation among rice genotypes.

Key words: *Simple sequence repeats (SSR), Nagina-22, PTB-7, drought, salinity, temperature, multiple abiotic stresses*

INTRODUCTION

Rice (*Oryza sativa* L.), a semi aquatic plant with an evolutionary advantage of growing under diverse water regimes (De Datta and Mikkelsen, 1985) starting from flooded conditions to dry land. Rice is grown on an area of 163 million hectares with the production of 758.9 million tonnes (499.2 million tonnes milled basis) of rice (FAO, 2017). The food production needs to be increased even from the drought prone areas with an increase of 40% from the difficult ecosystem to meet the targeted food production by 2025 (Pennisi, 2008). The irrigated and the rainfed rice ecosystem, which forms the major mainstay of food security in Asia has been highly sustainable with the environment having few adverse impacts. Increase in rice production should come from highly vulnerable, less productive drought-prone rainfed lowland and upland rice areas because there is limited scope for expanding irrigated rice area (Khush, 2009). These areas are neglected during green revolution period and concentrated on input responsive high yielding varieties. These areas are affected by different types of abiotic stresses like heat, drought, cold, salinity and induce severe cellular damage in plant species, including rice plants. There is a cross talk between abiotic stresses either individually or in combination, they cause morphological, physiological, biochemical, and molecular changes that adversely affect plant growth and productivity, and ultimately yield. Abiotic stress like drought, salinity, and low temperature manifest their effect through an osmotic stress, ultimately leading loss in turgor, disorganized membranes and denatured proteins triggering a cascade of events (Krasensky and Jonak, 2012). In addition, increased salinity of arable land is expected to have devastating global effects, resulting in up to 50% land loss by the middle of twenty-first century.

In rice two reproductive development stages, anthesis and microsporogenesis are very sensitive to high (>33°C) and low (15°C) temperatures (Jagadish et al., 2015). Extreme temperatures affect anther dehiscence, pollination, and pollen germination, leading to spikelet sterility. Water deficit or drought stress has similar effects and exacerbates the problem by reducing transpirational cooling and thereby increasing canopy and tissue temperature (Sheshshayee et al., 2011). Excess salt in soil adversely affects plant growth, development, and productivity when osmotic stress reduces water uptake by roots (Munns and Tester, 2008).

Adaptation mechanisms to these environmental cues in plants by appropriate physiological, developmental and biochemical changes through elaborate mechanisms of signal perception, transduction manifest in adaptive responses (Alcazar et al., 2003). However, tolerance or susceptibility to the abiotic stresses is a very complex phenomenon, in part because stress may occur at multiple stages of plant development and often multiple stresses simultaneously affect the plants during their development. Abiotic stress tolerance is quantitative in nature and governed by multiple loci by many genes; therefore, adapting to variable environmental stress is a highly intricate trait (Shinozaki and Shinozaki, 2006). Plants survival under environmental stresses depends on the severity of stress and the genetic background of the plant, even though plants constantly adapting these conditions by changing the physiology and metabolism (Pastori and Foyer, 2002). In addition, osmotic adjustment mechanism and redox metabolism help the plants to alter metabolism in such a way that protect plants from severity of abiotic stresses (Valliyodan and Nguyen, 2006; Munns and Tester, 2008). At the molecular level, gene expression is modified upon stress (Chinnusamy et al., 2007) and epigenetic regulation plays an important role in the regulation of gene expression in response to environmental stress (Hauser et al., 2011; Khraiwesh et al., 2011). Ali et al. (2017) stated that, low productivity of rice in rain fed areas is mostly correlated with decline in yield due to multiple abiotic stresses. Breeding programs are formulated so as to mitigate the effect of any single stress, whereas in field condition multiple stresses occur simultaneously. This provides a platform to work on and to exploit the tolerance level of rice genotypes to multiple abiotic stresses and to validate the molecular markers reported for various abiotic stresses in rice.

Earlier studies mainly focused on the effect of individual stress on morpho-physiological, biochemical, molecular and yield parameters in rice (Beena et al., 2012; Prince et al., 2015; Naresh et al., 2018; Beena et al., 2018a, Beena et al., 2018c). When abiotic stresses happen concomitantly in field condition, tolerant varieties identified of single stress need not necessarily tolerate the combined effect of stresses (Atkinson et al., 2013). Hence identification of multiple stress tolerant rice varieties is needed for climatic extremities with increasing population (Khush, 2009). Molecular markers, especially DNA-based markers, have been used extensively for the study of genetic diversity, unambiguous identification of germplasm and their protection. Exploitation of rice germplasm and identification of specific molecular markers linked to different abiotic stresses is highly critical to ensuring sustainable rice production and global food security in the ever-changing climatic conditions. Molecular marker technology is helpful in unraveling the genetic basis of complex traits within rice germplasm to identify major genes/QTLs for use in rice breeding (Beena, 2005; Beena, 2012; Swamy and Kumar, 2013; Swamy et al., 2014). With this background this study was undertaken to identify multiple abiotic stress tolerant rice varieties and to validate the reported SSR markers for abiotic stress tolerance.

MATERIALS AND METHODS

Plant material and germination assay

A laboratory experiment was conducted at the Department of Plant Physiology, College of Agriculture, Vellayani, Kerala Agricultural University during 2017-2019. Twenty rice (*Oryza sativa* L.) varieties composed of drought tolerant (Chomala, PTB-60, PTB-

55, PTB-30, PTB-7, CR Dhan307, Apo), salinity tolerant (Vyttila-3, Vyttila-4, Vyttila-5, Vyttila-6, Vyttila-7, Vyttila-8, Vyttila-9, Vyttila-10), high temperature tolerant (Nagina-22, NL-44) and three high yielding varieties (PTB-35, PTB-39 and MO-16) were used in this experiment. Seeds of different varieties were collected from Regional Agricultural Research Station, Pattambi and Rice Research Station, Vyttila, Kerala Agricultural University and NRRI, Cuttack, Odisha. Three independent lab studies were conducted for evaluating drought, salinity and high temperature tolerance. Seeds of each varieties were surface sterilized with 70% ethanol solution for 5 minutes. The seeds were then washed three times with sterilized distilled water. Germination assays were performed by paper towel method using 10 seeds per each towel. Each set of paper towel was moistened with 20 ml distilled water or uniform amounts of desired osmotic solutions to mimic drought stress and salinity.

Tolerance evaluation against individual stresses

Screening of rice genotypes for individual stress tolerance using paper towel method: In this part of study, drought stress was artificially induced by desired strengths of polyethylene glycol 6000 (PEG- 6000; Sigma Chemicals). Polyethylene glycol has been used to simulate water stress effects in plants (Swapna and Shylaraj, 2017). The experiment was laid out in a complete randomized design (CRD) with four levels of drought stress and control with three replications. Distilled water was used as a control (0 MPa) and osmotic potentials -1.0, -3.0, -5.0 and -7.0 bars were created by adding PEG-6000 @ 5, 10, 15 and 20 g per 100 ml distilled water. Four replicates of 50 seeds of each osmotic potential were used to assess the germination percentage. This experiment was carried out in growth chamber at 25±0.5°C and 80%±1 of relative humidity.

Salinity stress was artificially induced by desired strengths of NaCl (High Media). The experiment was laid out in a complete randomized design (CRD) with four levels of salinity stress and control with three replications 50 seeds each. Distilled water was used as a control (0 mM NaCl) and osmotic potentials of 100 mM NaCl, 150 mM NaCl, 200 mM NaCl, 250 mM NaCl were created by adding @ 5, 10, 15 and 20 g per 100 ml distilled water. This experiment was carried out in growth chamber at 25±0.5°C and 80%±1 of relative humidity.

High temperature was induced by keeping the moistened seeds in petri plates covered with two layers of moistened filter papers and kept in incubators at various temperatures 35°C, 40°C, 45°C, 50°C and 80%±1 of relative humidity. The experiment was laid out in a complete randomized design (CRD) with four levels of high temperature stress and control with three replications of 50 seeds each. From these three independent experiments, the number of germinated seed was recorded at 24 hours interval. The seedling height and seedling dry weights were measured on the 14th day. Seeds were considered germinated when both plumule and radicle extended to more than 2 mm from the seeds. Seedling vigour index was calculated from germination percentage and seedling height (root length + shoot length). Physiological traits like proline content as per the protocol by Bates et al. (1973) for drought, Na^+ K^+ ratio for salinity) by Zasoski and Buraum (1977), cell membrane stability trait by Blum and Ebercon (1981) for high temperature were studied on 14th day of germination.

Further experiment was carried out by selecting the genotypes that are capable of germinating under individual stress at possible highest extent. These selected nine genotypes were evaluated for combination of stresses.

Screening of rice genotypes for combination of stresses using paper towel method

In this experiment, seeds were exposed to combinations of above mentioned stresses *i.e.*, D_h (highest tolerated level from drought study) x S_h (highest tolerated level from salinity), D_h x T_h (highest tolerated level from temperature study), T_h x S_h and D_h x S_h x T_h. Rice seeds failed to germinate when a combination of all the stress (D_h x S_h x T_h) and combination of water and high temperature stress (D_h x S_h) was given.

Validation of rice genotypes using microsatellite (SSR) markers

In this experiment, all the twenty rice genotypes were screened with reported SSR markers for various traits. The documented SSR profiles were carefully examined for the polymorphism in banding pattern between the genotypes. All the 20 genotypes were analyzed for the identification of reported markers linked to stress tolerance such as drought, salinity and temperature. Reported microsatellite markers linked to drought, salinity and temperature were used to screen 20 rice varieties in order to analyze the presence of QTLs linked to stress tolerance in varieties which were selected as multiple abiotic stress tolerant based on phenotypic parameters. The sequence of RM primers used for screening is presented in Table 1.

SSR genotyping: Total genomic DNA of 20 rice genotypes was extracted from young leaves at 15 days old plants. The leaves were excised from each genotype, washed with distilled water to remove dust and any other foreign particles. The leaf samples were placed in small plastic bags individually and kept in container having cool environment, and carried immediately to the laboratory. The leaf sample then stored at -80°C until used for DNA extraction. Total genomic DNA was extracted using the method suggested by Dellaporta et al. (1983). The quantity and quality of DNA of each sample was determined by reading the absorbance at 260 nm and 280 nm in a spectrophotometer (ELICO, SL 21 UV-Vis spectrophotometer). The ratio between the readings at 260 nm and 280 nm (A 260/A 280) was used as an estimate of the purity of the DNA samples. Pure preparations of DNA have A260 nm/ A280 nm ratio between 1.7 and 1.8 (Sambrook and Russell, 2000). Quality was assessed by using gel electrophoresis with 5µl of crude DNA sample on agarose gel (0.8%). stained with ethidium bromide 0.5 µg/mL DNA. A total of 30 SSR markers were used, out of which nine markers were found to be polymorphic.

The PCR conditions (annealing temperature etc.) for SSRs were optimized through gradient PCR. A total of 20 µL final volume of PCR reaction was made. The DNA concentration for each reaction was 25ng/µL DNA, 10mM dNTPs, 1 µL forward and

Table 1. List of SSR markers used for screening among rice varieties.

Sl. no.	Marker	Forward primer	Reverse primer
1	RM 5749	GTGACCACATCTATATCGCTCG	ATGGCAAGGTTGGATCAGTC
2	RM 26212	GTCGCTCCTCTCCTCCAATCC	GCTCGCTGCTTCTAATCTCTTGC
3	RM 7076	CTCCACCAACAACTCGTATC	AAGCTATTCACAAGCAGCTC
4	RM 10793	GACTTGCCAACTCCTTCAATTCG	TCGTCGAGTAGCTTCCCTCTCTACC
5	RM 3412	AAAGCAGGTTTTCCTCCTCC	CCCATGTGCAATGTGTCTTC
6	RM 493	TAGCTCCAACAGGATCGACC	GTACGTAAACGCGGAAGGTG
7	RM 8094	AAGTTTGTACACATCGTATACA	CGCGACCAGTACTACTACTA
8	RM1287	GTGAAGAAAGCATGGTAAATG	CTCAGCTTGCTTGTGGTTAG
9	RM10843	CACCTCTTCTGCCTCCTATCATGC	GTTTCTTCGCGAAATCGTGTGG
10	RM349	TTGCCATTCGCGTGGAGGCG	GTCCATCATCCCTATGGTCG
11	RM 6100	TCCTCTACCAGTACCGCACC	GCTGGATCACAGATCATTGC
12	RM3042	CAAAAAGGAATCAATGTGAA	GGCTGTTGAGAGGTAGAGAA
13	RM7039	GCACATTTGCCATTCTACCG	GCCTTCCAGTGAGGTGACTC
14	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
15	RM224	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
16	RM243	GATCTGCAGACTGCAGTTGC	AGCTGCAACGATGTTGTCC
17	RM235	AGAAGCTAGGGCTAACGAAC	TCACCTGGTCAGCCTCTTTC
18	RM112	GGGAGGAGAGGCAAGCGGAGAG	AGCCGGTGCAGTGGACGGTGAC
19	RM241	GAGCCAAATAAGATCGCTGA	TGCAAGCAGCAGATTTAGTG
20	RM527	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG
21	RM507	CTTAAGCTCCAGCCGAAATG	CTCACCCTCATCATCGCC
22	RM447	CCCTTGTGCTGTCTCCTCTC	ACGGGCTTCTTCTCCTTCTC
23	RM528	GGCATCCAATTTTACCCCTC	AAATGGAGCATGGAGGTCAC
24	RM454	CTCAAGCTTAGCTGCTGCTG	GTGATCAGTGCACCATAGCG
25	RM348	CCGCTACTAATAGCAGAGAG	GGAGCTTTGTTCTTGCGAAC
26	RM256	GACAGGGAGTGATTGAAGGC	GTTGATTTCGCCAAGGGC
27	RM490	ATCTGCACACTGCAAACACC	AGCAAGCAGTGCTTTCAGAG
28	RM232	CCGGTATCCTTCGATATTGC	CCGACTTTTCCTCCTGACG
29	RM226	AGCTAAGGTCTGGGAGAAACC	AAGTAGGATGGGGCACAAGCTC
30	RM208	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC

Table 2. Variation in seedling vigour index of rice genotypes under different levels of drought stress.

Sl. no.	Variety	-1 bar PEG6000	-3 bar PEG 6000	-5bar PEG6000	Control
1	Chomala	2095 (3.317)ab	0.000 (0.000)h	0.000 (0.000)e	2213 (3.363)
2	MO-16	1921 (3.279)abcd	431.0 (2.621)de	0.000 (0.000)e	2070 (3.341)
3	PTB-35	2660 (3.421)a	1622 (3.209)ab	346.8 (2.541)b	2822 (3.471)
4	PTB-60	2208 (3.337)ab	1682 (3.225)ab	385.8 (2.587)b	2392 (3.402)
5	PTB-39	1240 (3.088)d	232.0 (2.368)f	32.40 (1.500)d	1708 (3.331)
6	PTB-55	1950 (3.287)abc	480.0 (2.683)d	0.000 (0.000)e	2152 (3.366)
7	PTB-30	1312 (3.113)cd	428.0 (2.633)de	124.8 (2.092)c	1819 (3.362)
8	PTB-7	1867 (3.264)abcd	1904 (3.279)a	998.0 (3.000)a	2559 (3.505)
9	CR Dhan307	2366 (3.369)ab	881.0 (2.946)c	0.000 (0.000)e	2464 (3.403)
10	Apo	1647 (3.213)bcd	0.000 (0.000)h	0.000 (0.000)e	1772 (3.272)
11	Vyttila-3	1908 (3.272)abcd	140.0 (2.103)g	0.000 (0.000)e	2344 (3.439)
12	Vyttila-4	1719 (3.228)abcd	296.0 (2.473)ef	0.000 (0.000)e	2563 (3.404)
13	Vyttila-5	1806 (3.251)abcd	397.0 (2.600)de	0.000 (0.000)e	2491 (3.392)
14	Vyttila-6	1797 (3.250)abcd	206.0 (2.317)f	0.000 (0.000)e	2653 (3.420)
15	Vyttila-7	1791 (3.249)abcd	438.0 (2.642)d	0.000 (0.000)e	2380 (3.372)
16	Vyttila-8	1836 (3.261)abcd	0.000 (0.000)h	0.000 (0.000)e	2317 (3.356)
17	Vyttila-9	1821 (3.256)abcd	1166 (3.067)bc	0.000 (0.000)e	2482 (3.390)
18	Vyttila-10	1996 (3.296)abc	1161 (3.065)bc	0.000 (0.000)e	2604 (3.410)
19	Nagina-22	712.2 (2.848)e	0.000 (0.000)h	0.000 (0.000)e	1926 (3.280)
20	NL-44	275.2 (2.434)f	0.000 (0.000)h	0.000 (0.000)e	2073 (3.311)
	Mean	**1746.00** **(3.202)**	**674.35** **(2.061)**	**377.56** **(0.586)**	**2447.00** **(3.379)**
	S.E. ±	**2.927**	**3.804**	**9.073**	**2.918**
	C.D. (0.05)	**0.195**	**0.164**	**0.111**	**NS**

reverse primer each, 1 unit of TaqDNA polymerase with 10X reaction buffer. The thermal profile starts with initial denaturation at 94°C for 3 min continued to 35 cycles of denaturation at 94°C for 1 minute, primer annealing 55°C for 1 min, extension 72°C for 1 min and final extension at 72°C for 5 min. After completion of amplification, PCR products were kept in a -20°C deep freeze. An aliquot of 10 µL PCR amplification products were loaded in an agarose gel of 3.5% containing 0.08 µg/ml of ethidium bromide for electrophoresis. The electrophoresis was carried out at 80 volts (2.5 V/cm) in 1X TBE (pH 8.0). Sizes of amplicons were determined by using 100bp DNA ladder. The gel was visualized under UV (312nm) transilluminator and documented in gel documentation system (Syngene G-box documentation system). The documented SSR profiles were carefully examined for the polymorphism in banding pattern between the genotypes.

Population structure: Population structure of 20 rice genotypes was estimated using a STRUCTURE software V2.3.4 based on Bayesian clustering algorithm (Pritchard et al, 2000). In order to conclude the optimum number of subpopulations, values for K = 2 to K = 8 were given using a burn length of 50,000 and run length of 50,000. Five independent runs yielded the reproducible results. The results were imported to STRUCTUREHARVESTER software to calculate exact value of 1K (Earl and Vonholdt, 2012).

Statistical analysis

The overall effects of treatment and rice genotypes and their interaction were analysed by means of two-way ANOVA with different stress levels and genotypes

Table 3. Variation in proline content (µg/g tissue) of rice genotypes under different levels drought stress.

Sl. no.	Variety	-1 bar PEG6000	-3 bar PEG6000	-5 bar PEG6000	Control
1	Chomala	20.04 (1.323)ef	0.000 (0.000)j	0.000 (0.000)d	6.320 (0.864)i
2	MO-16	22.91 (1.378)de	27.15 (1.449)ef	0.000 (0.000)d	6.691 (0.885)i
3	PTB-35	35.10 (1.557)ab	37.28 (1.583)a	42.55 (1.639)a	12.67 (1.135)defgh
4	PTB-60	35.19 (1.558)ab	36.36 (1.572)ab	38.34 (1.592)b	11.62 (1.101)h
5	PTB-39	27.95 (1.461)c	27.99 (1.462)de	34.39 (1.549)c	14.55 (1.191)bcd
6	PTB-55	29.50 (1.484)bc	29.91 (1.490)cde	0.000 (0.000)d	15.80 (1.225)ab
7	PTB-30	29.57 (1.472)c	23.90 (1.396)fg	31.87 (1.517)c	17.28 (1.261)a
8	PTB-7	36.42 (1.573)a	37.75 (1.588)a	42.23 (1.646)a	6.022 (0.846)i
9	CR Dhan307	30.94 (1.504)abc	32.66 (1.527)bc	0.000 (0.000)d	13.47 (1.160)cdefg
10	Apo	23.03 (1.380)de	0.000 (0.000)j	0.000 (0.000)d	11.99 (1.113)gh
11	Vyttila-3	28.14 (1.465)c	29.33 (1.482)cde	0.000 (0.000)d	12.35 (1.125)fgh
12	Vyttila-4	17.27 (1.261)f	18.78 (1.296)i	0.000 (0.000)d	16.82 (1.240)ab
13	Vyttila-5	21.62 (1.355)e	22.91 (1.378)gh	0.000 (0.000)d	18.36 (1.207)abc
14	Vyttila-6	27.32 (1.452)cd	31.83 (1.511)cd	0.000 (0.000)d	20.76 (1.182)bcdef
15	Vyttila-7	19.11 (1.303)ef	20.65 (1.335)hi	0.000 (0.000)d	16.69 (1.184)bcde
16	Vyttila-8	20.47 (1.348)ef	0.000 (0.000)j	0.000 (0.000)d	16.93 (1.157)cdefgh
17	Vyttila-9	21.32 (1.332)e	23.03 (1.380)gh	0.000 (0.000)d	18.23 (1.208)abc
18	Vyttila-10	19.99 (1.322)ef	19.99 (1.3222)i	0.000 (0.000)d	16.42 (1.141)defgh
19	Nagina-22	19.94 (1.321)ef	0.000 (0.000)j	0.000 (0.000)d	16.01 (1.116)gh
20	NL-44	19.14 (1.304)ef	0.000 (0.000)j	0.000 (0.000)d	15.86 (1.133)efgh
	Mean	**25.25**	**27.96**	**37.87**	**16.82**
	S.E.±	**2.714**	**2.375**	**4.088**	**2.471**
	C.D.(0.05)	**0.080**	**0.054**	**0.034**	**0.058**

were taken as fixed factors. Genotypes were treated as fixed factors because we were interested in the response of the specific genotypes used in this experiment. Design of experiment was CRD with treatment levels . Twenty rice genotypes were analysed with three replications each for the treatment levels. The statistical analysis were done using OPSTAT software (Two factorial CRD). Means were separated by least significant difference (LSD).

RESULT AND DISCUSSION

Screening of rice genotypes for individual stress tolerance using paper towel method

Evaluation of rice genotypes for seedling vigour index and proline content under varied drought

Table 4. Variation in seedling vigour index of rice genotypes under different levels of salinity.

Sl. no.	Variety	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	Control
1	Chomala	1932	243.4	226.6	0.000	2741
		(3.284)g	(2.382)m	(2.791)jk	(0.000)n	(3.438)
2	MO-16	2769	1737	1272	870	3111
		(3.442) abc	(3.240)b	(2.946)b	(2.940)a	(3.493)
3	PTB-35	2509	1,001	693.6	311	2985
		(3.399)cdef	(3.000)d	(2.721)d	(2.494)c	(3.475)
4	PTB-60	2723	493.6	364.0	145	3,077
		(3.435) abcd	(2.693)g	(2.463)f	(2.166)f	(3.488)
5	PTB-39	2564	238.8	230.4	63.0	3134
		(3.409) bcdef	(2.378)m	(2.575)hij	(1.806)i	(3.496)
6	PTB-55	2440	833.0	670.0	139	2961
		(3.387)def	(2.921)e	(2.867)d	(2.149)f	(3.471)
7	PTB-30	2706	1,019	858.0	322	3211
		(3.432) abcde	(3.008)d	(2.877)c	(2.509)c	(3.507)
8	PTB-7	2380	827.0	549.0	171	3040
		(3.376)f	(2.917)e	(2.739)de	(2.235)e	(3.483)
9	CRdhan307	2562	624.8	511.2	360	2647
		(3.408) bcdef	(2.796)f	(2.708)e	(2.557)b	(3.423)
10	Apo	1258	166.0	101.0	239	2103
		(3.096)h	(2.220)n	(2.004)m	(2.380)d	(3.323)
11	Vyttila-3	3055	1,133	999.0	822	3039
		(3.485) a	(3.054)c	(2.751)c	(2.915)a	(3.483)
12	Vyttila-4	2395	445.0	329.0	0.000	2685
		(3.379)ef	(2.648)h	(2.472)fg	(0.000)n	(3.429)
13	Vyttila-5	2775	316.2	271.8	64.8	3047
		(3.443) abc	(2.500)l	(2.296)ghi	(1.818)i	(3.484)
14	Vyttila-6	2558	172.8	147.6	114	2752
		(3.407)bcdef	(2.238)n	(2.337)l	(2.060)g	(3.440)
15	Vyttila-7	2481	355.8	319.2	10.4	3514
		(3.394)cdef	(2.551)jk	(2.505)fg	(1.056)m	(3.546)
16	Vyttila-8	2575	369.0	295.2	86.4	3563
		(3.410) bcdef	(2.567)j	(2.470)fgh	(1.941)h	(3.552)
17	Vyttila-9	2413	2276	1674	790	3447
		(3.382)def	(3.357)a	(3.223)a	(1.553)a	(3.537)
18	Vyttila-10	2452	421.2	205.8	34.8	2996
		(3.389)cdef	(2.624)hi	(2.313)ijk	(1.553)l	(3.476)
19	Nagina-22	2846	383.4	270.6	46.8	3236
		(3.454) ab	(2.584)ij	(2.432)gh	(1.679)k	(3.510)
20	NL-44	2663	322.8	189.6	53.4	3052
		(3.425) bcdef	(2.509)kl	(2.277)k	(1.735)j	(3.485)
	Mean	**2,502**	**668.9**	**508.8**	**257.9**	**3,017**
		(3.392)	**(2.709)**	**(2.611)**	**(1.945)**	**(3.477)**
	S.E.±	**0.758**	**0.772**	**1.791**	**1.179**	
	C.D.(0.05)	**0.054**	**0.044**	**0.098**	**0.048**	**NS**

stress condition:

There was significant difference for seedling vigour index among treatments and genotypes under diverse drought induction (Table 2). Among the treatments, control recorded the highest seedling viogur index of 2447. This treatment was followed by the treatment -1bar PEG6000 with an average seedling vigour index of 1746. None of the genotypes were germinated under -7bar PEG6000. Thus it was identified that -5bar PEG6000 was the highest tolerating level of drought stress.

Genotypes response to individual stress varied greatly. At the highest tolerating level of drought stress (-5barPEG6000), maximum seedling vigour index was recorded by PTB-7 (998.0) followed by PTB-60 (385.8) and PTB-35 (346.8), whereas 15 genotypes were not

Table 5. Variation in Na^+/K^+ ratio of rice genotypes under different levels of salinity.

Sl. no.	Variety	100mM NaCl	150mM NaCl	200mM NaCl	250mM NaCl	Control
1	Chomala	0.33 (0.124)	2.00 (0.477)bcde	3.50 (0.653)de	0.000 (0.000)i	0.16 (0.064)
2	MO-16	0.33 (0.124)	2.75 (0.574)bc	3.16 (0.619)def	5.25 (0.796)de	0.29 (0.111)
3	PTB-35	0.50 (0.176)	3.00 (0.602)b	6.00 (0.845)a	7.50 (0.929)bc	0.22 (0.086)
4	PTB-60	0.50 (0.176)	2.25 (0.512)bcd	4.50 (0.740)abcd	7.25 (0.916)bc	0.41 (0.149)
5	PTB-39	0.29 (0.111)	1.00 (0.301)de	4.16 (0.713)bcd	8.00 (0.954)abc	0.33 (0.124)
6	PTB-55	0.75 (0.243)	2.25 (0.512)bcd	5.00 (0.778)abc	9.50 (1.021)a	0.41 (0.149)
7	PTB-30	0.41 (0.149)	1.917 (0.465)bcde	3.75 (0.677)cd	8.50 (0.978)ab	0.25 (0.097)
8	PTB-7	0.50 (0.176)	1.00 (0.301)de	4.25 (0.720)abcd	9.00 (1.000)ab	0.75 (0.243)
9	CRdhan307	1.00 (0.301)	4.00 (0.699)ab	4.16 (0.713)bcd	4.50 (0.740)ef	0.50 (0.176)
10	Apo	0.75 (0.243)	5.75 (0.829)a	4.00 (0.699)cd	6.50 (0.875)cd	0.50 (0.176)
11	Vyttila-3	1.00 (0.301)	1.00 (0.301)de	2.75 (0.574)fg	3.25 (0.628)g	0.50 (0.176)
12	Vyttila-4	0.75 (0.243)	3.50 (0.653)ab	2.0 (0.477)gh	0.000 (0.000)h	1.00 (0.301)
13	Vyttila-5	0.75 (0.243)	2.50 (0.544)bc	5.50 (0.813)ab	6.50 (0.875)cd	1.00 (0.301)
14	Vyttila-6	0.75 (0.243)	3.50 (0.653)ab	2.50 (0.544)efg	2.83 (0.583)h	1.00 (0.301)
15	Vyttila-7	1.00 (0.301)	1.25 (0.352)cde	1.75 (0.439)ghi	3.50 (0.653)fg	1.00 (0.301)
16	Vyttila-8	0.75 (0.243)	1.25 (0.352)cde	1.25 (0.352)ij	3.50 (0.653)fg	1.00 (0.301)
17	Vyttila-9	1.00 (0.301)	1.25 (0.352)cde	1.75 (0.439)ghi	3.50 (0.653)fg	1.00 (0.301)
18	Vyttila-10	1.00 (0.301)	0.87 (0.272)e	1.33 (0.367)hij	4.5 (0.740)ef	1.00 (0.301)
19	Nagina-22	1.00 (0.301)	1.25 (0.352)cde	1.00 (0.301)j	3.00 (0.602)g	0.11 (0.045)
20	NL-44	1.00 (0.301)	2.25 (0.512)bcd	1.16 (0.334)ij	3.00 (0.602)g	0.29 (0.111)
	Mean	**0.71**	**2.37**	**3.171**	**5.53**	**0.588**
	S.E.±	—	**8.289**	**9.893**	**5.986**	—
	C.D. (0.05)	**NS**	**0.226**	**0.121**	**0.089**	**NS**

germinated under this condition (Table 2). At -3bar PEG6000, highest seedling vigour index was recorded by PTB-7(1904), followed by PTB60 (1682) and PTB35 (1622). At -1bar PEG6000, highest seedling vigour index was recorded by PTB35 (2660), followed by CR Dhan307 (2366), PTB60 (2208), Chomala (2095). There was no significant difference among the genotypes for seedling vigour index under control condition.

There was significant difference for proline content among treatments and genotypes for diverse drought stress condition (Table 3). Among the treatments, -5bar PEG 6000 recorded the highest average proline content of 37.87 µg/g tissue. Lowest proline content was recorded in control condition with an average of 16.82 µg/g tissue.

At the highest tolerating level of drought stress (-5bar PEG6000), highest proline content was recorded by PTB-35 (42.55 µg/g tissue). This genotype was on par with PTB7 (42.23 µg/g tissue) (Table 3). At -3barPEG6000, highest proline content was recorded by PTB-7(37.75 µg/g tissue). This genotype was on par with PTB35 (37.28 µg/g tissue). At -1bar PEG6000, highest proline µg/g tissue content was recorded by PTB7 (36.42 µg/g tissue), followed by PTB60 (35.19 µg/g tissue). This genotype was on par with PTB35 (35.10 µg/g tissue). Under control condition, highest proline content was recorded by PTB30 (17.28 µg/g tissue). This was followed by Vyttila 4(16.82 µg/g tissue). This genotype was on par with PTB55 (15.8 µg/g tissue).

Evaluation of rice genotypes for seedling vigour index and Na^+/K^+ ratio under varied salinity stress condition

There was significant difference for seedling vigour index among treatments and genotypes under diverse salinity condition (Table 4). Among the treatments, control recorded the highest seedling vigour index of 3017. This treatment was followed by the treatment 100 mM NaCl with an average seedling vigour index of 2502. There was a gradual reduction in seedling vigour from 100 mM NaCl (2502) to 250 mM NaCl (257.9).

At the highest tolerating level of salinity stress (250 mMNaCl), highest seedling vigour index was recorded by MO-16 (870). This genotype was on par with Vyttila 3(822) and Vyttila 9(790). Chomala and Vyttila 4 were not germinated under 250 mM NaCl (Table 4). At 200 mMNaCl, highest seedling vigour index was recorded by Vyttila9 (1674).This genotype was followed by MO-16 (1272). At 150 mMNaCl, Vyttila 9 recorded the highest seedling vigour index of

Table 6. Variation in seedling vigour index of rice genotypes high temperature stress of 35°C.

S. no.	Variety	Treatment (35°C)	Control
1	Chomala	1430 (3.155)ab	1728 (3.306)def
2	MO-16	927.0 (2.964)ab	1,446 (3.206)ghi
3	PTB-35	469.2 (2.602)bc	1145 (3.112)j
4	PTB-60	860.0 (2.934)ab	1264 (3.066)ij
5	PTB-39	712.8 (2.829)ab	1740 (3.174)def
6	PTB-55	464.0 (2.667)abc	1291 (3.172)hij
7	PTB-30	857.0 (2.933)ab	1548 (3.170)efg
8	PTB-7	1,423 (3.151)ab	2184 (3.253)bc
9	CRdhan307	905.0 (2.957)ab	1505 (3.262)fgh
10	Apo	94.20 (1.917)cd	1145 (3.107)j
11	Vyttila-3	0.000 (0.000)e	2272 (3.216)bc
12	Vyttila-4	1,815 (3.259)ab	1999 (3.351)cd
13	Vyttila-5	0.00 (0.000)e	1803 (3.242)de
14	Vyttila-6	2,224 (3.347)ab	2278 (3.357)bc
15	Vyttila-7	1033 (3.012)ab	1,609 (3.206)efg
16	Vyttila-8	1995 (3.300)ab	2211 (3.344)bc
17	Vyttila-9	723.0 (2.859)ab	1811 (3.258)de
18	Vyttila-10	91.00 (1.131)d	1608 (3.206)efg
19	Nagina-22	2584 (3.412)a	2807 (3.448)a
20	NL-44	2376 (3.376)ab	2555 (3.407)ab
	Mean	**1049** **(2.590)**	**1797** **(3.240)**
	S.E.±	**14.612**	**1.091**
	C.D.(0.05)	**0.790**	**0.074**

Table 7. Variation in cell membrane stability index (%) of rice genotypes under high temperature stress of 35°C.

Sl. no.	Variety	Treatment
1	Chomala	10.65 (0.998)h
2	MO -16	4.722 (0.750)i
3	PTB-35	44.71 (1.660)cde
4	PTB-60	24.19 (1.401)fg
5	PTB-39	77.07 (1.887)ab
6	PTB-55	35.49 (1.561)ef
7	PTB-30	38.28 (1.594)de
8	PTB-7	38.51 (1.597)de
9	CRdhan307	56.69 (1.757)bcd
10	Apo	7.234 (0.916)hi
11	Vyttila-3	0.000 (0.000)j
12	Vyttila-4	15.41 (1.212)g
13	Vyttila-5	0.000 (0.000)j
14	Vyttila-6	64.11 (1.814)abc
15	Vyttila-7	45.17 (1.663)cde
16	Vyttila-8	63.78 (1.811)abc
17	Vyttila-9	37.47 (1.585)def
18	Vyttila-10	66.66 (1.830)abc
19	Nagina-22	99.02 (2.000)a
20	NL-44	58.22 (1.771)bcd
	S.E. ±	**6.6**
	C.D.(0.05)	**0.190**

2276. This genotype was followed by MO-16 (1737). At 100 mM NaCl, Vyttila 3 recorded the highest seedling vigour index of 3055. This genotype was followed by Nagina-22 (2846). There was no significant difference among the genotypes for seedling vigour index under control condition.

There was significant difference in Na^+/K^+ ratio among the treatments and genotypes for diverse salinity stress condition (Table 5). Among the treatments, 250 mM NaCl recorded the highest average Na^+/K^+ ratio of 5.53. Lowest Na^+/K^+ ratio was recorded in control condition with an average of 0.588.

At the highest tolerating level of salinity stress (250 mMNaCl), highest Na^+/K^+ ratio was recorded by PTB-55 (9.5). Lowest Na^+/K^+ ratio was recorded by Vyttila 6 (2.83). This was followed by Nagina-22 and NL-44. Under 200 mM NaCl level, lowest Na^+/K^+ ratio was recorded by Nagina -22(1). This was followed by NL-44 (1.16). This genotype was on par with Vyttila -8(1.25). At 150 mM NaCl, lowest Na^+/K^+ ratio was recorded by Vyttila 10 (0.87). There is no significant difference among the genotypes for Na^+/K^+ ratio under both 100 mM NaCl and control condition.

Evaluation of rice genotypes for seedling vigour index and cell membrane stability index under high temperature condition

Seeds were not germinated at temperature 40°C, 45°C and 50°C. There was significant difference between the treatments. Highest seedling vigour index was recorded under control condition (1797) compared to the treatment-35°C (1049). There was significant difference among the genotypes for seedling vigour index under both treatment (35°C) and control condition. Under high temperature condition, Vyttila -3 and Vyttila-5 were not germinated (Table 6). Under both control and high temperature condition, Nagina-22 recorded the highest seedling vigour index of (2807), (2584) respectively. Under high temperature condition Nagina-22 recorded the highest cell membrane stability index of 99.02% (Table 7). This was followed by Vyttila-10, Vyttila-8 and Vyttila-6 and these genotypes were on par with each other.

Comparing the seedling vigour index under different stresses, highest seedling vigour index was recorded at temperature (35°C), this is not a severe stress for rice. But seeds were not germinated at high temperature 40°C, 45°C and 50°C. Otherwise highest seedling viogur index was recorded under different salinity level except for the variety PTB-7 (998) under drought condition.

In this study, there was significant variation for seedling vigour index and proline content among different levels of drought stress treatments. Seeds

Table 8. Variation in seedling vigour index of selected rice genotypes at highest tolerated level of drought and highest tolerated level of salinity.

Sl. no.	Variety	Treatment	Control
1	MO-16	0.000 (0.000)d	2818 (3.445)
2	PTB-35	209.2 (2.321)c	2712 (3.427)
3	PTB-60	0.000 (0.000)d	2799 (3.444)
4	PTB-7	1615 (3.207)a	2716 (3.428)
5	Vyttila-3	0.000 (0.000)d	2699 (3.426)
6	Vyttila-9	293.6 (2.469)b	2810 (3.432)
	Mean	**705.93** **(2.665)**	**2759** **(3.433)**
	S.E. ±	**2.256**	**2.897**
	CD(0.05)	**0.053**	**NS**

Table 9. Seedling vigour index under combined stress of highest tolerated level of Temperature (T_h) (35°C) and highest tolerated level of salinity (S_h) (250 mM NaCl).

Sl. no.	Variety	Treatment	Control
1	MO-16	1.000 (0.242)bc	2322 (3.366)a
2	Vyttila-3	0.000 (0.000)d	2100 (3.322)c
3	Vyttila-6	0.000 (0.000)d	1990 (3.299)d
4	Vyttila-9	0.000 (0.000)d	1806 (3.257)e
5	Nagina-22	3.467 (0.626)a	2222 (3.347)b
6	NL-44	1.067 (0.287)b	2148 (3.332)c
	Mean	**1.844** **(0.251)**	**2098** **(3.320)**
	S.E. ±	**7.974**	**8.654**
	CD(0.05)	**0.273**	**0.021**

were not germinated under -7bar PEG6000 and thus -5bar PEG 6000 was identified as the highest tolerating level of drought stress. Under this condition and less range of drought stress also, PTB-7, PTB-60 and PTB-35 were showed higher seedling vigour index. Similar study was conducted by Panda et al. (2019) and reported that among the folk rice genotypes of Odhisha, Nagina-22 showed highest seedling vigour index.

Among the treatments, -5bar PEG 6000 recorded the highest average proline content. Lowest proline content was recorded in control condition. Same genotypes recorded the highest proline content under various levels of drought stress. Hsu et al. (2003) reported that drought stress induction by PEG results in proline production in rice and there is 25 fold increase in proline for every 12h under stress. Beena et al. (2012) also reported an increase in proline (89.6%) across the RIL's of IR20 x Nootripathu as compared to control. Islam et al. (2018) studied the drought tolerance of 18 genotypes using 0, 5, 10, 15 and 20% PEG6000 and reported that seed germination and relative growth of seedlings decreased under high concentration.

At highest level of salinity stress, 250 mM NaCl, MO-16, Vyttila-3 and Vyttila-9 exhibited the highest seedling vigour index. There is gradual reduction in seedling vigour index from control to 250mM NaCl. Zhang et al. (2007) reported a similar kind of findings in lucerne crop under salinity stress. High levels of sodium adversely influence the acquisition of pottasium (Munns et al., 2010). There is significant difference in Na^+/K^+ ratio among the treatments and genotypes for diverse salinity stress condition. Among the treatments, 250 mMNaCl recorded the highest average Na^+/K^+ ratio of 5.53. Lowest Na^+/K^+ ratio was recorded in control condition with an average of 0.588. Vytilla-6, Nagina-22 and NL-44 recorded lowest Na^+/ K^+ ratio, so as to reduce sensitivity of the plants to salinity is due to the failure to prevent Na^+ and Cl^- from transpiration streams (Gorham et al., 1990). Similar results were reported by Bohra and Doerffling (1993).

In the present study, highest tolerating level of temperature stress was 35°C. Seeds were not germinated at different temperature levels of 40°C, 45°C and 50°C. Seedling vigour index was reduced under temperature stress condition. The maximum seedling vigour index was observed in Nagina-22 at 35°C. Iioh et al. (2014) reported that under temperature regimes seedling vigour index found to be decreasing with increasing temperature in rice, maize and sorghum. Among the varieties, cell membrane stability index was found to be maximum in Nagina-22 under temperature stress condition. Similar results were also reported by Prasad et al. (2006) and Beena et al. (2018a).

Screening of rice genotypes for combination of stresses using paper towel method

Among 20 genotypes, nine genotypes were selected for combination of stresses, of which three are tolerant to high level of salinity stress (Uma, Vyttila-3 and Vyttila-9), three for highest level of drought stress (PTB-7, PTB-60 and PTB-35) and three from highest tolerated temperature stress (Nagina-22, NL-44 and Vytilla-6). The selected genotypes from previously mentioned highest tolerated level of individual stress were subjected to combination of stresses. At highest tolerated level of drought (Dh) and highest tolerated level of salinity stress (Sh) PTB-7 (1615) recorded the highest seedling vigour index, followed by Vyttila-9 (293.6) and the least seedling vigour index was observed in PTB-35 (209.2) (Table 8). At highest tolerated level of Temperature (Th) and highest tolerated level of salinity (Sh), highest seedling vigour index was recorded by Nagina-22 (3.467). This is followed by NL-44 (1.067) and MO-16 (1.00) which are on par with each other (Table 9).

Highest tolerated level of drought (D_h) and highest tolerated level of salinity stress (S_h) PTB-7 recorded the highest seedling vigour index, followed by Vyttila-9 and the least seedling vigour index was observed in PTB-35. Combined stresses at their highest tolerable levels resulted in decrease in seedling vigour index, due to reduced water availability for seedling growth and establishment (Babu and Rosaiah, 2017), whereas, at the highest tolerated level of temperature (T_h) and highest tolerated level of salinity (S_h), highest seedling vigour index was recorded by Nagina-22. This is followed by NL-44 and MO-16 which are on par with each other. High temperature stress may cause severe damage to the proteins, disturb their synthesis, inactivate major enzymes and damage membranes. Heat stress could also have major effects on the process of cell divisions (Smertenko et al., 1997). Mokhberdoran et al. (2009) reported that rice cultivar Kalat at seedling stage stated that the deleterious effects of NaCl and PEG were more pronounced with increase in temperature. Metabolic adjustments in response to unfavourable conditions are dynamic and multifaceted and not only depend on the type and strength of the stress, but also on the type of genotypes used for the study.

Simple sequence repeats (SSR) are the DNA markers of choice for rice genetic assessment (*Oryza sativa* L.) owing to their abundance, elevated polymorphism, co-dominance and easy agarose gel electrophoresis assays (Singh et al., 2010). In the present investigation microsatellite markers or SSR markers (Simple Sequence Repeats) were used to characterize and to assess abiotic stress tolerance among 20 rice genotypes. Beena, 2011 and Prince et al., 2015 also reported that identification of trait linked molecular markers can improve rice breeding programme. Our results were supported by other studies. RM 6100 is associated with heat tolerance at flowering stage (Bharathkumar et al., 2014) and is located in chromosome 10. Buu et al. (2014) reported markers RM 7076, RM 3586, 26212 and RM 5749 were polymorphic for heat tolerance. Vu et al., 2012 reported SSR markers RM 1287, RM 8094, RM 3412, RM 493 and RM 140 were linked to the Saltol QTL on chromosome 1. The microsatellite marker, RM 8094

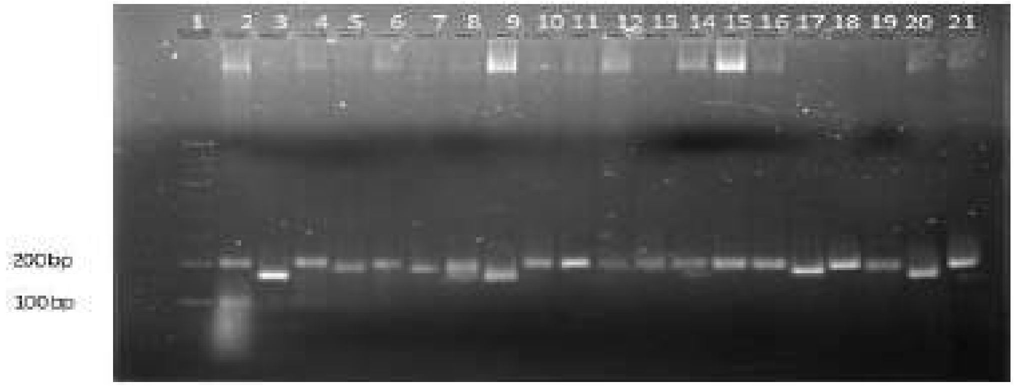

Plate 1. Polymorphism pattern of 20 rice genotypes using SSR marker RM8094.
Lane 1-100bp ladder, 2-Chomala, 3- MO-16, 4-PTB-35, 5-PTB-60, 6-PTB-39, 7-PTB-55, 8-PTB-30, 9-PTB-7, 10-CRdhan307,11-Apo, 12-Vyttila-3, 13-Vyttila-4, 14-Vyttila-5, 15-Vyttila-6, 16-Vyttila-7,17- Vyttila-8, 18-Vyttila-9, 19-N-22, 20-NL-44, 21-Vytilla-10

found in Saltol is considered as the superior marker for genetic diversity analysis. Rice genotypes with the Pokkali band type for locus RM 8094 marker were either highly tolerant or tolerant to salinity stress at the seedling stage (Nejad et al., 2008).

RM8085 was mapped on chromosome 1 and is linked to leaf rolling and leaf drying under drought stress (Salunkhe et al., 2011). The QTL on chromosome 9 is associated with spikelet fertility under stress and root and shoot traits (Yue et al., 2006). Major QTL on chromosomes 4 (Ctb1) and 8 (qCTB8) for cold tolerance at the booting stage were identified in a tropical japonica cultivar, Silewah, and markers have been used for introducing the tolerance gene (Ctb1) into japonica cultivars (Kuroki et al., 2007).

Validation of microsatellite markers

Screening of rice genotypes with 30 microsatellite markers revealed that, out of thirty microsatellite markers, eight markers were found to be polymorphic. RM8904 was associated with salinity tolerant trait. In this study, RM8904 showed clear polymorphism between salinity tolerant and susceptible genotypes (Plate 1). This marker clearly separated the genotypes Vyttila-3, Vyttila-4, Vyttila-5, Vyttila-6, Vyttila-7, Vyttila-8, Vyttila-9,Vyttila-10 and Nagina-22 from other genotypes approximately with a band size of 200bp. RM26212 and RM6100 were identified for high temperature tolerant trait in rice. In this study, RM26212 polymorphic between Nagina-22 and other genotypes with a band of size ~ 180bp (Plate 2). However, RM6100 clearly separated Nagina-22 and NL-44 from other varieties with approximate band size of 150bp (Plate 3). RM1287 was reported for salinity tolerant traits in rice. In this study, RM1287 showed distinct polymorphism PTB-7, Vyttila-3, Vyttila-4, Vyttila-5, Vyttila-6, Vyttila-7, Nagina-22 from other genotypes with appropriate band size of 150 - 220bp (Plate 4). RM5749 was reported for high temperature tolerant trait in rice. This marker showed clear and distinct polymorphism for Nagina-22 and NL-44 from other genotypes with approximate band size of 170bp (Plate 5).

Polymorphism information content: PIC of markers ranged from 0 to 0.69.The primer which showed the highest PIC was RM 26212 (0.69) followed by RM 5749 (0.67).

Population structure analysis

STRUCTURE software was used to analyze genetic structure of different populations and it was run for K = 8 based on SSR markers banding pattern. K is the number of significant populations in each main group. Results were analyzed using Evanno's method implemented in Structure HARVESTER. The results showed that the maximum delta K was detected at K = 5 (Fig. 1) therefore the number of sub-populations as obtained from the STRUCTURE analysis of the rice genotypes was observed to be five. The grouping of rice genotypes based on sub-populations is shown in Plate 6.

Plate 2. Polymorphism pattern of 20 rice genotypes using SSR marker RM 26212.
Lane 1-100bp ladder, 2-Chomala, 3- MO-16, 4-PTB-35, 5-PTB-60, 6-PTB-39, 7-PTB-55, 8-PTB-30, 9-PTB-7, 10-CRdhan307,11-Apo, 12-Vyttila-3, 13-Vyttila-4, 14-Vyttila-5, 15-Vyttila-6, 16-Vyttila-7,17- Vyttila-8, 18-Vyttila-9, 19-N-22, 20-NL-44, 21-Vyttila-10

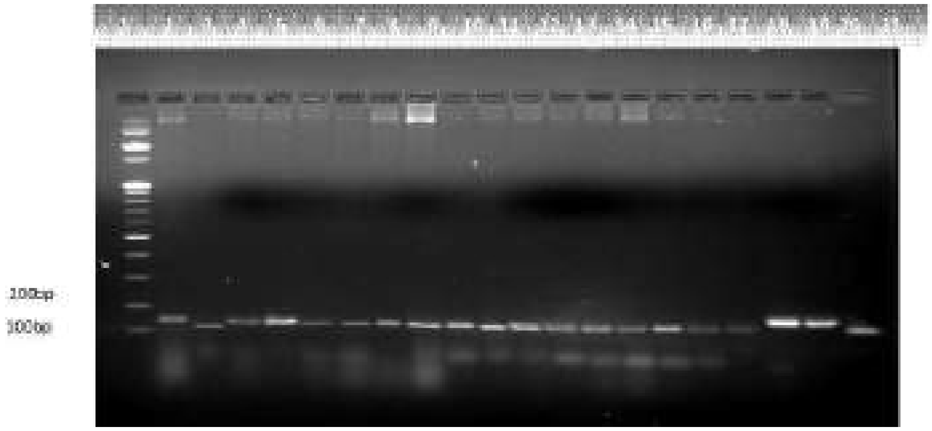

Plate 3. Polymorphism pattern of 20 rice genotypes using SSR marker RM6100.
Amplification pattern of 20 rice varieties obtained by SSR marker RM 6100(Lane 1 - 100bp ladder, Lane 2 - chomala, Lane 3 - MO-16, Lane 4 - PTB 35, Lane 5 -PTB 60, Lane 6 - PTB 39, Lane 7 - PTB 55, Lane 8 - PTB 30, Lane 9 - PTB 7, Lane 10 - CR dhan 307, Lane 11 - Apo, Lane 12 - Vyttila -3, Lane 13 - Vyttila -4, Lane 14 - Vyttila -5, Lane 15 - Vyttila -6, Lane 16 - Vyttila -7, Lane 17 - Vyttila -8, Lane 18 - Vyttila -9, Lane 19 - N-22, Lane 20 - NL -44, Lane 21 - Vyttila -10).

Five subpopulations obtained by STRUCTURE analysis were, SP1 consists of only one variety NL-44. SP2 consists of two varieties, Nagina-22 and Ptb-7. SP3 consists of varieties, Chomala, PTB-35, PTB-39, Vyttila-3, Vyttila -4, Vyttila-5, CR Dhan 307 and MO-16. SP4 consist of varieties- PTB-60, Vyttila 10, PTB-55, PTB-30 and Vyttila-6. SP5 consist of varieties Vyttila - 9, Vytilla-8, Apo and Vyttila-5 (Plate 7).

Five subpopulations obtained by STRUCTURE analysis were, SP1 consists of only one variety NL-44. It is NERICA Line from Africa, which is entirely different from indica varieties. SP2 consists of two varieties, Nagina-22 and Ptb-7 which have the common traits of drought and high temperature tolerance from previous studies (Beena et al., 2018c; Rejeth et al., 2020). In this study these two varieties showed salinity tolerance also. SP3 consists of varieties, Chomala, Ptb-35, Ptb-39, Vyttila-3, Vyttila -4, Vyttila-5, CR Dhan 307 and MO-16. In this group, except Chomala, all varieties are high yielding. SP4 consist of varieties- Ptb-60, Vyttila 10, Ptb-55, Ptb-30 and Vyttila-6. Among these, PTB60, PTB55 and PTB 30 are drought tolerant and Vyttila 10 and Vyttila 6 are salinity tolerant genotypes. SP5 consist of varieties Vyttila - 9, Vytilla-8, Apo and Vyttila-5. SP4

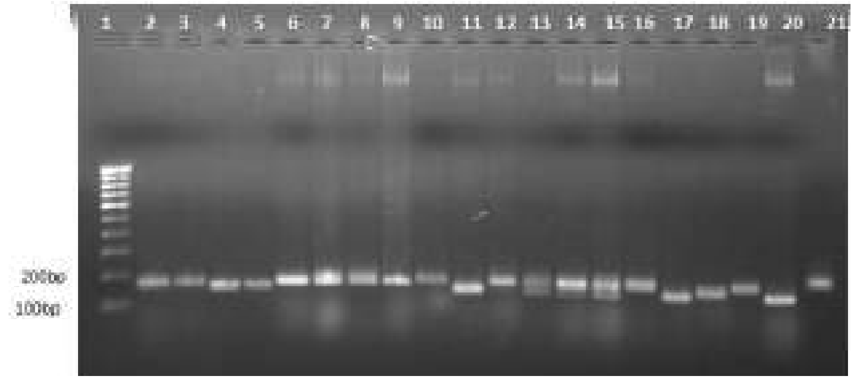

Plate 4. Polymorphism pattern of 20 rice genotypes using SSR marker RM1287.
Amplification pattern of 20 rice varieties obtained by SSR marker RM 1287 (Lane 1 - 100bp ladder, Lane 2 - chomala, Lane 3 - MO-16, Lane 4 - PTB 35, Lane 5 -PTB 60, Lane 6 - PTB 39, Lane 7 - PTB 55, Lane 8 - PTB 30, Lane 9 - PTB 7, Lane 10 - CR dhan 307, Lane 11 - Apo, Lane 12 - Vyttila -3, Lane 13 - Vyttila -4, Lane 14 - Vyttila -5, Lane 15 - Vyttila -6, Lane 16 - Vyttila -7, Lane 17 - Vyttila -8, Lane 18 - Vyttila -9, Lane 19 - N-22, Lane 20 - NL -44, Lane 21 - Vyttila -10)

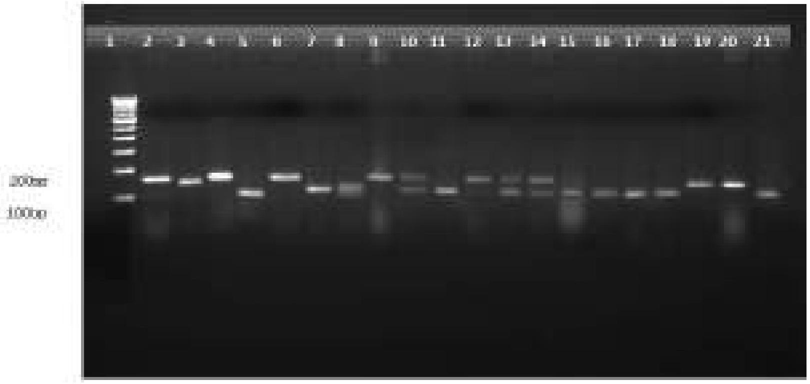

Plate 5. Polymorphism pattern of 20 rice genotypes using SSR marker RM5749.
Amplification pattern of 20 rice varieties obtained by SSR marker RM 5749 (Lane 1 - 100bp ladder, Lane 2 - chomala, Lane 3 - MO-18, Lane 4 - PTB 35, Lane 5 -PTB 60, Lane 6 - PTB 39, Lane 7 - PTB 55, Lane 8 - PTB 30, Lane 9 - PTB 7, Lane 10 - CR dhan 307, Lane 11 - Apo, Lane 12 - Vyttila -3, Lane 13 - Vyttila -4, Lane 14 - Vyttila -5, Lane 15 - Vyttila -6, Lane 16 - Vyttila -7, Lane 17 - Vyttila -8, Lane 18 - Vyttila -9, Lane 19 - N-22, Lane 20 - NL -44, Lane 21 - Vyttila -10)

and SP5 characterized by drought tolerant (Ptb-60, Ptb-55, Ptb-30, Apo) and saline tolerant varieties (Vyttila-5, 6, 8, 9, 10). This study also reveals the similar tolerance pattern or cross talk between salinity and drought. That may be the reason why saline and drought tolerant genotypes grouped in the same sub-population. Molecular and genomic studies have shown that several genes with various functions are induced by salinity, drought, and cold stresses, and that various transcription factors are involved in the regulation of stress-inducible genes. There are multiple stress perception and signaling pathways, some of which are specific, but others may crosstalk at various steps. Recently, progress has been made in identifying components of signaling pathways involved in salt, drought, and cold stresses (Mishra et al., 2016).

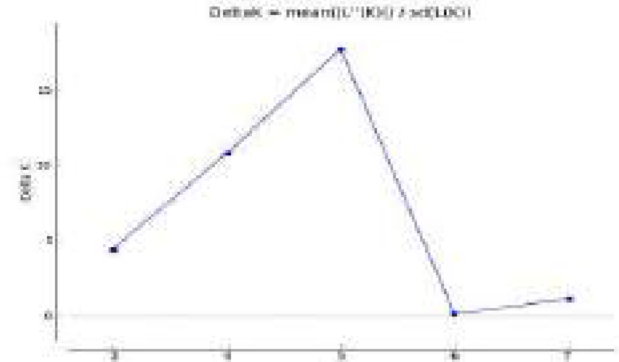

Plate 6. Estimates of subpopulations using delta K-values.

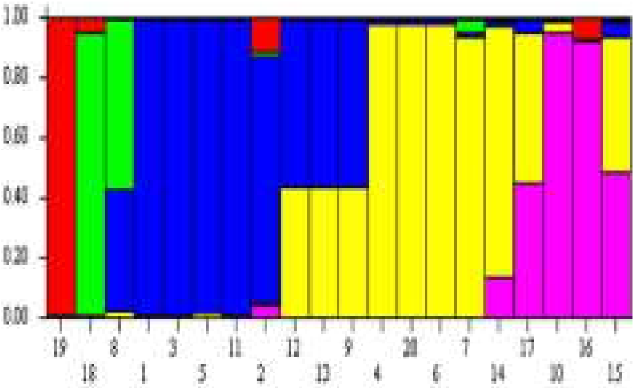

Plate 7. The summary plot of Q matrix estimates.

CONCLUSION

Under field conditions drought and heat stress occur in combination. Simultaneous occurrence of multiple stresses, increase the deleterious effect, such that the effect considerably exceeds the simple additive effects of the action alone. It has been suggested that variability in temperature extremes and water deficit events will be more critical in future climates. The present study based on phenotypic and genotypic analysis, the variety Nagina-22 was chosen the best tolerant variety for the combined stress salinity and temperature. PTB-7 was selected as the most tolerant variety for combined drought and salinity condition. Among the markers, RM6100, RM5749 and RM26212 were validated as markers linked to high temperature tolerance. RM8094 and RM1287 were validated as markers linked to salinity tolerance in rice. These markers can differentiate the tolerant genotypes from susceptible ones. Population structure analysis revealed the genetic relatedness and grouped these varieties into five subgroups. Natural stress tolerance is a very complex process involving numerous metabolites and metabolic pathways. Analyses of metabolic adjustments of plants with different levels of stress tolerance is very important.

Conflict of interest

All authors don't have any conflict of interest regarding this article.

REFERENCES

AbuQamar S, Luo H, Laluk K, Mickelbart MV, Mengiste T (2009). Crosstalk between biotic and abiotic stress responses in tomato is mediated by the AIM1 transcription factor. The Plant J. 58(2): 347-60

Ali J, Xu JL, Gao YM, Ma XF, Meng LJ, Wang Y, Pang YL, Guan YS, Xu MR, Revilleza JE, Franje NJ (2017). Harnessing the hidden genetic diversity for improving multiple abiotic stress tolerance in rice (*Oryza sativa* L.). PLoS One 12(3): p.e0172515

Almeida DM, Almadanim MC, Lourenço T, Abreu IA, Saibo NJ, Oliveira MM (2016). Screening for abiotic stress tolerance in rice: salt, cold, and drought. In: Environmental Responses in Plants. Humana Press, New York, NY pp. 155-182

Anjum SA, Wang LC, Farooq M, Hussain M, Xue LL, Zou CM (2011). Brassinolide application improves the drought tolerance in maize through modulation of enzymatic antioxidants and leaf gas exchange. Journal of Agronomy and Crop Science 197(3): 177-185

Atkinson NJ, Lilley CJ, Urwin PE (2013) Identification of genes involved in the response of Arabidopsis to simultaneous biotic and abiotic stresses. Plant Physiol.162: 2028-2041

Babu K, Rosaiah G (2017). A study on germination and seedling growth of Blcakgram (*Vigna mungo* L.) germplasm against Polyethylene glycol 6000 stress. IOSR J. Pharm. Bio. Sci. 5: 90-98

Bates LS, Waldren RP, Teare ID (1973). Rapid determination of free proline for water-stress studies. Plant soil. 39(1): 205-207

Battisti DS and Naylor RL (2009). Historical warnings of future food insecurity with unprecedented seasonal heat. Science 323: 240-244

Beena R (2005). Studies on physio- morphological traits and genetic markers associated with drought responses in rice (*Oryza sativa* L.). Ph.D. thesis submitted to Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Beena R (2012). An overview on QTLs linked to physio-morphological traits under water limited condition in rice (*Oryza sativa* L.). In: Advances in Plant Physiology, An International Treatise Series, Ed. Hemantaranjan A, Scientific Publishers 13: 233-249

Beena R (2013). Research paradigm and inference of studies on high temperature stress in rice (*Oryza sativa* L.). In: Advances in Plant Physiology, An International Treatise Series, Ed. Hemantaranjan A, Scientific Publishers 14: 497-511

Beena R, Thandapani V and Chandrababu R (2012). Physio-morphological and biochemical characterization of selected recombinant inbred lines of rice for drought resistance. Indian Journal of Plant Physiology. 17(2): 189-193

Beena R, Praveenkumar VP, Vighneswaran V, Narayankutty MC (2018c). Bulked line analysis: A useful tool to identify microsatellite markers linked to drought tolerance in rice. Indian Journal of Plant Physiology 23(1): 7-15

Beena R, Vighneswaran V, Sindumole P, Narayankutty MC, Voleti SR (2018a). Impact of high temperature stress during reproductive and grain filling stage in rice. Oryza-An International Journal on Rice 55(1): 126-133

Bharathkumar S, Pragnya PJ, Jitendra K, Archana B, Singh ON, Reddy JN (2014). Identification of rice germplasms associated with microsatellite (SSR) markers for heat tolerance at reproductive stage and expression of heat stress related gene. Indian Res. J. Genet. Biotech. 6: 424-27

Blum A and Ebercon A (1981). Cell membrane stability as a measure of drought and heat tolerance in wheat. Crop Sci. 21(1): 43-47

Bohra JS and Doerffling K (1993). Potassium nutrition of rice (*Oryza sativa* L.) varieties under NaCl salinity. Plant Soil. 152(2): 299-303

Buu BC, Ha PTT, Tam BP, Nhien T T, VanHieu N, Phuoc NT, Giang LH, Lang NT (2014). Quantitative trait loci associated with heat tolerance in rice (*Oryza sativa*

L.). Plant Breed. Biotechnol. 2(1): 14-24

Chinnuswamy V, Zhu J, Zhu JK (2007). Cold stress regulation of gene expression in plants. Trends Plant Sci. 12(10): 444-451 doi: 10.1016/j.tplants.2007.07.002

De Datta, SK, Mikkelse DS (1985) Potassium nutrition of rice. Potassium in agriculture pp. 665-699

Dellaporta SL, Wood J, Hicks JB (1983). A rapid method for DNA extraction from plant tissue. Plant Mol. Biol. Rep. 1: 19–21

FAO Rice Market Monitor (2017). vol.XX(1), Rome, Itali

Gorham JRG, Wyn Jones, Bristol A (1990). Partial characterization of the trait for enhanced K^+ - Na^+ discrimination in the D genome of wheat. Planta. 180: 590-597

Hoang T, Tran T, Nguyen T, Williams B, Wurm P, Bellairs, S, Mundree S (2016). Improvement of Salinity Stress Tolerance in Rice: Challenges and Opportunities. Agronomy 6(4): 54 doi:10.3390/agronomy6040054

Hsu SY, Hsu YT, Kao CH (2003). The effect of polyethylene glycol on proline accumulation in rice leaves. Biologia Plantarum 46(1): 73-78

Hauser F, Waadt R, Schroeder JI (2011). Evolution of abscisic acid synthesis and signaling mechanisms. Curr. Biol. 21(9): 346-355 doi: 10.1016/j.cub.2011.03.015

Iloh AC, Omatta G, Ogbadu GH, Onyenekwe PC (2014). Effects of elevated temperature on seed germination and seedling growth on three cereal crops in Nigeria. Scientific Research and Essays 9(18): 806-813

Islam MM, Kayesh E, Zaman E, Urmi TA, Haque MM (2018). Evaluation of rice (*Oryza sativa* L.) genotypes for drought tolerance at germination and early seedling stage. The Agriculturists 16(1): 44-54

Jagadish SVK, Murty MVR, Quick WP (2015). Rice responses to rising temperatures- challeges, perspectives and future directions. Plant, Cell and Environments. 38: 1686-1698

Jena KK, Mackill DJ (2008). Molecular markers and their use in marker-assisted selection in rice. Crop Sci 48(4): 1266-76

Khush GS (2009). What it will take to feed 5.0 billion rice consumers in 2030. Plant Mol. Biol. 59(1): 1-6

Khraiwesh B, Zhu JK, Zhu J (2011). Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta. 1819(2): 137-148 doi: 10.1016/j.bbagrm.2011.05.001

Krasensky J and Jonak C (2012). Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. Journal of Expt Bot 63(4): 1593-1608

Krishnan P, Ramakrishnan B, Reddy KR and Reddy VR (2011). High-temperature effects on rice growth, yield, and grain quality. Adv Agron.111: 87-206

Kuroki MK, Saito S, Matsuba N, Yokogami H, Shimizu I, Sato Y (2007). A quantitative trait locus for cold tolerance at the booting stage on rice chromosome 8. Theor. Appl. Genet. 115: 593-600

Lang NT (2002). Protocol for basics of biotechnology. Ho Chi Minh City (Vietnam): Agricultural Publishing House.

Marris E (2008). Water: more crop per drop. Nature 453: 273-277

Mishra S, Kumar S, Saha B, Awasthi J, Dey M, Panda SK, Sahoo L (2016). Crosstalk between Salt, Drought, and Cold Stress in Plants. Abiotic Stress Response in Plants, Edited by Narendra Tuteja, N. and Sarvajeet S. Gill,S.S. -VCH Verlag GmbH & Co. KGaA.1-803. pp 56-84

Mittler R (2006). Abiotic stress, the field environment and stress combination. Trends Plant Sci 11(1): 15-19

Mokhberdoran F, Nabavi SM, Sadrabadi K (2009). Effect of temperature, isoelectric concentrations of NaCl and PEG agents on germination and some seedling growth yield components in rice (*Oryza sativa* L.). Asian J. Plant Sci. 8(6): 409-416

Munns R and Tester M (2008). Mechanisms of salinity tolerance. Ann. Review of Plant Biol.59: 651-681

Munns R, Wallace PA, Teakle NL, Colmer TD (2010). Measuring soluble ion concentrations (Na^+, K^+, Cl^-) in salt-treated plants. J. Plant Stress Tolerance pp. 371-382

Naresh BP, Abida PS, Beena R, Valsala PA, Nazeem PA (2018). Morpho-physiological and proteomic analysis to identify and characterize the traditional rice genotypes for drought tolerance. Indian Journal of Plant Physiology 23: 785-795 doi: https://doi.org/10.1007/s40502-018-0405-5

Nejad G, Arzani A, Rezai AM, Singh RK, Gregorio GB (2008). Assessment of rice genotypes for salt tolerance using microsatellite markers associated with the saltol QTL. Afr J Biotechnol 7: 730-736

Panda D, Mishra SS, Mohanty SK, Behera PK, Lenka SK (2019). Data on genetic potentiality of folk rice

(*Oryza sativa* L.) genotypes from Koraput, India in reference to drought tolerance traits. Data in brief 25: 104363

Pastori GM and Foyer CH (2002). Common components networks and pathways of cross-tolerance to stress the central role of "redox" and abscisic acid-mediated. Plant Physiology 129: 460-468 doi:10.1104/pp.011021

Pennisi E (2008). The blue revolution, drop by drop, gene by gene. Sci. 320(5873): 171-173

Prasad PVV, Boote KJ, Allen Jr LH, Sheehy JE, Thomas JMG (2006). Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crops Res.95(2-3): 398-411

Prince KSJ, Beena R, Michael Gomez S, Senthivel S, Chandra Babu R (2015). Mapping consistent yield QTLs under drought stress in target rainfed environments. Rice 8:25

Pritchard JK, Stephens M, Rosenberg NA, Donnelly P (2000). Association mapping in structured populations. Am. J. Human Genet. 67(1): 170–181

Rachoski M, Gazquez A, Calzadilla P, Bezus R, Rodriguez A, Ruiz O, Menendez A, Maiale S (2015). Chlorophyll fluorescence and lipid peroxidation changes in rice somaclonal lines subjected to salt stress. Acta Physiol Plant 37: 117

Rejeth R, Manikanta CL, Beena R, Stephen R, Manju RV, Viji MM (2020). Water stress mediated root trait dynamics and identification of microsatellite markers associated with root traits in rice (*Oryza sativa* L.). Physiol Mol Biol Plants https://doi.org/10.1007/s12298-020-00809-y

Roychoudhury A, Paul S and Basu S (2013). Cross-talk between abscisic acid-dependent and abscisic acid-independent pathways during abiotic stress. Plant Cell Rep 32: 985-1006

Salunkhe AS, Poornima R, Prince KS, Kanagara JP, Sheeba JA, Amudha K (2011). Fine mapping QTL for drought resistance traits in rice (*Oryza sativa* L.) using bulk segregant analysis. Mol Biotechnol 49: 90-95

Sheshshayee MS, Shashidhar G Parsi, Madhura JN, Beena R, Prasad TG and Udayakumar M (2011). Drought phenotyping in crops: from theory to practice. (Eds. Philippe Monneveux and Jean-Marcel Ribaut). CGIAR Generation Challenge Programme/ CIMMYT

Singh H, Deshmukh RK, Singh A, Singh AK, Gaikwad K, Sharma TR, Mohapatra T and Singh NK (2010). Highly variable SSR markers suitable for rice genotyping using agarose gels. Mol. breed. 25(2): 359-364

Smertenko A, Draber P, Vicklicky V, Opatrny Z (1997). Heat stress affects organization of microtubules and cell division in Nicotyana tabacum cells. Plant Cell Envt. 20: 1534-1542

Vivitha P, Raveendran M, Vijayalakshmi D (2017). Introgression of QTLs controlling spikelet fertility maintains membrane integrity and grain yield in improved white ponni derived progenies exposed to heat stress. Rice Sci. 24(1): 32-40

Vu HTT, Le DD, Ismail AM, Le HH (2012). Marker-assisted backcrossing (MABC) for improved salinity tolerance in rice (*Oryza sativa* L.) to cope with climate change in Vietnam. Aust. J. Crop Sci 6(12): 1649

Yamaguchi-Shinozaki K, Shinozaki K (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu. Rev .Plant Bio. 57: 78

Yue B, Xue WY, Xiong LZ, Yu XQ, Luo LJ, Cui KH, Jin DM, Xing YZ, Zhang QF (2006). Genetic basis of drought resistance at reproductive stage in rice: Separation of drought tolerance from drought avoidance. Genet. 172: 1213-1228

Zasoski RJ and Burau RG (1977). A rapid nitric- perchloric acid digestion method for multielement tissue analysis. Commun. Soil Sci Plant Anal. 8(5): 425-436

Zhang S, Hu J, Zhang Y, Xie XJ, Knapp A (2007) Seed priming with brassinolide improves lucerne (*Medicago sativa* L.) seed germination and seedling growth in relation to physiological changes under salinity stress. Aust. J. Agri. Res. 58(8): 811-815

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Screening of rice genotypes for tolerance to soil acidity and related nutritional constraints

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ABSTRACT

A field experiment was conducted in the experimental farm of Zonal Agricultural Research Station, Dumka, Birsa Agricultural University, Ranchi, Jharkhand during kharif 2018 to evaluate location specific genotypes for tolerance to soil acidity and related nutrient constraints in rainfed medium low lands. The experimental findings have been interpreted in terms of grain yield, crop response (%) to lime, lime use efficiency (LUE), P and K-uptake by the crop. The experiment was laid out in split plot design in which fertilizer levels (two) [NPK (RD) i.e., @ (100:50:25) kg/ha and NPK (RD) + lime @3q/ha] were taken as main plots and rice genotypes (fourteen) as sub-plots. It was found that the grain yield and nutrient uptakes (P & K) by the crop were significantly influenced by lime (irrespective of vars.) and genotype differences (irrespective of lime levels). Significantly higher grain yield (55.20 q/ha), P-uptake (17.21kg/ha) and K-uptake (19.13kg/ha) were recorded with (NPK + Lime) (irrespective of vars.). The highest crop response to lime (26.9%) and lime use efficiency (4.74 kg grain /kg lime) were recorded with JKRH3333 and BINADHAN-75, respectively, whereas the maximum increase in P-uptake (19.1%) and K-uptake (33.0%) were obtained with BINADHAN-8 and PUP-223, respectively.

Key words: *Rice genotypes, crop response, lime use efficiency, P- uptake and K- uptake*

INTRODUCTION

Acid soils occupy approximately 30% of the total land in the world measuring about 3,950 million hectares and over 50 per cent of total arable soils (Kochian et al., 2004). These soils are distributed in America (40.9%), Asia (26.4%), Africa (16.7%), Europe (9.9%), Australia and New Zealand (6.1%) (von Uexkiill and Mutert, 1995). In India highly acidic soils (pH<4.5) covers 1.9 per cent of total geographical area (TGA). However, moderately acid soils (pH 4.5 to 5.5) cover an area of 24,414.6 thousand hectares which amounts 7.4 per cent TGA of the country. The extent of strongly acidic soils is largest in Arunachal Pradesh followed by Manipur, Sikkim, Tamil Nadu, Kerala, Chhattisgarh, Nagaland, Tripura and Assam. Moderately acidic soils cover highest area in Chhattisgarh (5930.1 thousand hectares) followed by Kerala and Assam. As far as Jharkhand is concerned, the extent of strongly to slightly acidic soil is 84.9 per cent (6771.7 thousand hectares) of TGA (Maji et al., 2012). Lower soil pH adversely affects the growth of many plants. Apart from low pH, toxic effect of some metal ions such as aluminum (Al3+) and iron (Fe^{3+}) also reduced the growth and development of plants (Vijarnsorn and Eswaran, 2002). It was reported that Al^{3+} reduce the uptake of water and nutrients remarkably by inhibiting root elongation (Ma et al., 2005). Recently, it was discovered that Al^{3+} induced secretion of organic acids from the root tips would alleviate Al toxicity by forming a chelate with Al and organic acids such as citrate, malate and succinate (Yang et al., 2006). Along with Al^{3+} toxicity, high concentration of ferric (Fe^{3+}) and ferrous (Fe^{2+}) also cause the inhibition of crop growth, however the mechanism of reduction of crop growth due to Fe toxicity has not yet been clear. Regarding manganese ion (Mn^{2+}), Izaguirre-Mayoral and Sinclair (2005) found a negative correlation between Mn and Fe content at cellular level. It could be deduced from their findings that Mn toxicity is not expressed clearly because Fe concentration is usually higher than Mn in many cases. Due to toxic effect of these metal ions crop cultivation is difficult without improvement of soil pH. Liming and application of rock phosphate are among the various means of improvement of soil pH. The effect of liming could not last for more than three years; hence farmers suffer from high cost of liming in rice cultivation. Moreover, liming occasionally causes the pollution of underground water and/or salt accumulation in the soil. Hence present study was carried out to identify the acid tolerant rice genotypes suitable for Dumka region of Jharkhand.

MATERIALS AND METHODS

A field experiment was conducted at Zonal Research Station, Dumka during *kharif* 2018 in medium lowland. The soil was acidic having pH 5.22, organic carbon 0.59%, available nitrogen 325.0 kg/ha, available phosphorus 21.7 kg/ha and available potassium 185.0 kg/ha. The soil belonged to the textural class sandy clay loam. The experiment was laid out in Split-plot design in which fertilizer levels (two) [NPK (RD) *i.e.*, @ (100:50:25) kg/ha and NPK (RD) + lime @3q/ha] were taken as main plots and rice genotypes (fourteen) as sub-plots. The seeds were sown on 4th July, 2018 and transplanting was done on 25th July 2018. Lime was applied at the time of final puddling. Of the recommended fertilizer dose, 50% of N, 100% of P and K were applied at the time of transplanting; the rest 50% N was applied in two splits-25% at 21 DAT and 25% at 42 DAT. The pH of soil was measured in 1:2.5 (Soil: Water) suspension with the help of glass electrode digital pH meter as described by Jackson (1973). Organic C was estimated by chromic acid wet digestion method given by Walkley and Black (1934). Available soil N was estimated by alkaline potassium permanganate method (Subbiah and Asija, 1956). Available soil P was determined by Bray P1 method (Bray and Kurtz, 1945) as described by Jackson (1973) using double beam spectrophotometer. Available potassium in soil was determined by flame photometer following neutral normal ammonium acetate method (Hanway and Heidal, 1952). Total P and K in grain were estimated by digestion with diacid mixture (HNO_3:$HClO_4$:: 4:1). Ammonium metavanadate method was followed to determine P-content in plant extract (Gericke and Kurmies, 1952). The crop was harvested and sun dried, then total produce was weighed and recorded as total biomass. The produce was then threshed and grains were separated, dried, and weighed. Grain yield was adjusted to a moisture concentration of 0.14 g H_2O g^{-1} fresh weight (Raja et al., 2014; Munda et al., 2016). Lime use efficiency (LUE), Phosphorus utilization efficiency (PUtE) and Potassium utilization efficiency (KUtE) were calculated using following equation -

LUE (kg grain per kg lime) = (Var. mean of grain yield (q per ha) x 100)/(Lime applied)

PUtE (kg grain per g P absorbed) = (Var.mean of grain yield (q per ha) x 100)/(Var.mean of P uptake (kg per ha) x 1000)

KUtE (kg grain per g K absorbed) = (Var.mean of grain yield (q per ha) x 100)/(Var.mean of K uptake (kg per ha) x 1000)

RESULTS AND DISCUSSION

Grain yield

It was found that grain yield of rice was significantly influenced by application of lime irrespective of varieties tested (Table 1). Significantly higher grain yield (55.20q/ha) was obtained with (NPK + Lime) over control. There were also significant variations in grain yield among different rice genotypes (irrespective of lime levels). The highest grain yield (67.67q/ha) was recorded with US-312, which was at par with BINADHAN-75(64.33 q/ha). The positive impact of lime on yield of rice varieties grown in acidic soil can be explained in terms of better Ca-nutrition of the crops as contributed by lime. The Rice vars. *viz*., US-312 (67.67 q/ha), BINADHAN-75 (64.33 q/ha), KRH-4 (59.42 q/ha), BINADHAN-17 (59.24 q/ha). PUP-221(58.55 q/ha) and PUP-223(56.28 q/ha) showed significant genotypic superiority over the local check MTU 7029 (47.20 q/ha) towards tolerance to soil acidity. The findings are in accordance with Saha and Jha (2006) and Saha et al. (2020).

Table 1. Grain yield of rice genotypes (q/ha) in acidic soil as influenced by lime.

Rice genotypes	Lime levels		Mean (vars.)
	Unlimed	Limed	
MTP-3	40.83	45.90	43.37
MTP-7	43.63	52.87	48.25
HRI -174	45.18	47.35	46.27
JKRH-3333	46.37	58.83	52.60
KRH-4	54.57	64.26	59.42
BINADHAN-8	41.42	47.49	44.46
BINADHAN-17	56.87	61.61	59.24
BINADHAN-75	57.22	71.43	64.33
TI-93	34.30	38.13	36.22
US-312	63.11	72.23	67.67
PUP-217	35.27	40.87	38.07
PUP-221	55.69	61.40	58.55
PUP-223	51.80	60.75	56.28
MTU 7029	44.73	49.67	47.20
Mean(Lime)	**47.93**	**55.20**	
CD at 5%	Lime	Var.	Lime X Var.
	4.61	**6.15**	**NS**

Table 3. K-uptake by rice grain (kg/ha) in acidic soil as influenced by lime.

Rice vars.	Lime levels		Mean(vars.)
	Unlimed	Limed	
MTP-3	14.79	17.56	16.18
MTP-7	14.95	18.29	16.62
HRI -174	14.46	16.11	15.29
JKRH-3333	15.82	20.40	18.11
KRH-4	19.01	21.19	20.10
BINADHAN-8	13.38	15.80	14.59
BINADHAN-17	19.86	22.20	21.03
BINADHAN-75	20.21	25.66	22.93
TI-93	13.79	15.82	14.81
US-312	20.73	23.05	21.89
PUP-217	11.38	13.06	12.22
PUP-221	19.38	22.51	20.94
PUP-223	15.42	20.50	17.96
MTU 7029	13.40	15.69	14.54
Mean(Lime)	**16.18**	**19.13**	
CD at 5%	Lime	Var.	Lime X Var.
	1.60	**2.19**	**NS**

Phosphorus and potassium uptake, Crop response to lime and lime use efficiency

P-uptake and K-uptake by rice grains in acidic soils were significantly influenced by lime and genotypes, while considering their individual effects separately (Table 2 & Table 3). Significantly higher P-uptake (17.21 kg/ha) was obtained with (NPK + Lime). Ca^{2+} and Mg^{2+} ions produced from liming materials might have released the $H_2PO_4^-$ ions adsorbed by Fe and Al-oxides through outer sphere complex, thereby increasing P-availability in the root zone. Significant variations in P-uptake were also observed among different rice genotypes taken in the experiment. The highest P-uptake (22.60 kg/ha) was recorded with US-312, which was at par with BINADHAN-75 (20.86 kg/ha) (Fig. 1). The rice vars. *viz*., US-312, BINADHAN-75, BINADHAN-17, KRH-4, PUP-221 and PUP-223 showed significant genotypic superiority over the local check MTU 7029 with respect to P-uptake. Addition of lime to NPK-fertilizers brought about significantly higher K-uptake (19.13 kg/ha) by rice grains. Liming of sandy clay loam soil, mostly dominated by low activity clays (*i.e*., 1:1 type) and also characterized by mixed mineralogical make-up, as in the case of given experimental site, results in increase in CEC because of their pH-dependent charge. This leads to an increase in quantity/intensity (Q/I) ratio. This type of soil is also characterized by low Q/I value. Thus, in such soils, a small increase in exchangeable K (Q-factor) produces a large increase in soil solution K^+ (I-factor), thereby leading to greater K-uptake by plant roots. The findings are in accordance with Imtiaz et al. (2010), Siddika et al. (2016) and Sharma et al. (2019). Rice genotypes, used as the test crops, showed significant variations in

Table 2. P-uptake by rice grain (kg/ha) in acidic soil as influenced by lime.

Rice genotypes	Lime Levels		Mean(vars.)
	Unlimed	Limed	
MTP-3	11.53	12.80	12.17
MTP-7	12.31	14.12	13.22
HRI -174	12.20	12.81	12.51
JKRH-3333	14.44	16.31	15.37
KRH-4	18.47	20.56	19.51
BINADHAN-8	12.96	15.43	14.19
BINADHAN-17	19.29	20.35	19.82
BINADHAN-75	19.63	22.08	20.86
TI-93	11.40	12.37	11.89
US-312	20.73	24.46	22.60
PUP-217	12.09	14.28	13.19
PUP-221	17.72	20.28	19.00
PUP-223	17.48	19.89	18.68
MTU 7029	12.96	15.19	14.07
Mean(Lime)	**15.23**	**17.21**	
CD at 5%	Lime	Var.	Lime X Var.
	1.29	**1.93**	**NS**

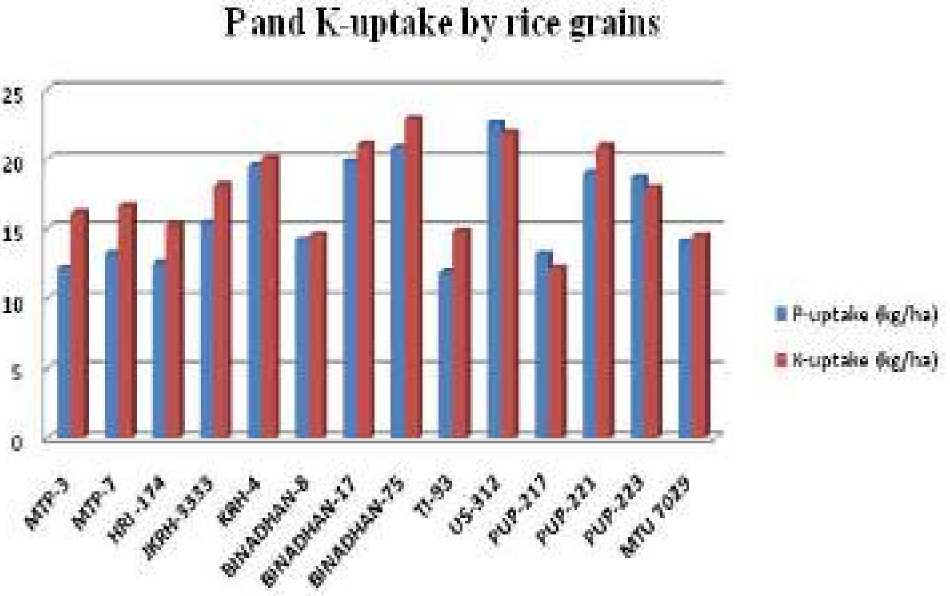

Fig. 1. P and K-uptake by rice grains.

K-uptake also. The highest K-uptake (22.93 kg/ha) was recorded with BINADHAN-75, which was at par with US-312 (21.89 kg/ha), BINADHAN-17 (21.03 kg/ha) and PUP-221 (20.94 kg/ha). The Maximum crop response to lime (26.9%) was recorded with JKRH-3333 whereas the highest lime use efficiency (4.74 kg grain/kg lime) was obtained with BINADHAN-75 (Table 4). The highest increase in P-uptake (19.1%) and K-uptake (33.0%) was recorded with BINADHAN-8 and PUP-223, respectively.

Phosphorus and potassium utilization efficiency

Because of the low mobility of P in the soil, soil exploration by roots is one major strategy for plant's P -acquisition (Lynch, 2011). The P- depletion zones around the roots reach usually not farther than a few millimeters and are even smaller in low P soil (Jungk and Claassen, 1989; Li et al., 1991). Phosphorus

Table 5. Nutrient (P & K) uptake and utilization efficiency of different rice genotypes.

Rice genotype	P-uptake (kg/ha)	P-utilization efficiency (PUtE) (kg/ha)	K-uptake (kg/g)	K-Utilization Efficiency (KUtE)(kg/g)
MTP-3	12.17	0.36	16.18	0.27
MTP-7	13.22	0.36	16.62	0.29
HRI -174	12.51	0.37	15.29	0.30
JKRH-3333	15.37	0.34	18.11	0.29
KRH-4	19.51	0.30	20.10	0.30
BINADHAN-8	14.19	0.31	14.59	0.30
BINADHAN-17	19.82	0.30	21.03	0.28
BINADHAN-75	20.86	0.31	22.93	0.28
TI-93	11.89	0.30	14.81	0.24
US-312	22.60	0.30	21.89	0.31
PUP-217	13.19	0.29	12.22	0.31
PUP-221	19.00	0.31	20.94	0.28
PUP-223	18.68	0.30	17.96	0.31
MTU 7029	14.07	0.34	14.54	0.32

Table 4. Crop response to lime and increase (%) in nutrient (P & K) uptake by different rice genotypes due to lime.

Variety	Response to lime (%)	Lime use efficiency (LUE) (kg grain/ kg lime)	Increase(%) in nutrient uptake due to lime	
			P	K
MTP-3	12.4	1.69	11.1	18.8
MTP-7	21.2	3.08	14.7	22.3
HRI -174	4.8	0.72	5.0	11.4
JKRH-3333	26.9	4.16	12.9	29.0
KRH-4	17.8	3.23	11.3	11.4
BINADHAN-8	14.7	2.02	19.1	18.1
BINADHAN-17	8.3	1.58	5.5	11.8
BINADHAN-75	24.8	4.74	12.5	26.9
TI-93	11.2	1.28	8.5	14.7
US-312	14.5	3.04	18.0	11.2
PUP-217	15.9	1.87	18.1	14.8
PUP-221	10.2	1.90	14.5	16.2
PUP-223	17.3	2.98	13.8	33.0
MTU 7029	11.0	1.65	17.3	17.0

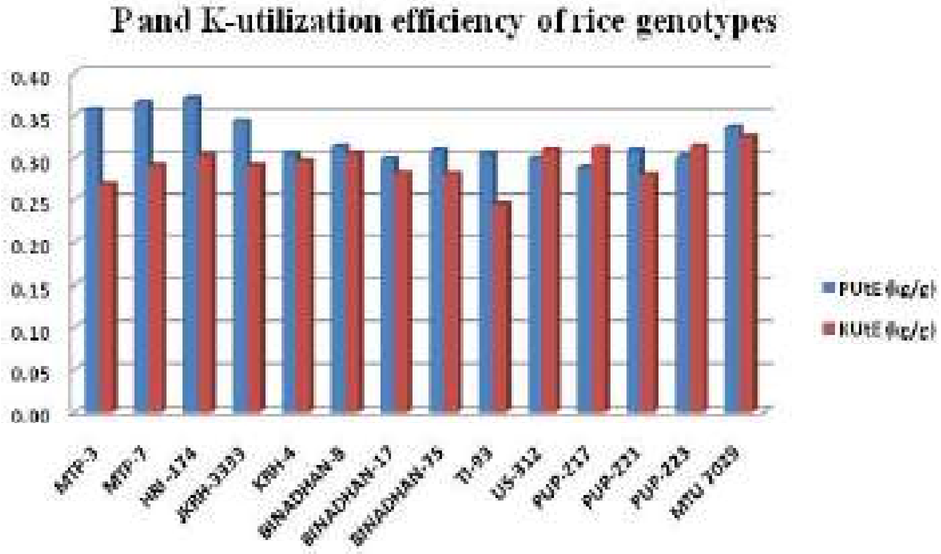

FIg. 2. P and K-Utilization Efficiency of Rice Genotypes.

utilization efficiency (PUtE) is the dry mass produced per unit P uptake (Moll et al., 1982). Sufficient genetic variability occurs among numerous crop species for better P-acquisition and utilization under P- stressed environment (Aziz et al., 2005). Phosphorus uptake efficiency (PUE) and utilization efficiency (PUtE) are two distinct characteristics of plants; the first represents the plant's capacity to take P from soil and the later explains how efficiently the plants have utilized the absorbed P to produce biomass. Genotypic variations in P and K-utilization efficiency (PUtE & KUtE) of Rice are shown in Table 5. The highest P-uptake (22.60 kg/ha) was recorded with US-312 whereas the highest PUtE (0.37 kg/g) was recorded with HRI-174. The K-efficient phenotype is a complex one comprising a mixture of uptake and utilization efficiency mechanisms. Differential exudation of organic compounds to facilitate release of non-exchangeable K is one of the mechanisms of differential K-uptake efficiency. Genotypes efficient in K-uptake may have a larger surface area of contact between roots and soil and an increased K-uptake at the root-soil interface to maintain a larger diffusive gradient towards roots. Better translocation of K into different organs, greater capacity to maintain cytosolic K^+ concentration within optimal ranges and increased capacity to substitute Na^+ for K^+ are the main mechanisms underlying K-utilization efficiency (Rengel and Daman, 2008). The highest K-uptake (22.93 kg/ha) was recorded with BINADHAN-75 whereas the highest KUtE (0.32 kg/g) was recorded with MTU 7029 (Fig. 2).

CONCLUSION

On the basis of the above experiment, it can be said that plant's uptake efficiency is important when a given genotype is exposed to several micro-environments because the former can be influenced by agronomic manipulations; on the other hand, nutrient utilization efficiency becomes the dominant factor when different genotypes of a given species are exposed to a particular micro-environment. Although plants often face nutritional constraints in their environment, they utilize a plethora of sophisticated mechanisms in an attempt to acquire sufficient amounts of the macro- and micronutrients required for proper growth, development and reproduction. In the present study highest crop response to lime (26.9%) was recorded with JKRH3333 and highest lime use efficiency (4.74 kg grain /kg lime) was recorded with BINDHAN-75, whereas BINDHAN-8 recorded maximum increase in P-uptake (19.1%).

REFERENCES

Aziz PT, Maqsood M, Mansoor T (2005). Differences in phosphorus absorption, transport and utilization by twenty rice (*Oryza sativa* L.) cultivars. Pakistan Journal of Agricultural Science 42: 8-15

Bray RH and Kurtz LT (1945). Determination of total, organic and available forms of phosphorus in soils. Soil

Science 59: 39-45

Gericke S and Kurmies B (1952). The colorimetric determination of phosphoric acid with ammonium vanadate, molybdate and their application in plant analysis. Zeitschrift für Pflanzenernährung, Düngung und Bodenkunde 59: 235-247

Hanway JJ and Heidel H (1952). Soil analysis methods as used in Iowa State College Soil Testing Laboratory. Iowa Agriculture 57: 1-31

Imtiaz M, Rashid A, Khan P, Memon MY and Aslam M (2010). The role of micronutrients in crop production and human health. Pakistan Journal of Botany 42: 2565-2578

Izaguirre-Mayoral ML and Sinclair TR (2005). Soybean genotypic difference in growth, nutrient accumulation and ultrastructure in response to manganese and iron supply in solution culture. Annals of Botany 96: 149-158

Jackson ML (1973). Soil Chemical Analysis, Indian Edition. Prentice - Hall India Pvt. Ltd., New Delhi

Jungk A and Claassen N (1989). Availability in soil and acquisition by plants as the basis for potassium and phosphorus supply to plants. Zeitschrift für Pflanzenernährung und Bodenke 152: 151-158

Kochian LV, Hoekenga OA and Pineros MA (2004). How do crop plants tolerate acid soils? Mechanism of aluminum tolerance and phosphorus efficiency. Annual Review of Plant Biology 55: 459-493

Li XL, George E and Marschner H (1991). Extension of the phosphorus depletion zone in VA-mycorrhizal white clover in a calcareous soil. Plant and Soil 136: 41-48

Lynch JP (2011). Root phenes for enhanced soil exploration and phosphorus acquisition: tools for future crops. Plant Physiology 156: 1041-1049

Ma JF, Nagao S, Huang CF and Nishimura M (2005). Isolation and characterization of a rice mutant hypersensitive to Al. Plant Cell Physiology 46: 1054-1061

Maji AK, Obi Reddy GP and Sarkar D (2012). Acid Soils of India - Their Extent and Spatial Variability, NBSS Publication No. 145, NBSS&LUP, Nagpur pp. 138

Moll RH, Kamprath EJ, Jackson WA (1982). Analysis and interpretation of factors which contribute to efficiency of nitrogen utilization. Agronomy Journal of American Society of Agronomy74: 562

Munda S, Nayak AK, Mishra PN, Bhattacharyya P, Mohanty S, Kumar A, Kumar U, Baig MJ, Tripathi R, Shahid M and Adak T (2016). Combined application of rice husk biochar and fly ash improved the yield of lowland rice. Soil Research 54(4): 451-9

Raja R, Nayak AK, Rao KS, Puree C, Shahid M, Panda BB, Kumar A, Tripathi R, Bhattacharyya P, Baig MJ and Lal B (2014). Effect of fly ash deposition on photosynthesis, growth and yield of rice. Bulletin of Environmental Contamination and Toxicology 93(1): 106-12

Rengel Z and Damon PM (2008). Crops and genotypes differ in efficiency of potassium uptake and use. Physiologia Plantarum 133(4): 624-636

Saha PB and Jha AK (2006). Participatory INM Technology Development at Dumka, Jharkhand. International Journal of Tropical Agriculture 24(1-2): 239-244 http://doi.org/10.13140/RG.2.2.12849.81760

Saha PB, Jha AK, Aind W and Chatterjee K (2020). Management of secondary nutrients and micronutrients in rice under rice-wheat cropping system in acid soil. Oryza 57(3): 235-239 http://doi.org/10.35709/ory.2020.57.3.8

Sharma S, Padbhusan R and Kumar U (2019). Integrated nutrient management in rice -wheat cropping system: an evidence on sustainability in the Indian Subcontinent through meta-analysis. Agronomy 9: 71

Siddika MA, Milan JA, Hoque TS and Hanif A (2016). Effect of different micronutrients on growth and yield of rice. International Journal of Plant and Soil Science 12: 1-8

Subbiah BB and Asija GL (1956). A rapid procedure for the determination of available nitrogen in soils. Current Science 25: 259-260

Vijarnsorn P and Eswaran H (2002). The soil resources of Thailand. First Press Ltd. Bangkok, Thailand pp. 55-84

Von Uexkiill HR and Mutert E (1995). Global extent, development and economic impact of acid soils. Plant and Soil 171: 1-15

Walkley A and Black CA (1934). An examination of degitjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. Soil Science 37: 29-37

Yang JL, Zhang L, Li YY, You JF, Wu P and Zheng SJ (2006). Citrate transports play a critical role in aluminum stimulated citrate efflux in rice bean (*Vigna umbellate*) roots. Annals of Botany 97: 579-584

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Effect of nutrient management strategies on productivity in rice-pigeon pea intercropping under drought prone rainfed ecology of Eastern India

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ABSTRACT

An experiment was undertaken to compare the productivity of rice- sole with rice-pigeon pea intercropping under different nutrient management (inorganic, organic and integrated nutrient management) strategies in drought prone rainfed (upland) ecology. The experimental data showed that, rice equivalent yield (REY) (2.83 and 3.22 t ha^{-1} under rice sole and rice -pigeon pea respectively) and yield attributing characters (grain panicle^{-1} (73) and test weight (24.3g) were higher under inorganic nutrient management followed by the integrated nutrient management (T_4) (2.25 t ha^{-1}, 2.64 t ha^{-1}, 64 and 22.7 g respectively). The yield gap between the inorganic and integrated nutrient management decreased over the three years, because, in the first year integrated nutrient management could not produce enough yield and difference was more which became narrow as this treatment produced comparatively more yield afterward. REY was more in rice-pigeon-pea system (varied from 1.13 to 3.22 t ha^{-1}) over the rice-sole (1.01 to 2.83 t ha^{-1}) across the nutrient management strategies. Thus it is evident that, rice- pigeon pea intercropping under integrated nutrient management in upland condition would be better choice in rainfed drought prone area to enhance farm income as well as system productivity.

Key words: *Crop productivity, intercropping, nutrient management, pigeon pea, rice*

INTRODUCTION

The rainfed drought prone upland areas of Eastern India is used mainly for short duration crops like upland rice, pulses, millets and oilseeds in *kharif* season. Crop cultivation in this upland ecology involves certain amount of risk as it solely depends on seasonal monsoon rainfall which is highly erratic in nature and may not support the second crop in sequence mainly due to moisture stress (Singh and Srivastava, 2018; Kumar et al., 2018). Due to vagaries of monsoon coupled with aberrant climate, rainfed agriculture is becoming highly risk prone particularly for resource poor farmers. Prevalent rice mono-cropping or rice-fallow system leads to deterioration of soil fertility and quality mainly due to nutrient imbalance as the crops are typically grown with improper nutrient management (Maiti et al., 2018), hence, almost all the upland soils are becoming low in fertility and consequently the productivity is also low (Maiti et al., 2018). Therefore, development and adoption of location specific intercropping or cropping system with proper nutrient management is essentially required for this fragile ecosystem.

In India about 20.4 million hectares area is under rainfed ecology, of which 16.2 million ha are present in Eastern India (Singh and Singh, 2000). Among the rice growing areas, upland rice contributes about 1/7th of total (upland rice accounts about 13 and 13.5% of total world and Indian rice growing areas respectively) where productivity enhancement is the major concern (Maiti et al., 2018). Rice is mainly grown in these areas for household need, so there is need to transform the system from subsistence to production (profit) oriented which should be sustainable too. Rice-fallow covers around 11.6 million ha in India and mainly spread in Bihar, Jharkhand, Chhattisgarh, etc. (Kumar et al., 2018). Mono cropping (rice-fallow) system must be replaced by selecting suitable intercropping or cropping system to enhance the system productivity. Selection of a crop in intercropping is very much important to get the maximum advantages by reducing the competition.

Pigeon pea is an important pulse crop in the *kharif* season for rainfed region, because of its drought tolerance and high protein content. Its initial slow growth and little spreading (horizontal) habit provide good scope for growing other short duration crops like rice, hence can be suitable choice for intercropping. It ensures some or even better yield to farmer under harsh/ unfavorable climate in case of failure or low yield from the main crop (Turner and Annamalai, 2012). Addition of legumes as intercrop also having several other advantages like nutrient cycling from deeper layer, reducing soil compaction, increasing soil organic matter etc. (Singh et al., 2005). Proper and balanced nutrient application, use of organic source of nutrient (FYM, compost etc.) and inclusion of legumes in cropping system are the desired farm practice to improve and sustain productivity, enhance nutrient use efficiency and to restore as well as improve soil fertility (Singh et al., 2005).

Although several studies evaluated the productivity of rice or rice- pigeon pea intercropping under fertilizers and manure application in several ecology (Singh and Srivastava, 2018; Singh et al., 2005; Kumawat et al., 2017) but limited information is available on the effect of different nutrient management options (inorganic, organic and integrated) on productivity of two system (rice-sole and rice-pigeon pea intercropping) together in this drought prone ecology. Hence, the present investigation was undertaken to compare the productivity of rice- sole with rice pigeon pea intercropping under different nutrient management options in drought prone upland ecology of Eastern India.

MATERIALS AND METHODS

Location and climate

The experiment was conducted at the research farm (latitude: 23° 57' N and longitude 85° 21'E) of Central Rainfed Upland Rice Research Station (ICAR-National Rice Research Institute), Hazaribag, Jharkhand. The experimental site is characterized as bunded upland. The region comes under sub-tropical zone and is characterized by warm climate and erratic rainfall during the *kharif* season resulting in 1 to 2 weeks of dry spells creating drought or moisture stress. The average annual rainfall of this region is around 1200 mm which mainly occurs during the monsoon months.

Experimental details

The experiment was initiated in *kharif* 2018 under rice-sole and rice-pigeon pea intercropping system with the combination of nutrient management options containing inorganic, organic and integrated nutrient management options (Table 1). Required amount of FYM (Farm Yard Manure) were applied during the land preparation; however AM (Arbuscular mycorrhiza) and PSB (Phosphorus Solubilizing Bacteria) were applied at the time of sowing. Inorganic fertilizers were applied within a week after germination where P (phosphorus) and K (Potassium) were applied once in a single dose and N (Nitrogen) in three split doses. Variety Sahbhagi Dhan and Birsa Arhar-1 were selected for rice and pigeon-pea, respectively. Sahabhagi Dhan is very popular short

Table 1. Details of nutrient management practices imposed in rice and rice-pigeon-pea intercropping.

Treatments	Details
T_1	Control
T_2	100% Recommended dose of fertilizers (RDF)*
T_3	50% RDF+Farm yard manure (FYM) @5 t ha^{-1}
T_4	50% RDF+FYM @5 t ha^{-1} + Arbuscular mycorrhiza (AM) 1.5 q ha^{-1}+ Phosphorus Solubilizing Bacteria (PSB)4 $kgha^{-1}$
T_5	50% RDF+Reside Incorporation (RI)
T_6	100% FYM @10 t ha^{-1}
T_7	100% FYM @10 t ha^{-1} + VAM 1.5 q ha^{-1}+ PSB 4 kg ha^{-1}
T_8	100% FYM @10 t ha^{-1} + RI

*RDF (60:30:30 $N:P_2O_5$: K_2O kg ha^{-1}) in rice and (40:30:30 $N:P_2O_5$: K_2O kg ha^{-1}) in rice -pigeon-pea intercropping.

duration (105 days) drought tolerant rice variety and Birsa Arhar-1 is a recommended variety of pigeon-pea in Jharkhand. Under rice sole system, paddy seeds were sown in line directly with 20 cm row to row distance where as in rice- pigeon pea system, rice was sown with 20 cm row to row distance and one line of pigeon pea was sown after every 4 rows of rice (4:1). The crop was grown with the different nutrient management strategies purely under rainfed condition. Weather parameter (monthly maximum and minimum temperature, rainfall) are presented as Fig. 1. The experiment was conducted in randomized block design with eight treatments combinations and three replications under two systems (rice sole and rice-pigeon pea intercropping).

Data collection and analysis

After each crop season the data on grain yield (rice and pigeon-pea) and yield attributes (plant height, number of grains panicle^{-1}, number of chaffs panicle^{-1}, test weight etc) were collected for rice from both the system. To compare the system productivity between rice-sole and rice- pigeon pea system, yield of pigeon pea was converted to rice equivalent yield (REY) and two systems were compared based on the REY, which was calculated based on minimum support price (MSP) 2020-21.The observed data were analyzed using SPSS-16.0, to find the effect of nutrient management practices on yield and yield attributing character under rice sole and rice-pigeon-pea system.

RESULTS AND DISCUSSION

Effect of nutrient management on yield

Table 2. Effect of nutrient management practices on yield.

	Rice sole		Rice pigeon-pea intercropping		
Treatments	Rice grain yield (t ha^{-1})	REY (t ha^{-1})	Rice grain yield (t ha^{-1})	Pigeon pea grain yield (t ha^{-1})	REY (t ha^{-1})
T_1	1.01^{g}	1.01^{g}	0.74^{e}	0.12^{e}	1.13^{f}
T_2	2.83^{a}	2.83^{a}	1.71^{a}	0.48^{a}	3.22^{a}
T_3	1.88^{c}	1.88^{c}	1.29^{c}	0.40^{b}	2.56^{b}
T_4	2.25^{b}	2.25^{b}	1.47^{b}	0.37^{c}	2.64^{b}
T_5	1.60^{d}	1.60^{d}	1.26^{c}	0.34^{c}	2.34^{c}
T_6	1.19^{f}	1.19^{f}	0.86^{d}	0.29^{d}	1.79^{d}
T_7	1.35^{e}	1.35^{e}	0.91^{d}	0.30^{d}	1.87^{d}
T_8	1.22^{ef}	1.22^{ef}	0.84^{de}	0.23^{e}	1.57^{e}

The values within a column followed by common letter are not significantly different by Duncan multiple range test (DMRT) at *P= 0.05*. T_1 : Control; T_2 :100% Recommended dose of fertilizers (RDF)*, T_3 : 50% RDF + Farm yard manure (FYM) @ 5 t ha^{-1}; T_4 :50% RDF +FYM @5 t ha^{-1} + Arbuscular mycorrhiza (AM) 1.5 q ha^{-1} + Phosphorus solubilizing bacteria (PSB) 4 kg ha^{-1}; T_5 :50% RDF + Reside incorporation (RI); T_6 :100% FYM @10 t ha^{-1}; T_7 :100% FYM @10 t ha^{-1} + VAM 1.5 q ha^{-1} + PSB 4 kg ha^{-1}; T_8 :100% FYM @10 t ha^{-1} +RI.

The three years pooled data (2018 to 2020) on grain yield showed that nutrient management had a significant impact on yield in rice sole and rice- pigeon-pea intercropping system (Table 2). In rice sole, the significantly highest yield (2.83 t ha^{-1}) was recorded in T_2 (100% RDF) followed by integrated nutrient management T_4 (2.25 t ha^{-1}) (50% RDF + FYM @5 t ha^{-1} + VAM 1.5 q ha^{-1} + PSB 4 kg ha^{-1}) and T_3 (1.88 t ha^{-1}) (50% RDF + FYM @ 5t ha^{-1}). Almost similar trend was observed in rice- pigeon pea system also in relation to different nutrient management strategies. Yield of rice and pigeon pea was significantly higher under T_2 (100% RDF) (1.71 and 0.48 t ha^{-1}) followed by the T_4 (50% RDF + FYM @ 5 t ha^{-1} + VAM 1.5 q ha^{-1} + PSB 4 kg ha^{-1}) (1.47 and 0.37 t ha^{-1}) respectively. Higher yield under inorganic nutrient management was because of the fact that,inorganic fertilizers provides readily available nutrients to plant as per demand and help to produce more yield as compared to integrated nutrient management, where some amount of nutrients are substituted by the organic sources, which provide the nutrients at relatively slower rate. Srivastava et al. (2014) also reported that application of 100% RDF increases effective number of tillers m^{-2}, grains panicle^{-1}, test weight and overall grain yield of rice over the 50% RDF. It was also reported that application of 100% RDF significantly improved the yield and yield attributes in pigeon pea (Singh and Pal, 2003 and Patil and Padmini, 2007). The yield under organic treatments were low (T_6, T_7, and T_8), where no inorganic fertilizers were applied however it was significantly higher than control (T_1). The lower yield in organic treatment might be due to the slower rate of nutrient transformation in soil (Myint et al., 2011) which leads to insufficient supply of nutrient to the plants at the early growth stages (Moe et al., 2019). It was also reported that, organic treatments (containing the FYM, PSB etc) having lower system yield and productivity as compared to integrated

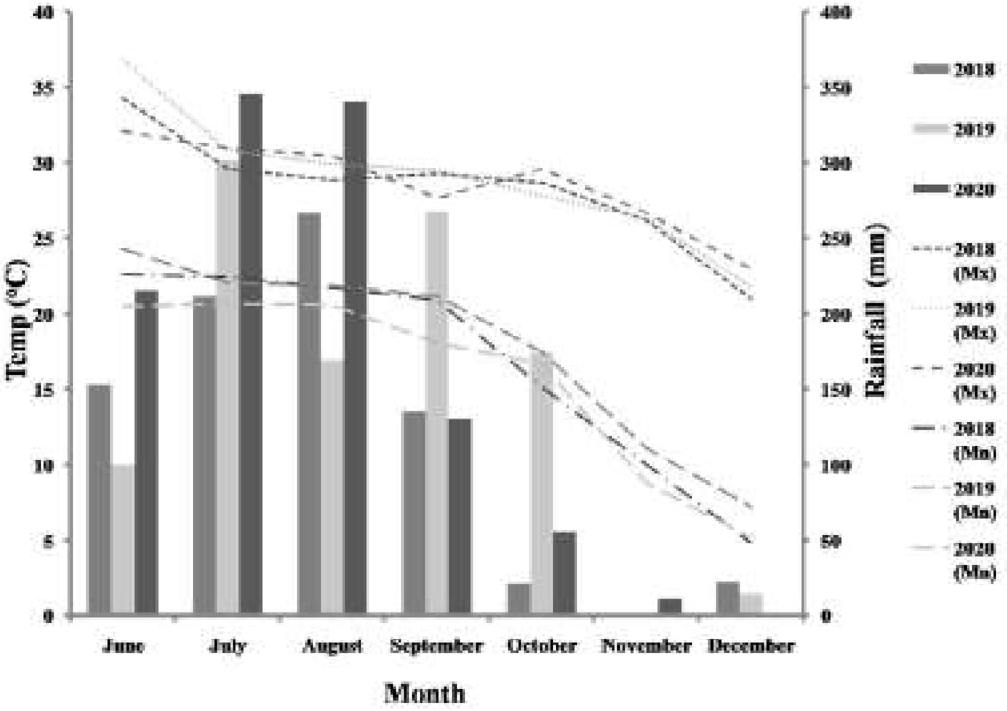

Fig. 1. Weather parameter (monthly average maximum and minimum temperature and rainfall) of CRURRS, Hazaribag during the crop growth period (2018-2020).

and inorganic treatments in rice based cropping system (Patra et al., 2017). Different worker had also reported the lower yield in organic treatments as compared to inorganic or integrated nutrient management practices. (Rao et al., 2014; Surekha and Satishkumar, 2014).

To compare both the system, yield of pigeon pea was converted to the rice equivalent yield (REY)

Table 3. Effect of nutrient management practices on yield over the period of three years (2018-2020).

Treatment (T)	Rice sole				Rice pigeonpea							
	Rice grain yield (t ha^{-1})				Rice grain yield (t ha^{-1})				Pigeon-pea grain yield (t ha^{-1})			
	Year (Y)			Mean	Year (Y)			Mean	Year (Y)			Mean
	2018	2019	2020		2018	2019	2020		2018	2019	2020	
T_1	0.49	1.21	1.34	1.01	0.17	0.89	1.15	0.74	0.09	0.10	0.18	0.12
T_2	1.63	3.18	3.69	2.83	0.32	2.28	2.52	1.71	0.38	0.46	0.59	0.48
T_3	0.75	2.33	2.58	1.88	0.27	1.69	1.90	1.29	0.35	0.39	0.46	0.40
T_4	0.68	2.71	3.37	2.25	0.30	2.01	2.09	1.47	0.32	0.36	0.42	0.37
T_5	0.66	2.11	2.03	1.60	0.34	1.62	1.83	1.26	0.26	0.32	0.43	0.34
T_6	0.45	1.47	1.64	1.19	0.19	1.05	1.34	0.86	0.27	0.30	0.31	0.29
T_7	0.45	1.66	1.94	1.35	0.19	1.21	1.32	0.91	0.28	0.31	0.33	0.30
T_8	0.50	1.39	1.77	1.22	0.26	1.00	1.26	0.84	0.18	0.24	0.26	0.23
Mean	0.70	2.01	2.29		0.25	1.47	1.67		0.26	0.31	0.37	
CD *(P= 0.05)*	T = 0.14; Y = 0.08; T X Y = 0.24				T = 0.11; Y = 0.07; T X Y = 0.19				T = 0.03; Y = 0.018; T X Y = 0.05			

T_1: Control; T_2 :100% Recommended dose of fertilizers (RDF)*, T_3 : 50% RDF + Farm yard manure (FYM) @ 5 t ha^{-1}; T_4 :50% RDF + FYM @5 t ha^{-1} + Arbuscular mycorrhiza (AM) 1.5 q ha^{-1} + Phosphorus solubilizing bacteria (PSB) 4 kg ha^{-1}; T_5 :50% RDF + Reside incorporation (RI); T_6 :100% FYM @10 t ha^{-1}; T_7 :100% FYM @10 t ha^{-1} + VAM 1.5 q ha^{-1} + PSB 4 kg ha^{-1}; T_8 :100% FYM @10 t ha^{-1} +RI.

Table 4. Effect of nutrient management practices on rice yield attributing character.

Treatments	Grain panicle^{-1}	Chaff panicle^{-1}	Test weight (g)	Plant height (cm)
T_1	55^{c}	10^{b}	21.79^{cd}	58.97^{e}
T_2	73^{a}	7^{c}	24.31^{a}	76.11^{a}
T_3	59^{bc}	10^{b}	21.50^{d}	73.18^{b}
T_4	64^{b}	13^{a}	22.71^{b}	70.01^{c}
T_5	57^{c}	12^{ab}	22.50^{bc}	71.55^{bc}
T_6	54^{c}	12^{ab}	20.40^{e}	61.25^{de}
T_7	48^{d}	13^{ab}	21.48^{d}	62.50^{d}
T_8	56^{c}	12^{ab}	21.05^{de}	61.04^{de}

The values within a column followed by common letter are not significantly different by Duncan multiple range test (DMRT) at *P= 0.05*. T_1 : Control; T_2 :100% Recommended dose of fertilizers (RDF)*, T_3 : 50% RDF + Farm yard manure (FYM) @ 5 t ha^{-1}; T_4 :50% RDF +FYM @5 t ha^{-1} + Arbuscular mycorrhiza (AM) 1.5 q ha^{-1} + Phosphorus solubilizing bacteria (PSB) 4 kg ha^{-1}; T_5 :50% RDF + Reside incorporation (RI); T_6 :100% FYM @10 t ha^{-1}; T_7 :100% FYM @10 t ha^{-1} + VAM 1.5 q ha^{-1} + PSB 4 kg ha^{-1}; T_8 :100% FYM @10 t ha^{-1} +RI.

and observed that, irrespective of the nutrient management options, REY is higher in the rice pigeon-pea inter cropping system as compared to rice sole (Table 2). It implies that growing rice with pigeon-pea will always be beneficial as compared to rice sole. Singh et al. (2014) also reported that growing rice with pigeon-pea is beneficial and having higher REY as compared to rice sole. The highest REY was recorded under T_2 (100% RDF) (3.22 tha^{-1}) followed by integrated nutrient management (T_3 and T_4). The REY in organic treatments (T_6, T_7 and T_8) under rice- pigeon pea were lower, however it is significantly better as compared to the control, where no inputs were applied. It might be due to fact that, in organic treatments nutrients release to the soil solution is slow as organic matter has to be first mineralized or broken down by microbes. It was reported that, organic matter maintains regular and continuous supply of nutrients to the soil (Sharma and Subehia, 2014). This might be the reason for higher REY as compared to the control where no inputs were applied.

Interactive effect of nutrient management and year on yield

The interactive effect of nutrient management and year are also significant for yield of rice and pigeon pea. From the yield data presented in Table 3, it was observed that in both the rice sole and rice-pigeon pea system, across the nutrient management practices, yield was low in the first year (2018) and significant improvement was observed in the subsequent years 2019 and 2020. The relative improvement of yield over the year showed that, compared to previous year yield improvement was more pronounced in the integrated nutrient managements options (T_4) (50% RDF +FYM @5 t ha^{-1} + VAM 1.5 q ha^{-1} + PSB 4 kg ha^{-1}) and the yield gap between inorganic and integrated nutrient management was gradually decreased in second (2019) and third (2020) years. It implies that integrated nutrient management has the potential for sustainable yield enhancement after certain period of time. Previous reporters also emphasized that improvement in productivity and sustaining them for a longer period must include an integrated nutrient management approach (Abid et al., 2020; Selim, 2020).

Effect of nutrient management on yield attributing characters

Yield attributing characters in rice like number of grains panicle^{-1}, number of chaffs panicle^{-1}, test weight and plant height was significantly affected by the nutrient management practices (Table 4). The significantly higher number of grains panicle^{-1}, test weight and plant height was recorded in T_2 (100% RDF) followed by the T_4 (50% RDF +FYM @5 t ha^{-1} +VAM 1.5 q ha^{-1} +

Table 5. Correlation of yield and yield attributing characters in rice.

	Grain yield	Grains panicle^{-1}	Chaffs panicle^{-1}	Plant height	Test weight
Grain yield	1				
Grains panicle^{-1}	0.79**	1			
Chaffs panicle^{-1}	-0.61**	-0.57**	1		
Plant height	0.64**	0.56**	-0.23	1	
Test weight	0.44**	0.48**	-0.34**	0.55**	1

**. Correlation is significant at the 0.01 level (2-tailed).

PSB 4 kg ha^{-1}) and T_3 (50% RDF + FYM @5 t ha^{-1})., but no definite trend was observed for the other nutrient management options. Primary yield attributing characters were directly related with the yield and it was well reflected in yield under particular nutrient management practices. Srivastava et al. (2014) also observed that full amount of RDF increased effective number of tillers m^{-2}, grains panicle^{-1}, test weight and overall grain yield of rice.

Correlation of yield with the yield attributes

Rice grain yield was highly correlated with the number of grains panicle^{-1} (0.79**), plant height (0.64**) and test weight (0.44**), and it was negatively correlated with the number of chaffs panicle^{-1} (-0.61**) (Table 5). Number of grains panicle^{-1} was also significantly correlated with the plant height (0.56**). Significant and positive correlations of yield attributing characters with yield stabilized the true relationship among them. Dwibedi et al. (2017) also found the good correlation of yield attributing parameters to the yield of rice.

CONCLUSION

It can be concluded that, under rainfed drought prone upland areas, growing pigeon-pea as an intercrop with rice under integrated nutrient management will be a better choice for the farmers not only to improve and sustain their farm income but also to enhance the system productivity on a long term basis. Under very severe drought stress condition even if the rice crop fails, pigeon pea being deep rooted drought tolerant crop will save the farmers from total crop failure. Use of integrated nutrient source (50% RDF + FYM @5 t ha^{-1} along with Arbuscular mycorrhiza and Phosphorus Solubilizing Bacteria) is also helpful in maintaining soil fertility and minimizing the yield loss.

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REFERENCES

Abid M, Batool T, Siddique G, Ali S, Binyamin R, Shahid MJ, Rizwan M, Alsahli AA and Alyemeni MN (2020). Integrated nutrient management enhances soil quality and crop productivity in maize-based cropping system. Sustainability 12(23): 10214 doi:10.3390/su122310214

Dwibedi SK, De GC and Dhua, SR (2017) . Relative performance of rice (*Oryza sativa*)-ratoon production system as influenced by date of sowing and system of cultivation of plant rice genotypes. Indian Journal of Agronomy 62(3): 247- 254

Kumar R, Mishra JS and Hans H (2018). Enhancing productivity of rice fallows of eastern India through inclusion of pulses and oil seeds. Indian farming. 68 (08) 7-10

Kumawat N, Kumar R, Morya J, Tomar IS and Meena RS (2017). Integrated nutrition management in pigeon pea intercropping system for enhancing production and productivity in sustainable manner- A review. Journal of Applied and Natural Sciences 9(4): 2143-2151

Maiti D, Mandal NP, Singh CV, Prasad SM, Bhagat S, Roy S, Banerjee A and Verma BC (2018). Climate Resilient Production Technologies for Rainfed Upland Rice Systems. In Rice Research for enhancing productivity, profitability and climate resilience. Published by ICAR-NRRI pp. 512-527

Moe K, Htwe AZ, Thu TTP, Kajihara Y, Yamakawa T (2019). Effects on NPK Status, Growth, Dry Matter and Yield of Rice (*Oryza sativa*) by Organic Fertilizers Applied in Field Condition. Agriculture 9(5): 109

Myint AK, Yamakawa T, Zenmyo T, Thao HTB and Sarr PS (2011). Effects of organic-manure application on growth, grain yield, and nitrogen, phosphorus, and potassium recoveries of rice variety Manawthuka in paddy soils of differing fertility. Communication in Soil Science and Plant Analysis 42: 457-474

Patil AB and Padmani DR (2007). Effect of integrated nutrient management on growth and yield of pigeon Pea (*Cajanus cajan* L. Milisp.) under rainfed conditions. International Journal of Agricultural Sciences 3(2): 202-204

Patra A K, Mishra K N, Garnayak L M and Mohanty, A K (2017). Influence of long-term organic nutrient management on soil quality and crop productivity in rice (*Oryza sativa*)-potato (Solanum tuberosum)-okra (Abelmoschus esculentus) cropping system under irrigated condition. Indian Journal of Agronomy 62 (3): 268-274

Rao AU, Murthy KMD, Sridhar TV, Raju SK and Lakshmi DA (2014). Studies on performance of organic farming and chemical farming in rainy season rice. International Journal of Plant, Animal and Environmental Sciences 4(4): 202-206

Selim,MM (2020) Introduction to the Integrated Nutrient Management Strategies and Their Contribution to Yield and Soil Properties. International Journal of Agronomy, Article ID 2821678, https://doi.org/10.1155/2020/2821678

Sharma U and Subehia SK (2014). Effect of long term integrated nutrient management of rice (*Oryza sativa* L.)-wheat (*Triticum aestivum* L.) on productivity and soil properties in North-western Himalaya. Journal of the Indian Society of Soil Science 62(3): 248-254

Singh CV, Maiti D, Mnadal NP, Kumar Y, Anantha MS, Variar M and Singh VK (2014). Crop diversification in upland rice areas. CRURRS Technology Bulletion, No. 1

Singh RS, and Srivastava GP (2018). Effect of fertilizer levels and pigeonpea based intercropping systems on yield, nutrient removal and economics in chhotanagpur region under rainfed condition. International Journal of Current Microbiology and Applied Science7: 3554-3561

Singh T and Pal M (2003). Growth parameters, yield attributes and yields of pigeon pea as influenced by cropping system and nitrogen + phosphorus level. Annals of Agricultural Research 24(4): 755-759

Singh VK, Dwivedi BS, Shukla AK, Chauhan YS and Yadav RL (2005). Diversification of rice with pigeon pea in a rice-wheat cropping system on a Typic Ustochrept: effect on soil fertility, yield and nutrient use efficiency. Field Crops Research. 92: 85-105.

Singh VP and Singh RK (2000). Rainfed Rice: A Sourcebook of Best Practices and Strategies in Eastern India. International Rice Research Institute pp. 292

Srivastava VK, Singh JK, Bohra JS and Singh SP(2014). Effect of fertilizer levels and organic sources of nitrogen on production potential of hybrid rice (*Oryza sativa*) and soil physic-chemical properties under system of rice intensification. Indian Journal of Agronomy 59(4): 24-29

Surekha K and Satishkumar YS (2014). Productivity, nutrient balance, soil quality, and sustainability of rice (*Oryza sativa* L.) under organic and conventional production systems. Communications in Soil Science and Plant Analysis 45(4): 415-428

Turner AG, and Annamalai H (2012). Climate change and the South Asian monsoon. National Climate Change 2: 587-595

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Assessment of suitable dose of calcium silicate to rice and its impact on soil properties in laterite soils of Odisha

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ABSTRACT

A field experiment was conducted in the central farm, Regional Research and Technology Transfer Station, Coastal Zone OUAT, Bhubaneswar during kharif 2020 using cv-Lalat variety of rice. The experiment was laid out in randomized block design (RBD) with ten treatments and three replications. In this experiment, Basic Oxygen Furnace (BOF) slag was used as a source of silica for application in rice crop. 200, 300 and 400 kg $SiO_2 ha^{-1}$ *was applied in combination with 50% Soil Test Dose (STD) and 75% STD in order to assess the efficacy of Silica with reduction in fertilizer dose by 50% and 25%. The result indicated that there was significant increase in number of effective tillers per plant and length of the panicle, but no significant increase was observed in case of 1000 grain weight over control. In case of grain yield, straw yield and harvest index, 100% STD registered maximum (36.9q/ha) grain yield which was 29.4% more than that of control. In case of 50% or 75% STD with 300kg* $SiO_2 ha^{-1}$ *grain yield was at par with 100% STD, which indicated that silica application has the capability to reduce the fertilizer dose. Harvest index was not significantly affected by Si treatments. However, maximum HI (0.478) was observed in 75% STD + 300 kg* $SiO_2 ha^{-1}$*. The present study showed increase in pH and organic carbon content in post-harvest soil which suggests that application of silica has the capacity to correct soil acidity and improve soil organic carbon in acidic laterite soils.*

Key words: *Silicon, rice, yield, soil properties, soil acidity*

INTRODUCTION

Silicon (Si) is the second most element abundantly available on the earth crust. Its content in soils varies greatly ranging from less than 1% to 45% by dry weight (Sommer et al.,2006). In regions with high rainfall, due to heavy weathering, less resistant silicates release silica which is rapidly leached out to the nearby streams. Although Si is present in the soil in large amounts, its availability to plants is limited. Due to the desilication process, subtropical and tropical soils are generally low in plant available Si and hence the plants would benefit from silicon fertilization (Korndorfer and Lepsch, 2001).

Silicon does not form a constituent of any cellular components but is primarily deposited on the walls of the epidermis and vascular tissues conferring strength, rigidity and resistance to pests and diseases. It also manages many abiotic stresses including physical stresses like lodging, drought, radiation, high temperature, freezing and chemical stresses like salt, metal toxicity and nutrient imbalance (Epstein,1994). An adequate supply of silica is essential if grasses and cereals are to give a good yield. Rice is the largest Si accumulator. Si plays a pivotal role in increasing yield, disease and pest resistance of rice. Many Indian farmers are not aware of the benefits of Si and its sources. The ideal Si source must have a relatively high content of Si; should provide sufficient water-soluble Si to meet the needs of the plant; be cost-effective; ease in availability; should have a physical nature that facilitates storage as well as application and not contain substances that will contaminate the soil (Gascho, 2001). With this backdrop, the present investigation was aimed to evaluate the influence of Si (BOF slag as source) with the objective find out a suitable dose of SiO2 to rice for increasing yield and yield attributing characters and to study about the amelioration effect of calcium silicate in acid soil.

MATERIALS AND METHODS

Experiment was conducted in the Regional Research and Technology Transfer Station, OUAT, Bhubaneswar during *kharif* 2020. Rice cv. Lalat (125-130 days)was selected for the research purpose since it suits for the medium land situation. In the experiment, BOF slag a byproduct of Jindal Steel Pvt. Ltd. was used as a source of silica for application to rice crop. The chemical composition of the BOF (Table 2) was CaO(49.5) , SiO$_2$(17), MgO(10.1), Al$_2$O3(3.8), FeO(12.8), MnO(1.43) , P$_2$O$_5$(2.99), K$_2$O(0.025), Na$_2$O(0.009), S(0.058). Treatment was imposed based on RBD design to the plot with different concentration of fertilizer doses given based on the soil test dose. 200, 300 and 400 kg SiO$_2$ha^{-1} was applied in combination with 50% STD and 75% STD in order to assess the effect of silica with reduction in fertilizer dose by 25% and 50%. Total 10 treatments with three replication were imposed to get the information more accurately. T$_1$ = Absolute control, T$_2$ = 50% STD, T$_3$ = 75% STD, T$_4$ = 100% STD, T$_5$ = T$_2$ + 200 kg SiO$_2$ ha^{-1}, T$_6$ = T$_2$ + 300 kg SiO$_2$ha^{-1}, T$_7$ = T$_2$ + 400 kg SiO$_2$ ha^{-1}, T$_8$ = T$_3$ + 200 kg SiO$_2$ha^{-1}, T$_9$ = T$_3$ + 300 kg SiO$_2$ ha^{-1} and T$_{10}$ = T$_3$ + 400 kg SiO$_2$ha^{-1}.

The nutrients were applied in form of urea, DAP and MOP and silicate slag as per the experimental plan. The requirement of nitrogen by the crop was supplemented in the form of DAP and urea in three splits *viz*., 25% basal, 50% at active tillering stage and finally 25% at panicle initiation stage. Required amount of phosphorus were applied as 100% basal in each plot. Total amount of potassium in form of Muriate of Potash (MOP) was applied in two splits i.e., 50% at basal and 50% at PI stage. Soil application of silicate slag was made @ 200, 300 and 400 kg SiO$_2$ ha^{-1} at the time of transplanting as per the treatment imposed.

Table 2. Characterization of (Basic Oxygen Furnace) BOF Slag.

Element	%	Element	%
CaO	49.5	MnO	1.43
SiO$_2$	17	P$_2$O$_5$	2.99
MgO	10.1	K$_2$O	0.025
Al$_2$O$_3$	3.8	Na$_2$O	0.009
FeO	12.8	S	0.058

The grains were cleaned after threshing and both grain and straw yields from net plot were recorded and expressed in kgha^{-1} after 3 days of thorough sun drying in order to reduce moisture content up to 14% in grain and 16% in straw. Harvest index (HI) was obtained by dividing the grain yield to the total biomass yield excluding the underground portion (roots). Then HI was converted into percentage data by multiplying with 100. (Donald, 1962)

HI = (Economic yield/ Biological yield) × 100

Soil and plant analysis was done by following standard procedures like Soil texture-Piper (1950), pH and electrical conductivity- Jackson (1973), organic carbon-Walkley and Black (1934), Available Nitrogen by alkaline potassium permanganate (KMnO$_4$) method (Subbiah and Asija, 1956), Available Phosphorus and Available Potassium by Page et al. (1982), The DTPA extractable Fe, Mn, Cu and Zn of the soil sample was estimated following the method by Lindsay and Norvell (1978) and concentration of elements was determined in AAS as described by Page et al. (1982). The Si content of digested sample was quantified colorimetrically through molybdenum blue method (Wei-

Table 3. Effect of Silica on number of effective tillers per plant, length of the panicle and 1000 grain weight.

Treatment	Number of effective tillers per plant	Length of the panicle (cm)	1000 grain wt.(gm)
T$_1$ = Absolute control	5.5	22.5	21.8
T$_2$ = 50% STD	6.1	23.2	22.1
T$_3$ = 75% STD	6.3	24.1	22.2
T$_4$ = 100% STD	8.4	25.8	23.1
T$_5$ = T$_2$ + 200 kg SiO$_2$/ha	6.5	24.5	22.4
T$_6$ = T$_2$ + 300 kg SiO$_2$/ha	7.1	25.0	22.7
T$_7$ = T$_2$ + 400 kg SiO$_2$/ha	6.6	24.7	22.4
T$_8$ = T$_3$+ 200 kg SiO$_2$/ha	6.9	24.9	22.5
T$_9$ = T$_3$+ 300 kg SiO$_2$/ha	7.4	25.8	23.0
T$_{10}$ = T$_3$ + 400 kg SiO$_2$/ha	7.3	25.4	22.8
SE m	0.18	0.26	-
CD (0.05)	0.55	0.78	NS

min et al. 2005).

RESULTS AND DISCUSSION

Effect of silica on growth attributing characters and yield of rice

The data presented in Table 3 indicates influence of silica on parameters like number of effective tillers per plant, length of the panicle and 1000 grain weight. Number of effective tillers per plant varied from 5.5 to 8.4 in which lowest number of tillers was observed in T$_1$ *i.e.*, Absolute control (no fertilizer) and significantly highest number of tillers was observed in T$_4$ *i.e.*,100% STD. Number of effective tillers increased as the fertilizer dose increased. In case of application of silica in combination with 50 and 75% STD, maximum number of tillers was obtained under application of 300 kg SiO$_2$ ha^{-1} which was reduced with increased application of silica. Data on effective tillers plant^{-1}, length of the panicle and 1000 grain wt. presented in Table 3 indicated that application of 100% STD was superior than other treatments but 75% STD + 300kg SiO$_2$ha^{-1} was also equally effective. Hence, by reducing the fertilizer dose by 25% and applying 300kg SiO$_2$ha^{-1} we can achieve the desired result. In our study, the increase in total number of effective tillers per plant might be attributed to increased availability of phosphorus and other beneficial effect of silicon on growth of paddy. These results are in confirmation with those reported by Sawant et al. (1994), Sudhakar et al. (2004), Singh et al. (2005), Singh et al. (2007), Muriithi et al. (2010), and Patil et al (2017), who also reported the beneficial role of Si fertilizer in increasing number of effective tillers per plant,length of the panicle varied from 22.5 cm to 25.8 cm. with lowest in T$_1$ (Absolute control). 100% STD (T$_4$) and 75% STD + 300 kg SiO$_2$ha^{-1} (T9) registered significantly higher panicle length which was 14.6% more than that of control. There was gradual increase in panicle length as the fertilizer dose increased. Application of 300kg SiO$_2$ha^{-1} registered highest panicle length when compared with 200 and 400kg SiO$_2$ha^{-1} irrespective of integration of fertilizer dose. There was no significant difference between 300 and 400 kg SiO$_2$ha^{-1} so far as length of the panicle is concerned. 1000 grain wt. varied from 21.8gm to 23.1 gm. Increased fertilizer dose did not have any significant effect on 1000 grain wt. It was lowest in absolute control *i.e.*, 21.8 gm and highest in 100% STD *i.e.*, 23.1 gm which was at par with 75% STD + 300kg SiO$_2$ha^{-1}. There was no significant difference between the treatments. Jawahar et al (2010) reported the effectiveness of Si fertilizer in promoting the assimilation of carbohydrates in panicles, which leads to increased number of filled grains. Application of Silica increases effective tillers per plant, length of panicles and 1000-grain weight which corroborates the findings of Gong et al. (2003) and Gholami and Falah (2013).The study shows an increase in length of panicle which is also in confirmation with Singh et al. (2007) and Shashidhar et al. (2008).

Our findings showed 1000-grain weight was not significantly affected by application of Silica which contradicts the findings of Prabhu et al. (2001), Singh et al. (2007), Dallagnol et al. (2014), Pati et al. (2017), which shows significantly increased 1000-grain weight with application of Si fertilizer.

The experimental data presented in Table 4 and Fig. 1 indicates influence of Silica on plant yield attributing characters like grain yield, straw yield and harvest index. Grain yield of rice revealed that there was a variation in yield from 28.5 qha^{-1} to 36.9 qha^{-1}. Grain yield was maximum (36.9 qha^{-1}) in T$_4$ (100% STD) which was almost 30% more than that of control (28.5 qha^{-1}) but it was at par (36.2 qha^{-1}) with T$_9$ (75% STD + 300 kg SiO$_2$ha^{-1}). All the treatments registered significantly higher yield over control. There was a continuous increase in yield as the dose of the fertilizer increased. Application of silica along with 50% STD/ 75% STD did not have any significant effect on yield

Table 4. Effect of Silica on grain yield, straw yield, and harvest index of paddy.

Treatment	Grain yield(q/ha)	Straw yield(q/ha)	Harvest index
T$_1$ = Absolute control	28.5	33.0	0.463
T$_2$ = 50% STD	32.8	36.8	0.472
T$_3$ = 75% STD	33.1	37.7	0.468
T$_4$ = 100% STD	36.9	40.8	0.475
T$_5$ = T$_2$ + 200 kg SiO$_2$/ha	33.7	38.0	0.470
T$_6$ = T$_2$ + 300 kg SiO$_2$/ha	35.5	39.1	0.476
T$_7$ = T$_2$ + 400 kg SiO$_2$/ha	34.3	38.4	0.472
T$_8$ = T$_3$ + 200 kg SiO$_2$/ha	34.9	38.8	0.473
T$_9$ = T$_3$ + 300 kg SiO$_2$/ha	36.2	39.6	0.478
T$_{10}$ = T$_3$ + 400 kg SiO$_2$/ha	35.6	39.3	0.476
SE m	1.37	1.32	-
CD (0.05)	4.09	3.94	NS

Table 1. Soil physico-chemical properties.

Sl. no.	Parameter	Value	Method followed
1.	Sand (%)	94	Bouyoucos Hydrometer Method (Piper, 1950)
	Silt (%)	2.6	
	Clay (%)	3.4	
	Textural class	Sandy	
2.	Bulk density(gcc-1)	1.58	Core sampler method (Dastane,1972)
3.	pH (1:2.5)	5.67	Glass electrode Beckman's Electronic pH meter (Jackson, 1973)
4.	EC (dSm^{-1})	0.16	Digital electrical conductivity Bridge method (Jackson, 1973)
5.	OC (g kg^{-1})	4.1 (Low)	Walkley and Black wet digestion method (1934)
6.	Av. N ($kgha^{-1}$)	178 (Low)	Alkaline KMnO4 method (Subbiah and Asija, 1956)
7.	Av. P (kg ha^{-1}) (Bray's)	13.5 (Low)	Bray and Kurtz's method (1945)
8.	Av. K (kg ha^{-1})	83 (Low)	Flame photometer method (Jackson, 1973)
9.	Av. S (kg ha^{-1})	6.8 (Low)	Williams and Steinbergs (1959)
10.	DTPA Extractable Fe (ppm)	78.8	Lindsay and Norvell, 1978
11.	DTPA Extractable Mn (ppm)	1.32	
12.	DTPA Extractable Cu (ppm)	0.21	
13.	DTPA Extractable Zn (ppm)	0.35	

increase however, yield was decreased when silica application went beyond 300 $kgha^{-1}$ (Fig. 3 and Fig. 4). Variation in straw yield ranged from 33 qha^{-1} to 40.8 qha^{-1}. Maximum straw yield was obtained in T_4 (100% STD) and minimum in control (33 qha^{-1}). Around 24% yield increase was observed due to application of fertilizer *i.e.*, 100% STD over control. Straw yield in 100% STD (T_4) was at par with 75% STD + 300 kg SiO_2ha^{-1} (T_9). There was significant increase in yield over control due to application of different doses of Silica along with 50% and 75% STD. Maximum grain and straw yield *i.e.*, 36.9 qha^{-1} and 40.8 qha^{-1} was obtained due to application of 100% STD followed by 36.2 qha^{-1} and 39.6 qha^{-1} due to application of silica. But harvest index was maximum (0.478) in 75%STD + 300kg SiO_2ha^{-1}. This indicates though 100% STD registered maximum yield but by reducing fertilizer dose by 25% along with supplemental dose of silica *i.e.*, 300 kg SiO_2ha^{-1} we could achieve statistically at par yield. In this study, a positive response of grain and straw yields to application of silica was observed. This improvement in grain and straw yields might be due to an enhanced growth, yield components and nutrient uptake of rice with the addition of SiO_2. Application of silica in different dose along with 50% or 75% STD did not have any significant effect on yield increase but

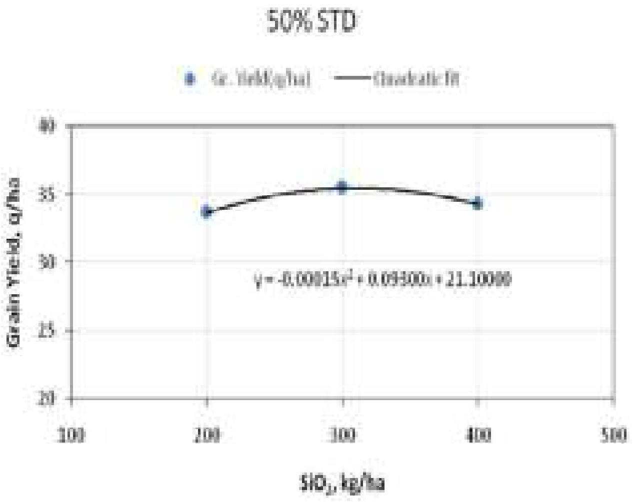

Fig. 3. Maximum yield vs optimum level Silica applied along with 50% STD fertilizer.

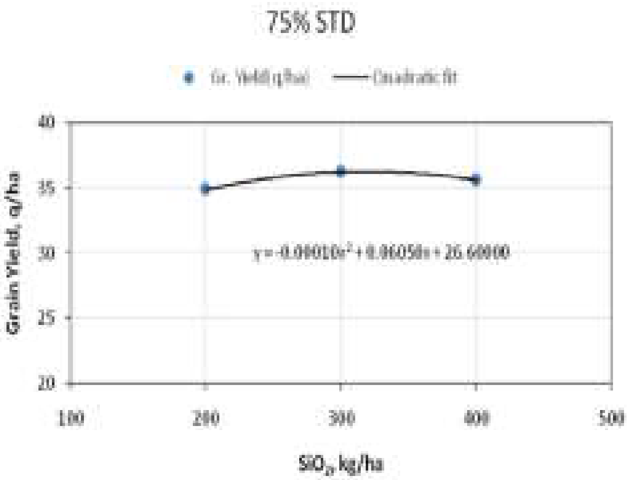

Fig. 4. Maximum yield vs optimum level silica applied along with 75% STD fertilizer.

straw yield was decreased over and above 300 kg SiO_2ha^{-1}. Harvest index was almost equal irrespective of treatments imposed. But maximum HI was obtained in T_9 (0.478) which was at statistically at par with T_4 (0.475) and T_{10} (0.476) and minimum in control (0.463). There was no significant difference between the treatments so far as HI was concerned. Deren et al. (1994) and Pati et al. (2017) also reported a significant increase in grain and straw yields of rice with increasing Si level. Though Harvest index was maximum due to application of 75% STD + 300 kg SiO_2ha^{-1} but no significant difference was obtained among the treatments. This contradicts the findings of Detmann et al. (2012) and Pati et al. (2017), who reported an increased harvest index of rice with Si application. The increases in both grain and straw yields might be attributed to the positive effect of Si in increasing growth and yield characteristics (Prakash et al., 2011 and Pati et al., 2017), enhancing the pollen viability and photosynthetic activity (Detmann et al., 2012), reducing abiotic and biotic stress, improving structural support and biomass (Meharg and Meharg, 2015) and improving nutrient uptake (Pati et al., 2017 and Crooks and Prentice, 2017).

Based on the quadratic regression equation ($y = 0.00020x^2 + 0.05876x + 28.62182$) the grain yield when compared with fertilizer dose (Fig. 2) (without silica application) it was observed that maxima will not be reached since a is > 0 however, there was steady increase in grain yield from control treatment to 100% STD and reached maximum *i.e.*, 36.9 qha^{-1}.

Table 5. Effect of Silica on soil pH, Electrical Conductivity and organic carbon content of post-harvest soil sample.

Treatment	pH	E.C(dS/m)	O.C (%)
T_1 = Absolute control	5.41	0.118	0.31
T_2 = 50% STD	5.20	0.121	0.42
T_3 = 75% STD	5.48	0.130	0.45
T_4 = 100% STD	5.64	0.135	0.48
$T_5 = T_2$ + 200 kg SiO_2/ha	5.71	0.120	0.41
$T_6 = T_2$ + 300 kg SiO_2/ha	5.85	0.124	0.44
$T_7 = T_2$ + 400 kg SiO_2/ha	5.91	0.136	0.48
$T_8 = T_2$ + 200 kg SiO_2/ha	5.89	0.142	0.42
$T_9 = T_3$ + 300 kg SiO_2/ha	5.90	0.146	0.43
$T_{10} = T_3$ + 400 kg SiO_2/ha	5.98	0.148	0.46
SE m	0.13	0.006	0.03
CD (0.05)	0.39	0.018	0.09

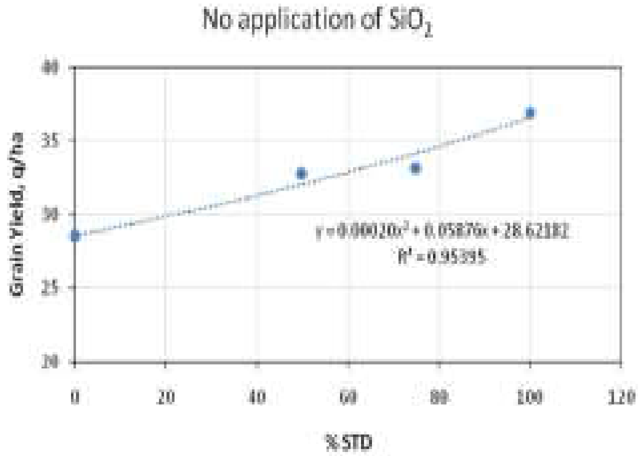

Fig. 2. Maximum yield vs optimum level of fertilizer applied.

Based on the quadratic regression equation ($y = -0.00015x^2 + 0.09300x + 21.1$) the vertex is the highest point on the graph of quadratic function (Fig. 3). The value of vertex ($x = -b/2a$) was 310 with Y max = a $(X\ max)^2$ + b (X max) + c = 35.51 Therefore. 310 $kgha^{-1}$ SiO_2 was the optimum level of Si fertilizer that could provide the maximum yield (35.51 qha^{-1}) in combination with 50% STD based on the quadratic regression model. It is evident that, more amount of Si fertilizer application could result in a decrease in the yield.

Based on the quadratic regression equation ($y = -0.00010x^2 + 0.06050x + 26.6$) the vertex is the highest point on the graph of quadratic function (Fig. 4). The value of vertex (x = -b/2a) was 336.1 with Y max = a $(X\ max)^2$ + b (X max) + c= 36.76. Therefore, 336.1 $kgha^{-1}$ was the optimum level of Si fertilizer that could provide the maximum yield (36.76 qha^{-1}) in combination with 75% STD based on the quadratic regression model. From this point, more amount of Si fertilizer application could result in a decrease in the yield.

Effect of silica on pH, electrical conductivity and organic carbon content of post-harvest soil

The data presented in Table 5 indicates effect of silica on pH, electrical conductivity and organic carbon in post-harvest soil. Soil pH presented in table revealed that there was a variation in pH value so far as different treatments are concerned. pH value decreased with T_2 (50% STD) over T_1 but increased with further application of fertilizer *i.e.*, T_3 (75% STD) and T_4 (100% STD) over control. pH value was recorded maximum (5.98) in T_{10}. Application of silicon in different doses along with T_2 (50% STD) and T_3 (75% STD) shows increase in pH value. However, this increase was not significant.

Variation in electrical conductivity ranged from 0.118 dSm^{-1} to 0.148 dSm^{-1}. Electrical conductivity was maximum in T_{10} (75% STD + 400 kg SiO_2ha^{-1}) and minimum in control (0.118 dSm^{-1}). There was gradual increase in electrical conductivity of post-harvest soil as the fertilizer dose increased. However, the increase was not significant. Application of silica in different doses along with T_2 (50% STD) or T_3 (75% STD) showed an increasing trend. The initial soil sample presented in Table 1 indicates acidic pH (5.67), E.C (0.16 dSm^{-1}) and low O.C (4.1 gkg^{-1}). These values when compared with post-harvest soil analysis indicated slight increase in pH, reducing E.C (0.14 dSm^{-1}) and increase in O.C (0.48%) The study shows increase in pH, electrical conductivity (EC) and Organic Carbon (OC) in post-harvest soil due to application of Silica.

The organic carbon content in post-harvest soil was presented in Table 5. There was a variation ranging from 0.31% to 0.48% of OC in post-harvest soil so far as different treatments are concerned. Maximum OC content (0.48%) in soil was recorded in T_4 (100% STD) and T_7 (50% STD + 400kg SiO_2ha^{-1}) as compared to lowest in absolute control T_1. This increase in OC content in soil was 55% more over control. With increase in fertilizer doses, the percentage of OC was also increased. All the treatments registered significantly higher OC content over Control. In case of application of silica, in combination with 50% and 75% STD, the percentage of OC increases but the increase was not significant. Application of 400kg SiO_2ha^{-1} registered maximum OC content in soil when compared with 200 and 300 kg SiO_2ha^{-1} irrespective of integration of fertilizer dose. This increase in soil pH could be attributed to the fact that silicate materials can increase soil reaction and help in correcting soil acidity by neutralizing exchangeable Fe, Al and Mn and other toxic elements (Sandhya, 2013). These results were also in line with that reported by Wallace (1993), Nwite et al. (2011); Qiang et al (2012); Guntamukkala et al. (2018).

The silicon application in soil resulted in increase in soil electrical conductivity (EC) after the experiment. This might be attributed to submergence, increase in solubility of salts present in the soil and due to the dissolution of silicon fertilizers. The increase in soil organic carbon (OC) was due to the reason that organic materials had direct impact on mineralization rate that increases soil organic carbon directly. This was in agreement with the findings of Njoku et al. (2011).

CONCLUSION

Application of Si in combination with chemical fertilizers resulted in higher number of effective tillers per plant, length of the panicle, grain yield and straw yield over chemical fertilizer alone. However, in case of 1000 grain wt. and harvest index, there was no significant difference between different doses of Si. This study also indicated an increase in pH and organic carbon in post-harvest soil due to application of silica as compared to initial soil test value. Hence it was suggested to go for application of silica for correcting soil acidity and improving soil organic carbon content in acidic laterite soils.

REFERENCES

Crooks R and Prentice P (2017). Extensive investigation into field-based responses to a silica fertilizer. Silicon, 9(2): 301-304

Dallagnol LJ, Rodrigues FA, Mielli MVB, Ma JF (2014). Rice grain resistance to brown spot and yield are increased by silicon. Tropical Plant Pathology, 39(1): 56-63

Detmann KC, Araújo WL, Martins SCV, Sanglard LMVP, Reis JV, Detmann E, Rodrigues FÁ, Nunes-Nesi A, Fernie AR, DaMatta FM (2012). Silicon nutrition increases grain yield, which, in turn, exerts a feed-forward stimulation of photosynthetic rates via enhanced mesophyll conductance and alters primary metabolism in rice, The New Phytologist 196(3): 752-762

Deren CW , Datnoff LE, Snyder GH and Martin FG (1994). Silicon concentration, disease response and yield components of rice genotypes grown on flooded organic histosols. Crop Science 34(3): 733-737

Donald CM (1962). In search of yield. 1. Aust. Inst. Agric. Sci. 238: 171-178

Epstein S (1994). Integration of the cognitive and the psychodynamic unconscious. American Psychologist 49(8): 709-724

Gascho GJ (2011). Silicon sources for agriculture. Silicon in Agriculture. Studies in Plant Science (8): 197-207

Guntamukkala Babu Rao, Poornima Yadav PI and Elizabeth K Syriac (2018). Effect of Silicon on Soil Physico-chemical Properties in Laterite derived Paddy Soils of Kerala, J Krishi Vigyan 6(2): 75-77

Gong H, K Chen, G Chen, S Wang and C Zhang (2003). Effects of silicon on growth of wheat under drought. Journal of Plant Nutrition 26: 1055-63

Gholami Y and A Falah (2013). Effects of two different sources of silicon on dry matter production, yield, and yield components of rice, Tarom Hashemi variety and 843 lines. International Journal of Agriculture and Crop Sciences 5: 227-31

Jackson ML (1973). Soil Chemical Analysis. Prentice Hall of India Private Limited, New Delhi

Jawahar S and Vaiyapuri V (2010). Effect of sulphur and silicon fertilization on growth and yield of rice. Int. J. Curr. Res. 9: 036-038

Lindsay WL and Norvell WA(1978). Development of DTPA soil test for Zn, Fe, Mn and Cu. Soil Sci. Soc. Am. J. 42: 421-428

Korndorfer GH and Lepsch I (2001). Effect of silicon on plant growth and crop yield. Silicon in Agriculture. L.E. Datnoff, G.H. Snyder, and G.H. Korndorfer (Editors) pp. 133-145

Meharg C, Meharg AA (2015). Silicon, the silver bullet for mitigating biotic and abiotic stress, and improving grain quality, in rice. Environmental and Experimental Botany 120: 8-17

Muriithi C, Mugai E, Kihurani A W, Nafuma C J and Amboga S (2010). Determination of silicon from rice by-products and chemical sources on rice blast management. 12th KARI Scientific Conference Proceedings

Njoku C, Mbah CN and Okonkwo CI (2011). Effect of rice mill wastes application on selected soil physical properties and maize yield on an ultisol in Abakaliki, southeastern Nigeria. Journal of Soil Science and Environmental Management 2(11): 375-383

Nwite JC, Obalum SE, Igwe CA and Wakatsuki T (2011). Properties and potential of selected ash sources for improving soil conditions and Sawah rice yields in a degraded in land valley in southeastern Nigeria. World Journal of Agricultural Sciences 7(3): 304-310

Orlov DS (1985). Humus acids of soils / D.S. Orlov Published for the United States Dept. of Agriculture and the National Science Foundation, Washington, D.C., by Amerind Pub. Co New Delhi

Page AL, Miller RH and Keeney DR (1982). Methods of Soil Analysis, Part-2, Second Edition, Soil Sci. Soc.Am. J., Madison, Wisconsin, USA

Patil AA(2018). Effect of silicon application along with chemical fertilizers on nutrient uptake and nutrient availability for rice plants, International Journal of Chemical Studies 6(1): 260-266

Piper C (1950). Soil and Plant Analysis. International Public Inc., New York

Prabhu AS, Barbosa Filho M P, Filippi MC, Datnoff LE and Snyder GH (2001). Silicon from rice disease control perspective in Brazil. Studies in Plant Science 8: 293-311

Prakash NB, Chandrashekar N, Mahendra C, Patil SU, Thippeshappa GN, Laane HM (2011). Effect of foliar spray of soluble silicic acid on growth and yield parameters of wetland rice in hilly and coastal zone soils of Karnataka, South India. Journal of Plant Nutrition 34(12): 1883-1893

Qiang FU, Hong H, Ming WU and Zheng CU (2012). Silicon-mediated amelioration of Fe2+ toxicity in rice (*Oryza sativa* L.) roots. Pedosphere 22(6): 795-802

Sommer M, Kaczorek D, Kuzyakov Y and Breuer J (2006). Review Article Silicon pools and fluxes in soils and landscapes-a review. J. Plant Nutr. Soil Sci. 169: 310-329

Shashidhar HE, Chandrashekhar N, Narayanaswamy C, Mahendra AC and Prakash NB (2008). Calcium silicate as silicon source and its interaction with Nitrogen in aerobic rice. IVth Silicon in Agriculture Conference Wild Coast Sun, KwaZulu-Natal, South Africa. pp. 93

Sandhya K (2013). Diatomaceous earth as a source of Silicon on growth and yield of Rice (*Oryza sativa* L.). M.Sc. (Ag) Thesis, University of Agricultural Sciences, Bengaluru pp. 164

Sawant AS, Patil VH and Sawant NK (1994). Rice hull ash applied to seedbed reduces dead hearts in transplanted rice. IRRN19(4): 21-22

Singh AK, Singh R and Singh K (2005). Growth, yield and economics of rice (*Oryza sativa*) as influenced by level and time of silicon application. Indian Journal of Agronomy 50(3): 190-193

Singh K, Singh R, Singh KK and Singh Y (2007). Effect of silicon carriers and time of application on rice productivity in a rice-wheat cropping sequence. IRRN 32(1): 30-31

Sudhakar PC, Singh JP and Singh K (2004). Effect of silicon sources and fertility levels on transplanted rice. IRRN 29(2): 61-63

Subbiah BV and Asija GL (1956). A rapid procedure for the estimation of available nitrogen in soil. Curr. Sci. 25: 259-260

Wei-min et al. (2005). Rapid Determination of Silicon Content in Rice .Rice Science 12(2): 145-147

Wallace A (1993). Participation of silicon in cation-anion balance as a possible mechanism for aluminium and iron tolerance in some gramineae. Journal of Plant Nutrition. 16: 547-553

Walkley and Black (1934). An examination of Digestion method for determining soil organic matter and a proposed modification ofthe chromic acid titration method. Soil Sci. 37: 29-37

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Weed control, yield and quality of small-grain scented rice as influenced by spacing and weed management in lower *gangetic* plains

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ABSTRACT

The effect of planting density and weed management on weed control, yield and quality of a non-Basmati type short-grained scented rice (cv. Radhunipagal) was studied at 'C' Block Farm of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal. The field experiment was conducted in a split-plot design comprising 2 spacings (15 cm × 15 cm and 20 cm × 15 cm) in main plots and 6 weed control practices [weedy check, 2 hand weeding (HW), Butachlor as pre-emergence (PE) + HW, Butachlor (PE) + 2,4-D Na-salt as post-emergence (PoE), HW + 2,4-D Na-salt (PoE), mechanical weeding + HW) in sub-plots during rainy (kharif) season of 2013 and 2014. Mean number of total weeds in 1 m2 field was 94.3 and 118.8 at 28 and 56 days after transplanting (DAT); and weed control efficiency varied among 5 weed management practices over weedy check as: 49.0-67.3% at 28 DAT and 56.5-72.8% at 56 DAT. Close planting (15 cm × 15 cm) resulted in 9.1% greater grain yield (2.27 vs. 2.08 t/ha), higher net income (Rs. 23,700 vs. 20,900/ha) and B:C ratio (1.79 vs. 1.73) over wider spacing (20 cm × 15 cm). Manual weeding twice at 21 and 42 DAT recorded better weed control efficiency (67.3 and 72.8% at 28 and 56 DAT), grain yield (2.51 t/ha), milling recovery (65.9%) and net return (Rs. 25,900/ha). But chemical weed control [Butachlor (PE) + 2,4-D Na-salt (PoE)] was adjudged as less expensive option (Rs. 27,300/ha) for Radhunipagal rice with moderate grain yield (2.16 t/ha), higher protein content (7.45%) and B: C ratio (1.90).

Key words: *Aromatic rice, grain quality, growth attributes, spacing, weed control, yield*

INTRODUCTION

Radhunipagal, meaning 'cook maddening aroma' (Watt, 1891), is a tall-indica type small-grained non-Basmati aromatic rice of West Bengal, whose early records of cultivation were found in district gazetteers (Hunter, 1875 and 1876) and book (Mukherji, 1901) during pre-independence period. The important characteristics of the variety include: long duration (145-155 days), tall stature (125-135 cm), small grain with straw colour and purple spot at tip, low test weight (10 g), short-bold type kernel and strong aroma (Ghosh, 2019). It is traditionally grown for a long period in *rahr* and gangetic alluvium region of South Bengal covering the districts of Birbhum, Bankura, Burdwan, Hooghly, Nadia, etc. Farmers in native areas cultivate Radhunipagal rice in medium or medium-lowlands following traditional methods intermixed with a few untested modern technologies in recent times. With morpho-genetic characterization of the variety (Pal et al., 2020) and considering its market potentiality, the RKVY project on 'Bengal Aromatic Rice' of Bidhan Chandra Krishi Viswavidyalaya (BCKV) and Government of West Bengal initiated the process of area expansion, technology-supported group cultivation and production-based marketing of Radhunipagal rice in the state.

Planting geometry depends on various factors such as plant type, season, age of seedlings and planting time. Spacing determines the plant population in unit area, thereby influencing the input-use efficiency and yield of the crop. Besides, weeds pose a major problem in rice production, as they compete with the crop particularly in early and mid-growth stages and also affect the grain quality to some extent. An estimate of IRRI showed that weed growth in unweeded plots reduced the yield by 34% in transplanted rice, 45% in direct seeded rainfed lowland rice and 67% in upland rice (De Datta, 1981). As timely weed control has become one of the crucial factors for realizing desired level of productivity, the efficient weed management is necessary to control different types of weeds occurring throughout the cropping period. Manual weeding in rice field is common in eastern India, but alternative chemical method are also gradually adopted due to scarcity of labourers and increased wages in recent times (Duary et al., 2015). In the context, integrated weed management (Butachlor 1.0 kg/ha fb hand weeding) was found equally effective to two hand weeding in Basmati rice cultivation in Uttar Pradesh, India (Kishore et al., 2016). On the other hand, chemical weed control either once with application of Ethoxysulfuron (Sunrice 15 WG) in transplanted aromatic rice field during aman season (Chowdhury et al., 2015) or twice with pre-emergence (Clean Master 18 WP) and post-emergence (Tiraz 10 WP) could result in greater grain yield over other weed management options in aromatic rice cultivation in Bangladesh (Islam et al., 2018). Keeping these in view, an effective, timely and economic weed control strategy along with optimum planting density needs to be developed for higher productivity and better grain quality of Radhunipagal rice in lower Gangetic plains of West Bengal.

MATERIALS AND METHODS

Experimental details

A field experiment was conducted during rainy (*kharif*) season of 2013 and 2014 at 'C' Block Farm (22°57' N, 88°22' E and 9.75 m above m.s.l.) of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Kalyani, Nadia,West Bengal. The soil of medium-lowland field was sandy-loam (order Entisol), neutral in reaction (pH 6.8), medium in organic C (0.57%) and available N (330 kg/ha), high P (45 kg/ha) and medium K (239 kg/ha).

The experiment was laid out in a split-plot design with 3 replications, where two spacing *viz*., 15 cm × 15 cm and 20 cm × 15 cm were assigned in main plots and 6 weed control practices *viz*., weedy check, 2 hand weeding (HW) at 21 and 42 days after transplanting (DAT), Butachlor 1.5 kg/ha as pre-emergence (PE) at 2 DAT followed by (*fb*) 1 HW at 42 DAT, Butachlor (PE) 1.5 kg/ha at 2 DAT *fb* 2,4-D Na-salt 1.5 kg/ha as post-emergence (PoE) at 42 DAT, 1 HW at 21 DAT *fb* 2,4-D Na-salt (PoE) 1.5 kg/ha at 42 DAT, and 1 mechanical weeding (MW) by paddy weeder at 21 DAT *fb* 1 HW at 42 DAT in sub-plots.

Seeds of Radhunipagal paddy collected from RKVY project on 'Bengal Aromatic Rice' of BCKV were sown 18-20 kg/ha in wet nursery. 28 days old seedlings 2-3/hill were transplanted as per spacing schedule at a shallow depth (3-4 cm) in puddled field in second fortnight of July. A uniform fertilizer dose consisting of 2 t FYM, 40 kg N, 20 kg P and 20 kg K/ha was applied across all the experimental plots. Weed control practices were adopted as per treatment schedule, while weedy check plots remained infested with native population of weeds till harvest. The crop was raised largely with south-west monsoon rain, but need-based irrigation was also given at critical stages. The crop was harvested manually, when 80-85% panicles became mature in the field.

Data collection and statistical analysis

Species-wise densities of weeds were counted in a quadrate (1 m × 1 m) in each plot at 28 and 56 DAT. The weed density data were transformed with square-root transformation [$\sqrt{(x + 0.5)}$] before statistical analysis. Weeds in quadrate were cut at ground level, washed with tap-water, sun-dried, oven-dried at 60-75±2°C for 48 hours and then weighed. Weed control efficiency was calculated by the following formula:

$$WCE = \frac{DW_c - DW_t}{DW_c} \times 100$$

where WCE, weed control efficiency; DW_c, dry weight of weeds in control plots; DW_t, dry weight of weeds in treated plots.

Growth attributes like tiller production, dry matter accumulation and crop growth rate (Watson, 1958) were recorded at different stages, while final plant height, yield attributes, grain and straw yield at maturity. Grain quality parameters like milling recovery (%) using Rice Milling Unit (Satake make, Japan) following standard method and protein content (total nitrogen × 5.95) (Sadasivam and Manickam, 1996) were determined at Aromatic Rice Laboratory of BCKV.

$$\text{Milling}\ (\%) = \frac{\text{Weight of milled rice}\ (g)}{\text{Weight of rough rice}\ (g)} \text{ x } 100$$

Aroma of milled rice was estimated in 0.1 N KOH solution following the method of Nagaraju et al. (1991), and the intensity of aroma was scored in a 3-point scale (1: mild, 2: medium, and 3: strong) by a panel of experts.

The cost of cultivation, gross return, net income and benefit:cost ratio were calculated based on the market prices of inputs and produces along with the related wages during the years of investigation.

The pooled analysis of recorded data was done using the Fisher's 'Analysis of Variance' technique as described by Gomez and Gomez (1984) and the mean differences were compared at 5% level of significance.

RESULTS AND DISCUSSION

Weed population and control efficiency

The puddled field with standing water suppressed the germination and growth of weeds to some extent, but common grasses, sedges and broad-leaf weeds appeared at different stages. The diversity of weed species included common grass *viz*., cut grass (Leersia hexandra), barnyard grass [*Echinochloa crusgalli* (L.) P. Beaur], jungle rice [*Echinochloa colona* (L.) Link]; sedges viz. globe finger rush (*Fimbristylis miliacea*), purple nut sedge (*Cyperus rotundus* L.), small flower umbrella sedge (*Cuperus difformis*), flat sedge (*Cyperus iria* L.); and board-leaf weeds *viz*., perennial water primerose (*Luduigia parviflora*), false daisy (*Eclipta alba*, Hassk.), etc. Similar weed flora in transplanted rice (cv. Pusa 1176) field at Pusa, Bihar was reported by Raj et al. (2016). Mean number of

Table 1. Effect of spacing and weed control method on weed population in scented rice (cv. Radhunipagal) field during rainy (*kharif*) season (pooled data of 2 years).

Treatment	Grass (no./m^2)		Sedge (no./m^2)		Broad leaf (no./m^2)		Total weeds (no./m^2)	
	28 DAT	56 DAT	28 DAT	56 DAT	28 DAT	56 DAT	28 DAT	56 DAT
Spacing								
15 cm × 15 cm	5.86b (35.80)	6.62a (45.97)	5.03b (25.39)	5.84b (35.04)	4.88b (23.98)	5.78b (34.00)	9.32b (89.81)	10.52b (115.19)
20 cm × 15 cm	6.05a (37.71)	6.73a (47.39)	5.26a (27.63)	6.02a (37.11)	5.24a (27.55)	6.11a (37.81)	9.81a (98.71)	10.86a (122.31)
CD (P<0.05)	0.11	NS	0.07	0.17	0.10	0.08	0.04	0.08
Weed control method								
Weedy check	8.75a (76.08)	10.01a (99.71)	6.41a (40.67)	8.43a (70.70)	6.68a (44.24)	7.97a (63.00)	13.30a (176.70)	15.29a (233.38)
2 HW at 21 and 42 DAT	4.87d (23.28)	5.16e (26.21)	4.42c (19.19)	4.85e (23.01)	4.39c (18.87)	4.93f (23.91)	7.92e (62.34)	8.58e (73.13)
Butachlor (PE) 1.5 kg/ha *fb* HW at 42 DAT	5.75bc (32.67)	5.43d (28.99)	5.10b (25.62)	5.25d (27.19)	4.87b (23.27)	5.17c (26.35)	9.06c (81.66)	9.10d (82.52)
Butachlor (PE) 1.5 kg/ha *fb* 2,4-D Na-salt(PoE) 1.5 kg/ha	5.81b (33.32)	6.74b (44.89)	5.20b (26.61)	5.78b (32.98)	4.96b (24.17)	6.07b (36.40)	9.32b (86.51)	10.71b (114.36)
HW at 21 DAT fb 2,4-D Na-salt (PoE) 1.5 kg/ha	4.94d (23.96)	6.74b (44.89)	4.54c (20.34)	5.80b (33.22)	4.51c (19.95)	6.02c (35.81)	8.54d (73.08)	10.72b (114.48)
MW 21 DAT *fb* HW at 42 DAT	5.63c (31.22)	5.96c (35.31)	5.21b (26.65)	5.46c (29.37)	4.95b (24.07)	5.51d (29.96)	9.26b (85.24)	9.74c (94.64)
CD (P<0.05)	0.16	0.10	0.18	0.13	0.16	0.15	0.13	0.11

Figures in the parentheses are original values. Data were transformed through square-root ($\sqrt{(x + 0.5)}$ method. DAT, Days after transplanting; *fb*, Followed by; HW, Hand weeding; MW, Mechanical weeding; PE, Pre-emergence; PoE, Post-emergence

Table 2. Effect of spacing and weed control method on weed dry weight, weed control efficiency and growth attributes of scented rice (cv. Radhunipagal) during rainy (*kharif*) season (pooled data of 2 years).

Treatment	Total weed dry weight (g/m^2)		Weed control efficiency (%)		Plant height at harvest (cm)	Tillers (no./m^2) at 56 DAT	Dry matter accumulation (g/m^2)		CGR at 28-56 DAT (g/m^2/ day)
	28 DAT	56 DAT	28 DAT	56 DAT			28 DAT	56 DAT	
Spacing									
15 cm × 15 cm	11.5b	14.0b	47.9b	54.4a	146.9a	300.5a	259.4a	401.6a	5.08a
20 cm × 15 cm	12.8a	14.9a	50.0a	55.5a	146.1a	233.2b	199.5b	307.0b	3.84b
CD (P<0.05)	0.32	0.23	1.66	NS	NS	12.63	5.94	6.27	0.40
Weed control method									
Weedy check	23.8a	32.1a	0.0f	0.0e	140.6c	243.9c	211.5d	322.8e	3.97c
2 HW at 21 and 42 DAT	7.8e	8.8e	67.3a	72.8a	154.0a	283.8a	250.3a	381.9a	4.70ab
Butachlor (PE) 1.5 kg/ha *fb* HW at 42 DAT	10.0c	10.2d	58.1c	68.2b	146.3b	268.0b	227.8b	345.6d	4.21bc
Butachlor (PE) 1.5kg/ha *fb* 2,4-D Na-salt(PoE)1.5kg/ha	12.0b	11.6c	49.0e	63.7c	147.0b	262.0b	232.0b	359.9c	4.57ab
HW at 21 DAT *fb* 2,4-D Na-salt (PoE) 1.5kg/ha	10.5c	13.9b	55.9d	56.5d	145.7b	266.2b	222.5c	347.4d	4.46b
MW at 21 DAT *fb* HW at 42 DAT	8.7d	10.1d	63.4b	68.5b	145.5b	277.0a	232.5b	368.2b	4.84a
CD (P<0.05)	0.50	0.41	1.78	0.85	2.37	7.03	7.13	3.30	0.29

DAT, Days after transplanting; *fb*, Followed by; HW, Hand weeding; MW, Mechanical weeding; PE, Pre-emergence; PoE, Post-emergence.

total weeds in 1 m^2 area was 94.3 and 118.8 at 28 and 56 DAT, respectively. The weed flora comprised 39.0 and 39.3% grasses, 28.1 and 30.4% sedges, and 27.4 and 30.2% broad-leaf weeds at 28 and 56 DAT, respectively in the experimental field (Table 1).

Spacing had significant influence on weed population in the study, because it affected the crop-weed competition particularly at early and mid-early stages. Close spacing (15 cm × 15 cm or 44 hills/m^2) resulted in less population of grasses, sedges and broad-leaf weeds at 28 and 56 DAT compared to wide-spaced plots (20 cm × 15 cm) (Table 1). Similarly, Mahata (2014) reported lowest population of weeds in plots of close spacing (20 cm × 10 cm) compared to widely spaced ones (15 cm × 15 cm and 20 cm × 15 cm) in Gobindabhog rice field. Although all five weed management treatments reduced the total weed density compared to weedy check; but hand weeding twice was most effective to keep the population of weeds at the lowest level compared to chemical (Butachlor *fb* 2,4-D Na-salt) or integrated weed management practices (Butachlor *fb* hand weeding, hand weeding *fb* 2,4-D Na-salt, and mechanical weeding *fb* hand pulling). Although hand pulling caused maximum reduction in total weed density (62.34/m^2 at 28 DAT and 73.13/m^2 at 56 DAT) compared to other weed control methods, but it involved intense labour thereby increasing the cost of cultivation. The lower efficiency of mechanical method compared to hand pulling might be due to inefficient weeding in intra-row spaces by the wheel hoe used in the experiment. Kishore et al. (2016) reported that two hand weeding or Butachlor *fb* hand weeding were similarly effective for suppression of weed growth in Basmati rice field. But Mandal et al. (2013) reported that Pyrazosulfuron-ethyl in combination with cono-weeder recorded the lowest weed population and dry weight at 40 DAS/DAT in Basmati 370 rice field in lateritic belt of West Bengal.

Planting density had significant effect on weed control efficiency (WCE) of scented rice (cv. Radhunipagal) at 28 DAT only (Table 2), but WCE varied among 5 weed management practices (manual weeding twice, Butachlor fb hand weeding, Butachlor *fb* 2,4-D Na-salt, hand weeding *fb* 2,4-D Na-salt and mechanical weeding fb hand pulling) over the weedy check as: 49.0-67.3% at 28 DAT and 56.5-72.8% at 56 DAT. Hand weeding twice recorded highest WCE at early and mid-phases of crop growth, which was followed by the treatment comprising mechanical and manual weed control.

Growth attributes

Close spacing (15 cm × 15 cm) resulted in significantly greater tiller production at 56 DAT (300.5/m^2) and dry matter accumulation (259.4 and 401.6 g/m^2 at 28 and 56 DAT) due to more number of hills per unit area (44/m^2) than the wider spacing (20 cm × 15 cm, 33/m^2) in the study (Table 2). Field observation revealed that the crop in widely spaced plots (33 hills/m^2) produced greater number of tillers at 56 DAT (7.1 vs. 6.8/hill) due to less plant to plant competition and better input use efficiency than the closely planted crop (44 hills/m^2). Among six weed management practices, manual weeding twice at 21 and 42 DAT recorded the highest plant height, maximum tiller number and dry matter production in unit area throughout the cropping period; while the undisturbed weed growth in weedy check plot retarded the growth of Radhunipagal rice plants, that was reflected by poor growth attributes. The pre-emergence application of Butachlor 1.5 kg/ha *fb* either hand weeding or 2,4-D Na-salt could not control all the weeds in the field leading to lesser values of growth attributes compared to other weed control methods, excluding weedy check. Mechanical weeding *fb* hand weeding recorded the highest crop growth rate at 28-56 DAT (4.84 g/m^2/day) being at par with hand weeding twice at 21 and 42 DAT and chemical weed control through Butachlor *fb* 2,4-D Na-Salt in the study.

Yield components and grain yield

Close square planting (15 cm × 15 cm) resulted in greater number of panicles (299/m^2), but recorded less filled grains/panicle (121.6) compared to wider spacing (217/m^2 and 125.4) (Table 3). Hand pulling twice in Radhunipagal rice field favoured the production of panicles (270/m^2) and filled grains/panicle (127.5) compared to other weed control methods and weedy check adopted in the experiment. Test weight remained unaffected due to variation in planting density and weed control practices in the experiment.

Closer spacing (15 cm × 15 cm) resulted in significantly 9.1% greater yield of Radhunipagal rice (2.27 vs. 2.08 t/ha) compared with wider spacing (20 cm × 15 cm), which could be supported by Singh et al. (2012). Among six weed management practices, manual weeding twice favoured the panicle production (270/m^2) and filled grains/panicle (127.5), which led to maximum grain yield (2.51 t/ha). Based on grain yield, six weed control methods could be arranged as: manual weeding twice (2.51 t/ha) > Butachlor *fb* hand weeding (2.23 t/ha) > mechanical weeding *fb* hand pulling (2.19 t/ha) > Butachlor *fb* 2,4-D Na-salt (2.16 t/ha) > hand weeding *fb* 2,4-D Na-salt (2.10 t/ha) > Weedy check (1.88 t/ha). Superiority of hand weeding with regard to grain yield (2.51 t/ha) over integrated and chemical weed control methods could be ascribed to less competition of weeds due to almost elimination of weeds from the field, and better crop growth and yield components. Similarly, Chander and Pandey (2001) reported that hand weeding significantly increased the

Table 3. Effect of spacing and weed control method on yield components and yield of scented rice (cv. Radhunipagal) during *kharif* season (pooled data of 2 years).

Treatment	Panicle/m^2	Field grains/ panicle	Grain yield (t/ha)			Straw yield (t/ha)
			2013	2014	Pooled	
Spacing						
15 cm × 15 cm	299a	121.6b	2.24a	2.29a	2.27a	5.64a
20 cm × 15 cm	217b	125.4a	2.15a	2.02b	2.08b	5.43b
CD (P<0.05)	20.0	1.51	NS	0.05	0.05	0.09
Weed control method						
Weedy check	232b	117.9d	1.95c	1.80	1.88c	5.19b
2 HW at 21 and 42 DAT	270a	127.5a	2.47a	2.55a	2.51a	5.63a
Butachlor (PE) 1.5 kg/ha *fb* HW at 42 DAT	262a	122.1c	2.27b	2.19ab	2.23b	5.54a
Butachlor (PE) 1.5kg/ha *fb* 2,4-D Na-salt (PoE) 1.5 kg/ha	263a	124.0bc	2.14bc	2.18ab	2.16b	5.68a
HW at 21 DAT *fb* 2,4-D Na-salt (PoE) 1.5 kg/ha	260a	124.6b	2.18b	2.01b	2.10b	5.61a
MW at 21 DAT *fb* HW at 42 DAT	261a	125.2a	2.15bc	2.23ab	2.19b	5.57a
CD (P<0.05)	11.7	2.43	0.17	0.38	0.20	0.22

DAT, Days after transplanting; *fb*, Followed by; HW, Hand weeding; MW, Mechanical weeding; PE, Pre-emergence; PoE, Post-emergence. Selling price in district market, Grain Rs.1800/q (2013) and 1950/q (2014); Straw Rs.1800/q(2013 and 2014) grain yield of scented rice (cv. Pusa Basmati 1) compared with herbicides and weedy check at New Delhi. But Anwar Bhat (2017) found that combined application of Pretilachlor + Pyrazosulfuron recorded considerably higher grain yield of rice (var. Jhelum) than Butachlor and weedy check in temperate conditions of Kashmir. There was no definite trend of variation in straw yield among five different weed control methods because they were more or less equally good to produce similar straw yield (5.54-5.68 t/ha) over the weedy check (5.19 t/ha). Due to stiff competition between weeds and Radhunipagal rice plants in weedy check plots, the lowest values of yield attributes, grain and straw yields were recorded.

Grain quality

Planting density could not affect the grain quality parameters like milling recovery and protein content of Radhunipagal rice in the investigation (Table 4). Manual weeding twice usually resulted in highest milled rice yield (65.9%) closely followed by the use of herbicides (65.4%) but significantly greater over mechanical fb manual weeding (64.1%) in Radhunipagal rice field in the investigation. Akbar et al. (2011) reported higher percentage of normal kernels of fine rice variety (cv. Super Basmati) in hand pulling and mechanical hoeing compared to herbicidal treatments and weedy check at Faisalabad, Pakistan. The chemical weed control method (Butachlor *fb* 2,4-D Na-salt) recorded the highest protein content (7.45%) followed by integrated weed management (hand weeding *fb* 2,4-D Na-salt) in the study. On the contrary, Kumar et al. (2007) found that three hand weedings could result in highest protein content of Pusa Sugandha 3 rice compared to other weed control methods at New Delhi. The intensity of aroma was not significantly influenced by spacing and weed management, but aroma scores among treatments noted in the study indicated that Radhunipagal rice generally had medium-strong aroma (score ± 2.35).

Economics

The total cost of cultivation for closely spaced (15 cm × 15 cm) Radhunipagal rice crop was slightly higher (Rs. 30,000 vs. 29,000/ha) than widely spaced one (20 cm × 15 cm) due to variation in seed rate (20 vs. 18 kg/ha) and labour required for row-transplanting (40 vs. 36 mandays/ha) in the study (Table 4). Among weed control practices, manual weeding twice resulted in highest cost of cultivation (Rs. 32,400/ha), while minimum expenditure (Rs. 27,000/ha) was noted with weedy check. The cost of cultivation in second year was higher mainly due to increase in input cost and labour wages than first year.

Gross income included the values of both grain and straw, where return from grain and straw of Radhunipagal paddy in the experiment would be summarized in the ratio of 3.5-4:1. The variation in gross return among 6 weed control methods ranged between Rs. 45,500/ha (weedy check) and Rs. 58,300/ha (hand weeding twice); while the closely planted paddy (15

Table 4. Effect of spacing and weed control method on grain quality and economics of scented rice (cv. Radhunipagal) during *kharif* season (pooled data of 2 years).

Treatment	Milling (%)	Protein (%)	Aroma (score)	Total cost of cultivation (×10^3 Rs./ha)	Gross income (×10^3 Rs./ha)	Net return (×10^3 Rs./ha)	B:C ratio
Spacing							
15 cm × 15 cm	64.9a	6.97a	2.36a	30.0	53.7	23.7	1.78
20 cm × 15 cm	65.3a	7.08a	2.33a	29.0	49.9	20.9	1.73
CD (P<0.05)	NS	NS	NS				
Weed control method							
Weedy check	65.3a	6.73b	2.31a	27.0	45.5	18.5	1.69
2 HW at 21 and 42 DAT	65.9a	6.87b	2.42a	32.4	58.3	25.9	1.80
Butachlor (PE) 1.5 kg/ha *fb* HW at 42 DAT	64.9ab	7.00b	2.35a	29.9	52.8	23.0	1.78
Butachlor (PE) 1.5 kg/ha *fb* 2,4-D Na-salt(PoE) 1.5 kg/ha	65.4a	7.45a	2.34a	27.3	51.8	24.5	1.90
HW at 21 DAT *fb* 2,4-D Na-salt (PoE) 1.5 kg/ha	64.9ab	7.09ab	2.30a	30.4	50.4	20.0	1.66
MW at 21 DAT *fb* HW at 42 DAT	64.1b	7.03b	2.36a	30.2	52.2	22.0	1.73
CD (P<0.05)	1.07	0.41	NS				

cm × 15 cm) recorded higher gross return (Rs. 53,800 vs. 49,900/ha) and income (Rs. 23,700 vs. 20,900/ha) compared to widely spaced crop (20 cm × 15 cm). Manual weeding twice resulted in maximum net return (Rs. 25,900/ha) closely followed by chemical weed control (Butachlor 1.5 kg/ha *fb* 2,4-D Na-salt), and these two weed management practices recorded higher B:C ratio (1.80 and 1.90) than other methods in the experiment. However, Singh et al. (2008) also reported that two hand weeding increased the grain yield of Pusa Basmati 1, but it had lower net return and B:C ratio on account of higher cost involved.

CONCLUSION

Based on the findings, it can be concluded that close planting (15 cm × 15 cm) resulted in 9.1% greater grain yield (2.27 vs. 2.08 t/ha), higher net income (Rs. 23,700 vs. 20,900/ha) and B:C ratio (1.79 vs. 1.73) over wider spacing (20 cm × 15 cm). Manual weeding twice recorded better weed control efficiency, higher grain yield (2.51 t/ha), milling recovery (65.9%) and net return (Rs. 25,900/ha) compared to chemical or integrated weed management adopted in the study. However, chemical weed control [Butachlor (PE) + 2,4-D Na-salt (PoE)] might be a less expensive option (Rs. 27,300/ha) for Radhunipagal rice with moderate grain yield (2.16 t/ha), higher protein content (7.45%) and B:C ratio (1.90).

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REFERENCES

Akbar N, Jabran EK and Ali MA (2011). Weed management improves yield and quality of direct seeded rice. Australian Journal of Crop Science 5(6): 688-694

Anjum SA, Tanveer M, Akbar N, Ashraf U, Ali I, Zohaib A and Manzoor N (2017). Comparative efficacy of various weed control measures in weed dynamics, yield and profitability of direct seeded fine rice. Pakistan Journal Agricultural Science 54(1): 129-134

Anwar Bhat A, Hussain M, Ganai MA, Jehangir IA and Teli NA (2017). Bioefficacy of pyrazosulfuron and bensulfuron methyl in combination with pretilachlor against weeds in transplanted rice (*Oryza sativa* L.) under temperate conditions of Kashmir. Journal of Crop and Weed 13: 178-182

Chander S and Pandey J (2001). Effect of rice (*Oryza sativa*) culture, nitrogen and weed control on nitrogen comprtition between scented rice and weeds. Indian Journal of Agronomy 46: 68-74

Chowdhury IF, Ali MH, Karim MF, Masum SM and Rahman A (2015). Weed control strategies affecting yield potential of aromatic rice. Pakistan Journal of Weed Science Research 21(4): 453-466

Duary B, Mishra MM, Dash R and Teja KC (2015). Weed management in low land rice. Indian Journal of Weed Science 47(3): 224-232

De Datta SK (1981). Principles and Practices of Rice Production. John Willey and Sons, New Delhi

Ghosh M (2019). Aromatic Rice of West Bengal: Diversity, Production and Marketing (in Bengali). Mehanati Publications, Hooghly, West Bengal, India

Gomez KA and Gomez AA (1984). Statistical Procedures for Agricultural Research. John Willey and Sons, Singapore

Hunter WW (1875). Statistical Account of Bengal: Districts of 24 Parganas and Sundarbans, Vol. I, Trubner & Co., London

Hunter WW (1876). Statistical Account of Bengal: Districts of Midnapur and Hugli (including Howrah), Vol. III, Trubner & Co., London

Islam AKMM, Hia MAUH, Sarkar SK and Anwar MP (2018). Herbicide based weed management in aromatic rice of Bangladesh. Journal of Bangladesh Agricultural University 16(1): 31-40

Kishore R, Dwivedi A, Singh R, Naresh RK, Kumar V, Bankoti P, Sharma DK and Yadav N. (2016). Integrated effect of population and weed management regimes on weed dynamics, performance and productivity of basmati rice (*Oryza sativa* L.). Paddy, Water and Environment 1-13

Kumar A, Shivay YS and Pandey J (2007). Effect of crop establishment methods and weed control practices on weed dynamics, productivity, nutrient removal by weeds vis-à-vis crop and quality of aromatic rice (*Oryza sativa*). Indian Journal of Agricultural Science 77(3): 179-183

Mahata D (2014). Improvement of production and storage system for Gobindabhog rice in West Bengal. Ph. D. Thesis, Department of Agronomy, Bidhan

Chandra Krishi Viswavidyalaya, Mohanpur, Nadia, West Bengal, India

Mandal MK, Duary B and De GC (2013). Effect of crop establishment and weed management practices on weed growth and productivity of Basmati rice. Indian Journal of Weed Science 45(3): 166-170

Mukherji NG (1901). Handbook of Indian Agriculture, Hare Press, Calcutta, India

Nagaraju M, Mohanty KK, Chaudhury D and Gangadharan C. (1991). A sample screening technique to detect scent in rice. Oryza 28: 109-110

Pal AK, Ghosh M, Das B, Roy K, Dolui S and Ghose TK (2020). Morpho-genetic characterization of scented Radhunipagal rice landraces of South Bengal. Indian Journal of Plant Genetic Resources 33(2): 154-160

Raj R, Kumar A, Kumar V, Singh CB and Pandey UC (2016). Herbicide options for controlling weeds in transplanted rice (*Oryza sativa*) under North Eastern Plains Zone. Indian Journal of Agronomy 61: 197-203

Sadasivam S and Manickam A (1996). Biological Methods. 2nd Ed. New Age International Publishers, New Delhi, India

Singh N, Kumar D, Thenua OVS and Tyagi VK. (2012). Influence of spacing and weed management on rice (*Oryza sativa*) varieties under system of rice intensification. Indian Journal of Agronomy 57(2): 138-142

Singh P, Singh P and Singh SS (2008). Production potential and economic analysis of direct wet seeded aromatic rice (*Oryza sativa* L.) cv. Pusa Basmati 1 as influenced by fertility levels and weed management practices. Oryza 45(1): 23-26

Watson DJ (1958). The dependence of net assimilation rate on leaf area index. Annals of Botany (N.S.) 22: 22-54

Watt G (1891). A Dictionary of the Economic Products of India (Vol. V), Cosmo Publications, Delhi, India

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Effect of irrigation and nitrogen management on water productivity and nutrient uptake of aerobic rice

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ABSTRACT

Water and nitrogen fertilizer are the two important inputs for rice cultivation. A field experiment was carried out in split plot design, with three water management treatments, irrigation at (i) 75 % Cumulative Pan Evaporation (CPE), (ii) 100% CPE, (iii) 125% CPE in the main plot, and four nitrogen management strategies, (i) 100% nitrogen applied through chemical fertiliser, (ii) 75% through chemical fertiliser + 2.5 t ha^{-1} vermicompost, (iii) 50% through chemical fertiliser + 5.0 t ha^{-1} vermicompost, and (iv) 25% through chemical fertiliser +7.5 t ha^{-1}vermicompost in subplot. The grain yield, straw yield, water productivity, nutrient uptake and economics of aerobic rice were considerably affected by irrigation and nitrogen management. Crops receiving irrigation at 125% CPE had the highest grain yield (3618 kg ha^{-1}), which was comparable to crops receiving irrigation at 100 % CPE. Regardless of irrigation and nitrogen management, the straw yield of aerobic rice ranged from 4127 to 5092 kg ha^{-1}. The highest total NPK uptake by grain was recorded with nitrogen at 100 % N through fertiliser (N_1), which was significantly greater than what was obtained from other nitrogen treatments and irrigation management at 100 % CPE (I_{100}) and 125 % CPE (I_{125}) was significantly better than irrigation at 75 % CPE (I_{75}). Irrigation management at 125 % CPE yielded the highest gross return, net return, and B: C ratio of aerobic rice, which was comparable to 100 % CPE and 100 % N through fertiliser, 75 % N through fertiliser + 2.5 t ha^{-1} vermicompost, and significantly higher than other nitrogen treatments.

Key words: *Aerobic rice, irrigation, nitrogen, water productivity, NPK uptake, economics*

INTRODUCTION

Among all the cultivated crops, rice (*Oryza sativa* L.) is the most important food grain crop on the world, and it provides a staple diet for more than half of the world's population. Rice is not only a staple food, but it is also a major economic crop and a big source of employment and money in rural areas. India is first in the world in rice acreage, with 43.78 million hectares, and second only to China in overall paddy production, with 118.4 million tonnes in 2019-20 (Anonymous, 2019-20). Rice offers 30-75 % of total calories to more than 3 billion Asians (Khush, 2004). Due to competition among agricultural, industrial, environmental, and home users, a water crisis is brewing (Ramana et al.,, 2007). When there is an erratic rainfall for storage, water supplies will be vulnerable as a result of climate change (Chan and Chong, 2009). Lack of fresh water will pose issues for rice production in the near future as a results direct seeded rice or aerobic rice culture is becoming a more popular alternative to transplanting in India, and it now covers about one-third of the country's total rice acreage. Producing more rice with less water is thus a daunting issue for the region's food, economic, social, and water security. The chances of increasing the rice-growing area in the near future are limited. To maintain rice production when water catchment areas are shrinking and water supply is insufficient, now it is necessary to develop alternative cultivars and growing methods with high water use efficiency. To meet rising rice demand with dwindling resources, rice yields must be improved per unit area while using less water.

The name "aerobic rice" was invented by the International Rice Research Institute, while the notion of aerobic rice was initially developed in China (Bouman and Tuong, 2001). Rice water productivity is 32-88 % higher in aerobic circumstances than under flooded conditions (Bouman et al., 2005). Aerobic rice systems, in which the crop is developed by direct sowing in non-flooded, non-puddled fields, are one of the most promising ways to save water (Singh et al., 2008). Aerobic rice is special because in transplanted paddy for land preparation a large amount of water is required (Nayak et al., 2015). When rice is grown under flooded condition, 78 per cent of the roots die at the flowering stage as compared to that of under aerated condition (Barison, 2002). Practically in all conditions, nitrogen is the most important and limiting nutrient for rice development and yield (Yan et al., 2022); as a result, rice receives an enormous amount of the total nitrogen given to crops (Spiertz, 2010). Nitrogen fertiliser in rice is expensive all over the world, but it is quickly lost through several channels such as runoff, volatilization, leaching, and denitrification before it is used by the rice crop, nitrogen utilisation efficiency is often low (30-40%) in traditional rice agriculture (Leon and Kohyama, 2017). The management of nitrogenous fertiliser has a significant impact on rice production, as it maximises yield attributing features (Guo et al., 2017 and Nayak et al., 2015). Nitrogen also significantly enhances the consumption of phosphate and potassium (Brady, 1999). Therefore, the current study was conducted with the following objectives:

- ♦ To assess the effect of irrigation and nitrogen management on yield and water productivity
- ♦ To investigate the nutrient uptake of aerobic rice under different irrigation and nitrogen management
- ♦ To work out economics of aerobic rice under irrigation and nitrogen management.

MATERIALS AND METHODS

The purpose of this study was to see how irrigation and nitrogen management affected yield, NPK uptake, and economics of aerobic rice and the experiment was conducted in the PSB farm Sriniketan. The experimental site falls under sub-humid tropical zone of Eastern India, in the western section of West Bengal. Summer or pre-*kharif* (March to June), rainy or *kharif* (July to October), and winter or *rabi* (November to February) are the three main seasons in this zone, with a total rainfall of 833.36 mm received over the cropping period. The soil texture of experimental field was sandy loam, with a pH of 6.1, organic carbon of 0.49 mg kg^{-1}, available N of 136 kg ha^{-1}, available P of 11.5 kg ha^{-1}, and available K of 160.5 kg ha^{-1}.

This experiment with split plot design was carried out with three irrigations (I_{75}=Irrigation at 75 % of CPE, I_{100}= irrigation at 100 % of CPE and I_{125}= irrigation at 125 % of CPE), and four nitrogen levels N_1=100 % N through fertiliser (100 kg ha^{-1}), N_2= 75 % N through fertiliser + 2.5 t ha^{-1} vermicompost, and N_3= 50 % N through fertilizer + 5.0 t ha^{-1} vermicompost and N_4= 25 % N through fertilizer +7.5 t ha^{-1} vermicompost in the sub plots. The initial tillage operation was performed to clear the land of all weeds and other plant remains from the previous crop. After 15 days of the first ploughing, the field received a second tillage and light irrigation to aid in the germination of weed seeds and previous crop seeds. To get rid of weeds and previous crops, the third ploughing was done three days before seeding. On 6th April, 2016, after thorough site preparation, Shabhagi Dhan at a seed rate of 45 kg ha^{-1} was sown under optimal soil moisture conditions. Thinning and gap filling were done 20 days after sowing to maintain an optimal and homogeneous plant population.

Full dose of phosphorus (50 kg P_2O_5 ha^{-1}) and half dose of potash (25 kg K_2O ha^{-1}) were put in the rows about 4-5 cm deep at the base and the remaining half dose of potash (25 kg K_2O ha^{-1}) was administered at 60 DAS. Half of the nitrogen dose was delivered at 20 DAS, and the remaining half dose was applied in two equal doses at 40 DAS and 60 DAS. According to the treatment, vermicompost was applied at the time of final land preparation. Irrigation water volume of each plot was calculated by multiplying the USWB Open Pan Class A evaporimeter reading from the net plot area. 90° V-notch weirs were installed in the pucca channel of the experimental field to determine the amount of irrigation water.

The rate of discharge through 90° V-notch was calculated as per the formula:

$$Q = 0.0138 \times H^{5/2}$$

where, Q is the rate of discharge (litre per second) and H is the head of the crest (cm). The time of irrigation for every plot was calculated by using given depth of irrigation, area of the plot and discharge rate and the formula is given below:

$$T = A * D/Q$$

Where, Q is the discharge rate (litre per second), A is the plot area (m^2), D is the CPE value (mm) and T is the irrigation time (sec or min).

I_{100}: Irrigation at 100% CPE means that the amount of irrigation water to be given is equal to the amount of water that actually evaporated (as measured by a USWB Open Pan Class A Evaporimeter) during the irrigation interval, and the time of irrigation is determined by a visible symptom, such as the first top leaf tip rolling (Parthasarathi et al., 2012).

I_{75}: 75 per cent of I_{100}

I_{125}: 125 per cent of I_{100}

Plants were collected from the delimited net plot area, bundled into bundles, and carried to the threshing floor for drying and threshing. The plants were collected and dried in the open for 3-4 days to reduce the moisture content to roughly 14%. The seeds were cleaned and sundried after threshing, and their weight was measured in kilogrammes plot^{-1} and represented as kg ha^{-1}. The NPK content of grain and straw was calculated in the lab. The total intake at harvest was estimated by summing the grain and straw uptake numbers.

Nutrient uptake by grain/straw (kg ha^{-1}) =(Nutrient content in grain/straw (%) x Grain /Straw yield/ (kg /ha))/100

Total nutrient uptake (kg ha^{-1}) = Nutrient uptake by grain (kg ha^{-1}) + Nutrient uptake by straw (kg ha^{-1})

The cost of cultivation (ha^{-1}) was calculated using the market prices for various agro inputs such as labour, fertiliser, vermicompost, pesticides, herbicides, irrigation, and other necessary items. For each treatment, the gross return (ha^{-1}) was calculated by converting the harvested produce into monetary terms using the current market price. The net return (ha^{-1}) is computed by deducting the cost of cultivation from each treatment's gross return. Benefit: Cost ratio was determined with dividing gross return by total cultivation costs.

B: C ratio = (Gross return)/(Total cost of cultivation)

To draw a meaningful conclusion, the experimental data acquired for various parameters under research were subjected to statistically analysed by ANOVA provided by Gomez and Gomez (1984). Least significant difference (LSD) values at a 5% level of significance were used to examine the variation in the treatments mean.

RESULT AND DISCUSSION

Water productivity

The water requirement of aerobic rice grown at different irrigation and nitrogen management was calculated by adding effective rainfall during crop period and amount of irrigation water applied. The data on water productivity in aerobic rice cultivation was statistically analysed, whereas water requirement was the average mean value during the experiment and presented in the Table 1 irrespective of irrigation regimes and nitrogen management.

The result showed that water productivity of aerobic rice was significantly influenced by various irrigations management during the experiment.

Table 1. Effect of irrigation and nitrogen management on water productivity.

Treatments	Grain yield (kg ha^{-1})	Water use (mm)	Water productivity (kg/ha-mm)
Irrigation management			
I_{75}	2998	348.08	8.61
I_{100}	3502	464.11	7.55
I_{125}	3618	580.08	6.24
SEm (±)	**80**	--	**0.19**
CD at 5%	**314**	--	**0.76**
Nitrogen management			
N_1	3655	464.09	8.09
N_2	3740	464.09	8.34
N_3	3426	464.09	7.65
N_4	2670	464.09	5.78
SEm (±)	**68**	--	**0.14**
CD at 5%	**204**	--	**0.41**

Table 2. Effect of irrigation and nitrogen management on NPK uptake of aerobic rice.

Treatments	Nutrient uptake by grain (kg ha^{-1})			Nutrient uptake by straw (kg ha^{-1})			Total NPK uptake by grain	Total NPK uptake by straw
	N	P	K	N	P	K	(kg ha^{-1})	(kg ha^{-1})
Irrigation management								
I_{75}	41.0	17.2	8.3	25.5	6.1	77.6	66.6	109.2
I_{100}	54.5	23.0	11.8	30.9	10.1	109.8	89.3	150.9
I_{125}	53.6	21.8	10.4	30.6	8.0	108.4	85.9	147.1
SEm (±)	1.0	0.7	0.6	0.7	0.4	2.4	1.4	3.0
LSD at 5%	3.9	2.6	2.2	2.8	1.7	9.2	5.7	11.9
Nitrogen management								
N_1	56.6	23.9	11.8	34.7	9.6	117.4	92.2	161.6
N_2	55.5	22.9	11.5	30.1	9.2	105.0	89.9	144.3
N_3	49.1	20.6	9.7	26.8	7.7	89.1	79.3	123.6
N_4	37.7	15.4	7.8	24.4	5.7	83.1	60.9	113.2
SEm (±)	1.6	0.6	0.6	0.9	0.5	4.2	2.1	5.0
LSD at 5%	4.7	1.8	1.7	2.7	1.6	12.6	6.4	14.8

Nitrogen management played an important role for increasing per cent of water productivity in aerobic rice. The maximum value was recorded with N_2 and there was no significant difference between N_1 and N_2 and N_2 also was at par with N_3 and N_3 with N_4.

Grain and straw yield

The effect of various treatments had profound effect on the grain yield and straw yield of aerobic rice (Fig. 1 and Fig. 2) during the experiment. Result showed that I_{125} recorded the highest grain yield of 3618 kg ha^{-1} which was at par with I_{100} (3502 kg ha^{-1}). I_{100} and I_{125} recorded the higher grain yield, might be due to higher growth and yield attributes as well conducive situation for efficient water uptake which boost their growth and yield attributes through supply of more photosynthates at the reproductive sink. Lowest grain yield of 2998 kg ha^{-1} with 75% of CPE (I_{75}) could be due to the significant reduction in photosynthetic rate as a result reduced production of assimilates for growth of panicles and filling of rice grains; ultimately yield was drastically decreased. This result was in

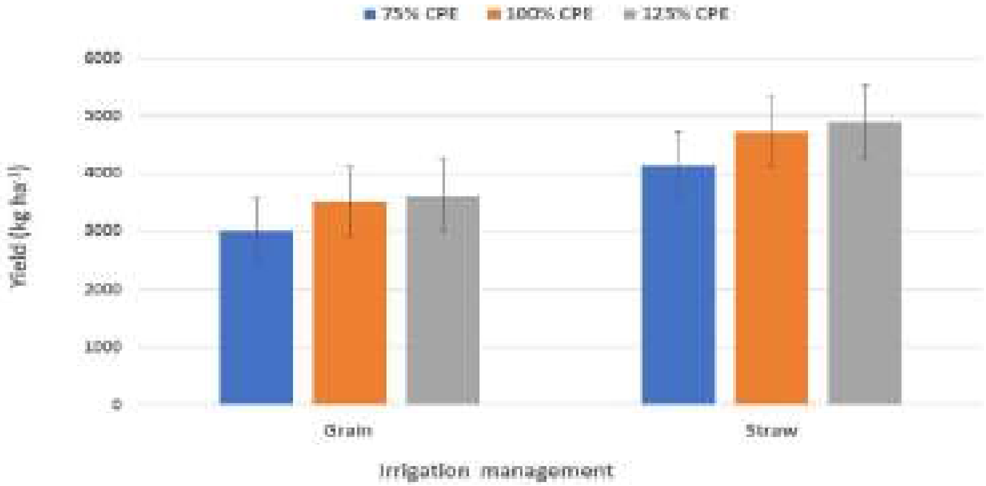

Fig.1. Grain yield and straw yield (kg ha^{-1}) of aerobic rice as influenced by irrigation management.
75% CPE- 75% Cumulative pan evaporation, 100% CPE-100% Cumulative pan evaporation, 125% CPE- 125 % Cumulative pan evaporation.

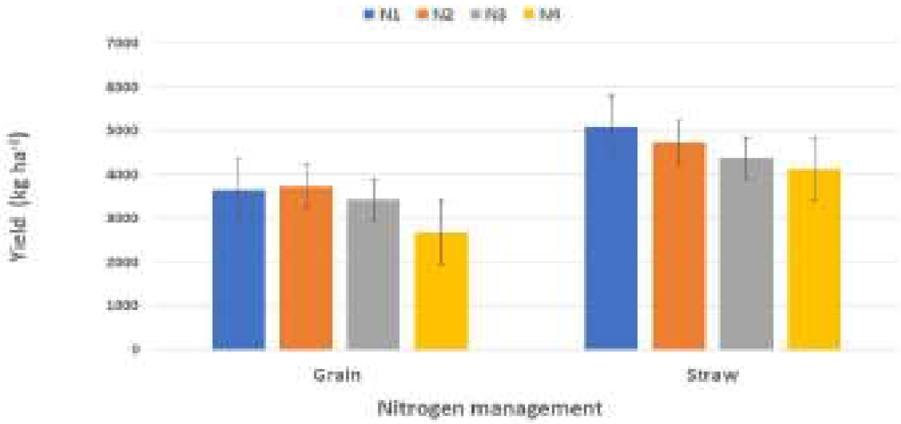

Fig. 2. Grain and straw yield (kg ha^{-1}) as influenced by nitrogen management.
N_1-100 % nitrogen was obtained by fertiliser, N_2-75 % through fertiliser + 2.5 t ha^{-1} vermicompost, N_2-50 % through fertiliser + 5.0 t ha^{-1} vermicompost, N_4 -25 % through fertiliser +7.5 t ha^{-1} vermicompost.

collaborates with the findings of Mandal et al. (2013), Duary and Pramanik (2019) and Mondal and Pramanik (2020).

The maximum grain yield of 3740 kg ha^{-1} was recorded with N_2 which was at par with N_1 (3655 kg ha^{-1}). Significant increase in grain yield N application due to enhance uptake of N, P and K in plant and ultimately accelerate photosynthetic activities resulting in better growth and yield and these findings are similar with those of Seema et al. (2014) and Nayak et al. (2015).

The highest straw yield was recorded with I_{125} (4888 kg ha^{-1}) followed by I_{100} (4723 kg ha^{-1}) and I_{75} (4137 kg ha^{-1}). The N_1 recorded maximum straw yield of 5092 kg ha^{-1}. There was no significant difference between N_2 (4734 kg ha^{-1}) and N_1 and N_2 also was at par with N_3 (4377 kg ha^{-1}).

NPK uptake by grain

Irrigation and nitrogen management showed significant influence on nitrogen (N), phosphorus (P) and potassium (K) uptake by grain (Table 2). Results showed that I_{125} and I_{100} recorded significantly higher N, P and K uptake over I_{75}. N_1 recorded highest N uptake in grain but it was at par with N_2, however, N_2 recorded significantly higher N uptake than N_3 and N_4. Higher amount of N might have helped in inducing good vegetative growth and root system which increased higher uptake of NPK in grain. These findings are in close conformity with those of Seema et al. (2014) and Nayak et al. (2015). I_{100} recorded higher total NPK uptake in grain (89.3 kg ha^{-1}) over I75 (66.6 kg ha^{-1}). N_1 recorded highest NPK uptake (92.2 kg ha^{-1}) over N_2 and N_4 (60.9 kg ha^{-1}).

NPK uptake by straw

As illustrated in Table 2, N, P and K uptake in straw significantly influenced with irrigation managements. I_{125} and I_{100} recorded significantly higher N, P and K uptake than I_{75}. Result showed that I_{100} recorded significantly higher NPK (150.9 kg ha^{-1}) uptake over I_{75} (109.2 kg ha^{-1}). Among nitrogen treatment N_1 recorded significantly higher N, P and K uptake in straw. The highest NPK uptake (161.6 kg ha^{-1}) by straw was recorded with N_1 whereas N_4 recorded significantly lowest NPK uptake (113.2 kg ha^{-1}). This might be due to higher straw yield under I_{125} as well as higher inorganic nitrogen level.

Economics

The data on gross return, net return and B: C ratios in aerobic rice cultivation were statistically analysed with respect to cost of cultivation irrespective of irrigation and nitrogen management (Table 3).

The highest cost of cultivation (Rs. 51693) was observed with I_{125} whereas lowest value (Rs. 49162) was observed with I_{75}. The maximum gross return ha^{-1} (Rs. 688491), net return ha^{-1} (Rs. 17156) and B: C ratio (1.53) were obtained from the I_{125} irrigation

Table 3. Effect of irrigation and nitrogen management on economics of aerobic rice.

Treatments	Cost of cultivation (Rs. ha^{-1})	Gross return (Rs. ha^{-1})	Net return (Rs. ha^{-1})	B:C
Irrigation management				
I_{75}	49162	57175	8013	1.38
I_{100}	50428	66634	16206	1.53
I_{125}	51693	68849	17156	1.53
SEm (±)	---	**1462**	**1462**	**0.04**
LSD at 5%	---	**5741**	**5741**	**0.15**
Nitrogen management				
N_1	28423	69791	41368	2.45
N_2	43093	70688	27595	1.64
N_3	57763	64816	7053	1.12
N_4	72433	51584	-20849	0.71
SEm (±)	---	**1137**	**1137**	**0.03**
LSD at 5%	---	**3377**	**3377**	**0.09**

treatment, but it was at par with I_{100}, however I_{100} recorded higher gross return, net return and B: C ratio than I_{75}.

The highest cost of cultivation (Rs. 72433 ha^{-1}) was observed with N_4 whereas lowest value (Rs. 28423 ha^{-1}) was observed with N_1. Maximum gross return recorded at N_2 which was significantly higher than N_3 and N_4. But there was no significance difference between N_1 and N_2. N_1 recorded significantly higher net return than N_2, N_3 and N_4.

Among all the treatments N_4 treatment recorded highest cost of cultivation due to use of more amount of vermicompost (7.5 t vermicompost per ha @ Rs 6/kg). The higher gross return ha^{-1}, net returns ha-1 and B: C ratio with I_{125} and I_{100} might be due to higher productivity of rice under irrigation at I_{125} of CPE and I100 of CPE. Similar findings obtained by Shekara et al. (2010), Murthy and Reddy, (2013) and Nayak et al. (2015). Higher gross return, net return and B:C ratio was recorded with application of nitrogen at 100 % N through fertilizer might be due to higher grain and straw yield with higher nitrogen levels. Similar findings obtained by Murthy et al. (2012) and Pradhan et al. (2014).

CONCLUSION

The current study shows that irrigation and nitrogen management have an impact on aerobic rice yield, NPK uptake and economics. Data obtained from this experiment showed that aerobic rice needs to be irrigated at 100 % CPE with the administration of nitrogen 100 % N using inorganic fertilizer to obtain economically optimal yield and nutrient uptake in red and lateritic soils of West Bengal.

REFERENCE

Anonymous (2019-20). Annual Report, 2020-21. Department of Agriculture, Cooperation & Farmers Welfare

Barison J (2002). Nutrient-use efficiency and nutrient uptake in conventional and intensive (SRI) rice cultivation systems in Madagascar. Master's thesis, Crop and Soil Sciences Department, Cornell University, Ithaca, NY

Bouman BAM, Peng S, Castaneda AR and Visperas RM (2005). Yield and water use of irrigated tropical aerobic rice systems. Agricultural Water Management74: 87-105

Bouman BAM and Tuong TP (2001). Field water management to save water and increase its productivity in irrigated rice. Agricultural Water Management 49(1): 11-30

Brady NC (1999). The nature and properties of soils. Prentice Hall of India Pvt. Ltd. Delhi

Chan CS and Chong AH (2009). Effect of seasonal weather on crop evapotranspiration and rice yield. Paper presented at the 14th MANCID annual conference. 14 -15 Feb. Kuching, Sarawak

Duary S and Pramanik K (2019). Response of aerobic rice to irrigation and nitrogen management in red and lateritic soil of West Bengal. Journal of Crop and Weed 15(1): 108-113

Gomez KA and Gomez AA (1984). Statistical Procedures for Agricultural Research. International Rice Research Institute. John Wiley and Sons, Second Edition, New York pp. 139-240

Guo J, Hu X, Gao L, Xie K, Ling N, Shen Q and Guo S (2017). The rice production practices of high yield and high nitrogen use efficiency in Jiangsu, China. Scientific reports 7(1): 1-10

Khush GS (2004). Harnessing science and technology for sustainable rice-based production systems. FAO Rice Conference 04/CRS.14.Rome, Italy, 12-13 February

Leon A and Kohyama K (2017). Estimating nitrogen and phosphorus losses from lowland paddy rice fields during cropping seasons and its application for life cycle assessment. Journal of Cleaner Production

164: 963-979

Mandal KG, Kundu DK, Thakur, Kannan AK, Brahmanand PS and Kumar A (2013). Aerobic rice response to irrigation regimes and fertilizer nitrogen rates. Journal of Food, Agriculture and Environment 11: 1148-1153

Mondal B and Pramanik K (2020). Effect of irrigation regimes and method of zinc application on growth and yield attribute of aerobic rice in lateritic soil. Plant Archives 20(1): 1213-1219

Murthy KVR and Reddy DS (2013). Effect of irrigation and weed management practices on nutrient uptake and Economics of production of Aerobic rice. IOSR Journal of Agriculture and Veterinary Science 5: 15-21

Murthy KVR, Reddy DS and Reddy GP (2012). Response of rice varieties to graded levels of nitrogen under aerobic culture. Indian Journal of Agronomy 57: 367-372

Nayak BR, Pramanik K, Panigrahy N, Dash AK and Swain SK (2015). Yield, nitrogen uptake and nitrogen use efficiency indices of aerobic rice (*Oryza sativa* L.) Under various irrigation regimes and nitrogen levels. International Journal of Bio-resource, Environment and Agricultural Sciences 1: 8-13

Parthasarathi T, Vanitha K, Lakshamanakumar P and Kalalyarasi D (2012). Aerobic rice mitigating water stress for the future climate change. International Journal of Agronomy and Plant Production 3: 241-54

Pradhan A, Thakur A and Sonboir HL (2014). Response of rice varieties to different levels of nitrogen under rainfed aerobic system. Indian Journal of Agronomy59:76-79

Ramana AV, Reddy DS and Reddy K (2007). Influence of sowing time and nitrogen levels on growth, yield and N uptake of rainfed upland rice varieties. The Andhra Agricultural Journal 54(3&4): 114-120

Seema, Krishna M and Devi MTT (2014). Effect of nitrogen and weed management on nutrient uptake by weeds under direct aerobic rice. The Bioscan 9: 535-537

Shekara BG, Sharnappa and Krishnamurthy N (2010). Effect of irrigation schedules on growth and yield of aerobic rice (*Oryza sativa* L.) under varied levels of farmyard manure in Cauvery command area. Indian Journal of Agronomy 55(1): 35-39

Singh S, Ladha JK, Gupta RK, Bhushan L and Rao AN (2008). Weed management in aerobic rice system under varying establishment methods. Crop Protection 27: 660-671

Spiertz H (2010). Food production, crops and sustainability: restoring confidence in science and technology. Current Opinion in Environmental Sustainability 2(5-6): 439-443

Yan J, Wu Q, Qi D and Zhu J (2022). Rice yield, water productivity, and nitrogen use efficiency responses to nitrogen management strategies under supplementary irrigation for rain-fed rice cultivation. Agricultural Water Management 263: 107486

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Effect of seedling age on productivity and profitability in traditional rice landraces

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ABSTRACT

A field experiment was conducted at Tamil Nadu Agricultural University, with an objective to study the effect of seedling age on productivity and profitability in rice landraces under irrigated rice ecosystem. The experiment was laid out in split plot design with three replications. The treatments in main plot were three age of seedlings viz., 15, 20 and 25 days old seedlings and in sub plot eight rice landraces viz., Chandikar, Kuliyadichan, Kuruvaikalanjiyam, Norungan, Nootripathu, Black Kavuni, Red Kavuni, Njavara and CO(R) 50 (one high yielding variety). Observations were recorded on growth parameters such as plant height, tiller numbers and dry matter production. The yield attributes like productive tillers, total spikelet, filled grains, unfilled grains, spikelet sterility percentage, thousand grain weight, grain yield and straw yield were recorded at the time of harvest. From the study it was observed that 15 days old seedlings in all the eight rice landraces and one high yielding variety, recorded higher growth and yield attributes. In rice landraces, Red Kavuni recorded higher grain, straw yield and B:C ratio. Based on quality parameters, CO(R) 50 rice variety and landraces viz., Red Kavuni, Black Kavuni, Nootripathu, Chandikar recorded optimum values and were suitable for cooking purpose.

Key words: *Rice landraces, age of seedlings, Red kavuni, quality parameters*

INTODUCTION

Transplanting is a common practice in rice cultivation. Improper planting technique is one of the important factors limiting rice yield. The age of seedlings is an important factor which determines grain yield as it has a tremendous influence on the tiller production, grain formation and yield contributing characteristics in rice. The recommended age of seedlings is 18-21 days for short duration, 21-25 days for medium duration and 25-30 days for long duration varieties under irrigated transplanted rice eco-system and 12-14 days old seedlings with mat nursery in SRI system of planting (CPG, 2012).

The red rice varieties are having appealing red colour, has more complex taste and contains more nutrition, fibre-filled bran compared to normal rice. In the red rice, colour is confined to the bran layer, ranges from light red to dark red, a tinge of red remains even after a high degree of milling and contains polyphenols, anthocyanin and possesses antioxidant properties. The inner portion of red and white rice is alike and white. The zinc and iron content of red rice is 2-3 times higher than that of white rice (Ramaiah and Rao, 1953). American scientists also reported high amount of iron content in the Chinese red varieties of "Bloody Sticky" and "Dragon Eyeball" (Rhoades, 2008). Njavara is a rice variety endemic to Kerala, famed for its use in Ayurveda. There are two types of Njavara rice based on differences in glume colourviz., black glumed of 60-90 days maturity and tolerant to drought conditions whereas yellow glumed matures in 60-90 days and is susceptible to lodging and diseases (Ashraf and Lokanadan, 2017). Kavuni is a rice variety endemic to Tamil Nadu, cultivated in the outskirts of Tanjore. There are three types of Kavuni on the basis of kernel colour *viz*., Black Kavuni, Red Kavuni and Local Kavuni (Ashraf et al., 2017).

The most significant characteristics among the traditional varieties are the medicinal and nutritional traits which the consumers prefer. Sathya et al. (2007) while evaluating the biodiversity of traditional rice varieties in Tamil Nadu documented some of the rice varieties available and their unique characteristics and reported that nearly 400 varieties of landraces of paddy had been in vogue in Tamil Nadu. More than one hundred different varieties of rice had prevailed in North Arcot district alone of Tamil Nadu, of which Munagada (submerged) warrants a special mention and Vaigunda a fast growing traditional rice surpassing weeds.

The current paradigm of rice research is shifting towards farmer-centered one, with development along the principles of food sovereignty and biodiversity based rice ecosystems, thus provide more diverse and nutritious sources of food, complementary to rice. Hence, the present study is planned to evaluate the best performing landrace and to find the effect on grain yield due to different seedling age.

MATERIALS AND METHODS

A field study was conducted in wetland during samba season of 2014-15 at Tamil Nadu Agricultural University, Coimbatore with an objective to study find the effect of different seedling age on yield in rice landraces under transplanted irrigated rice ecosystem. The location of the study area is situated at 11°N latitude, 77°E longitude and at an altitude of 426.7 m above mean sea level. The soil of the experimental field was deep clay loam, moderately drained and grouped under *Vertic Ustochrep* taxonomical classification belonging to *Noyyal* series. In the experimental soil the available nitrogen was low, available phosphorus was medium and potassium was high. All packages of practices were carried out as per recommendation of CPG, 2012. The experiment was laid out in Split Plot Design with three replications. The treatments in main plot were three age of seedlings *viz*., 15 (A_1), 20 (A_2) and 25 (A_3) days old seedlings and in sub plot eight rice landraces with one high yielding variety *viz*., Chandikar (V_1), Kuliyadichan (V_2), Kuruvaikalanjiyam (V_3), Norungan (V_4), Nootripathu (V_5), Black Kavuni (V_6), Red Kavuni (V_7), Njavara (V_8) and CO(R) 50 (V_9) were used in the field experiment. Observations were recorded on growth parameters such as plant height, tiller numbers and dry matter production. The yield attributes like productive tillers, total spikelet, filled grains, unfilled grains, spikelet sterility percentage, thousand grain weight, grain yield and straw yield were recorded at the time of harvest.

Quality parameters

Physical characters

Kernel length and breadth

Brown rice length and breadth was measured by using vernier caliper and expressed in mm (Kaul, 1970).

Length/Breadth (L/B) ratio

It is the ratio between kernel length and kernel breadth used to determine the grain shape as suggested by Kaul (1970).

$$\text{L/B ratio} = \frac{\text{Kernal length (mm)}}{\text{Kernal breadth (mm)}}$$

Hulling percentage

About 250 g of cleaned raw rice with 14 per cent moisture content was hulled in experimental huller (rubber roller) and husk and brown rice were separated. The weight of brown rice was recorded and the hulling percentage was calculated as suggested by Khush et al. (1979)

$$\text{Hulling percentage} = \frac{\text{Weight of brown rice (g)}}{\text{Weight of rough rice (g)}} \times 100$$

Milling percentage

The brown rice obtained after hulling was subjected to milling and the milled rice and bran were separated. The weight of the milled rice was recorded and then the milling percentage was calculated as suggested by Khush et al. (1979).

$$\text{Milling percentage} = \frac{\text{Weight of milled rice (g)}}{\text{Weight of rough rice (g)}} \times 100$$

Cooking characteristics

Kernel Length after Cooking (KLAC)

Ten normal milled rice grains were pre soaked for 10 minutes and placed directly in boiling water for their optimum cooking time. Then the length of cooked rice was measured in mm with a graduated cardboard and the average was worked out (Azzez and Shafi, 1966).

Kernel Breadth after Cooking (KBAC)

As in the case of kernel length after cooking, breath of cooked rice was measured and the average was computed (Azzez and Shafi, 1966).

Linear Elongation Ratio (LER)

Using the length of the brown rice and the length of kernel after cooking, linear elongation ratio was calculated as suggested by Azzez and Shafi (1966).

$$\text{LER} = \frac{\text{Length of cooked rice (mm)}}{\text{Length of raw rice (mm)}}$$

Breadth wise Expansion Ratio (BER)

Using the breadth of the brown rice and the breadth of kernel after cooking, breadth wise expansion ratio was calculated as suggested by Azzez and Shafi (1966).

$$\text{BER} = \frac{\text{Breadth of cooked rice (mm)}}{\text{Breadth of raw rice (mm)}}$$

Volume expansion ratio

The volume of initial milled rice was measured by water displacement method in a graduated measuring cylinder. Then it was cooked in boiling water and the cooked rice volume was measured again by water displacement method (Juliano, 1984).

$$\text{Volume expention ratio} = \frac{\text{Volume of cooked rice (ml)}}{\text{Volume of raw rice (ml)}}$$

Bio-chemical characters

Amylose content

The simplified procedure reported by Juliano (1972) was used for the estimation of amylose content. 0.1 g milled rice flour was put into a 100 ml volumetric flask and 1 ml of 95 per cent ethanol and 9 ml of 1 M Sodium hydroxide was added. The samples were kept overnight. The volume was made up to 100 ml. About 5 ml of the starch solution was added into a test tube and 1 ml of 1 M acetic acid, 2 ml of iodine solution (1 g iodine and 2 g potassium iodide in 100 ml aqueous solution) were added and the volume was made up to 100 ml with distilled water. The contents were shaken well and the absorbance of the solution was measured at 620 nm with a spectrophotometer. Amylose concentration was obtained by plotting the absorbance in the standard curve. For standard curve 100 mg of potato amylose was taken in a volumetric flask and wetted with 1 ml of 95 per cent ethanol and 9 ml of 1 M sodium hydroxide. The sample was kept overnight. The volume was made up to 100 ml. 1, 2, 3, 4 and 5 ml of solution was taken and placed in different test tubes and this 1 ml of 1 M acetic acid and 2 ml of iodine solution were added and the volume was made up to 100 ml. The absorbance was read at 620 nm and the standard curve was plotted.

Gelatinization Temperature (GT)

Gelatinization temperature was estimated based on alkali spreading value (ASV) of milled rice as developed by Little et al. (1958) was used to score alkali spreading value. Six whole milled kernels of each variety were placed in petri plates containing 10 ml of 1.7 per cent KOH solution. The kernels were arranged in such a way to provide space between kernels for spreading. The plates were covered and incubated at room temperature for 23 hrs. The appearance and disintegration of kernels was rated visually based on a 7 point numerical spreading scale.

Gel Consistency (GC)

Gel consistency was analyzed based on the method described by Cagampang et al. (1973). 100 mg milled rice flour was weighed, 0.2 ml of 95 per cent ethanol containing 0.025 per cent thymol blue and 2 ml of 0.2 N KOH was added. Contents were mixed using a vortex genie mixer. The test tubes were covered with glass marbles in order to prevent steam loss and to reflux the samples. The samples were cooked in a vigorously boiling water bath for eight minutes to make the contents reach two third of the height of the tube. The test tubes were removed from the water bath and kept at room temperature for five minutes. The tubes were kept in an ice water bath for 20 minutes and laid horizontally on a table, lined with millimeter graphing paper. The total length of the gel was measured in mm from the bottom of the tube after one hour. The test classified the rice into three categories.

1. Very flaky rice with hard gel consistency (length of gel = < 40 mm)
2. Flaky rice with medium gel consistency (length of gel = 40-60 mm)

Table 1. Details of landraces/rice variety in the experiment.

Sl. no.	Varieties	Special characters
1.	*Chandikar*	Drought tolerant, with crop duration 110-120 days, tall statured and moderately tolerant to lodging.
2.	*Kuliyadichan*	Drought tolerant, with crop duration 100-105 days, medium to tall statured (height increases with moisture availability).
3.	*Kuruvaikalanjiyam*	Drought tolerant, with crop duration 110-115 days, tall statured (height increases with moisture availability), lodging and has loose panicle.
4.	*Norungan*	Drought tolerant, with crop duration 110 -115 days, tall statured, lodging even under drought condition, shattering, loose panicle. Hardy plant type, sturdy culm and thick, dark green broad leaves.
5.	*Nootripathu*	Drought tolerant, with crop duration 100 -110 days, semi tall statured, lodging and loose panicle. Hardy plant type, sturdy culm and thick, dark green broad leaves.
6.	*Blackkavuni*	Used for medicinal purpose with crop duration 130-135 days.
7.	*Redkavuni*	Used for medicinal purpose with crop duration 130-135 days.
8.	*Njavara*	Medicinal rice variety, used in Ayurveda and susceptible to lodging.
9.	CO(R)50	Medium tall with new plant type characteristics, Green leaf sheath, Long compact droopypanicle with crop duration 130-135 days.

3. Soft rice with soft gel consistency (length of gel = > 60 mm)

RESULTS AND DISCUSSION

Effect on plant height

The plant height is a direct index to measure the growth and vigour of the plants. In the study, the plant height gradually increased from tillering to maturity stage. The incremental increase of plant height was higher from active tillering to flowering. This reflected on the general growth behaviour of rice crop, with peak increment in the plant height between panicle initiation and flowering stage.

In the present investigation, plant height was found to be higher with 25 days old seedlings 41.1 cm during early stage of growth. This might be due to the transplantation of old seedlings which had gained height at nursery stage. But at later stages, 15 days old seedlings produced taller plants. It recorded maximum plant height 79.7 cm at panicle initiation, 104.3 cm at flowering and 123.8 cm at maturity stages. Taller plant height was observed with 15 days old seedlings in all the rice landraces *viz*.,Chandikar, Kuliyadichan, Kuruvaikalanjiyam, Norungan, Nootripathu, Black Kavuni, Red Kavuni, Njavara and CO(R) 50 rice variety. It was due to the fact that young seedlings had higher vigour and more root growth which stimulated cell divisions causing more stem elongation thus resulting in increased plant height which was also reported earlier by Ancy Francis, (2007).

Among the rice landraces, Red Kavuni produced maximum plant height 46.6 cm at initial tillering stage, 83.8 cm at panicle initiation and 137.5 cm at maturity stage, as compared to other varieties. At flowering stage, Kuliyadichanrecorded maximum plant height of 113.3 cm which was on par with Nootripathu with plant height 111.5 cm. Kuruvaikalanjiyam recorded lower plant height of 33.7 cm at initial tillering stage, 67.8 cm at panicle initiation, 80.4 cm at flowering and 100 cm at maturity stage, respectively, but it was on par with CO(R)50 rice variety at all the growth stages. Variation in plant height among the varieties might be due to the differences in their genetic makeup. This result was in accordance with those of Khatun (2001) and Das et al. (2012) who observed variable plant height in the rice varieties.

Effect on number of tillers

The tillering capacity is the more important feature in rice cultivars and varieties. In general, tiller production is slow in beginning, increases steadily, attains its peak and then starts to decline. The total number of tillers per unit area was significantly affected by age of seedlings. However, 15 days old seedlings produced more number of tillers compared to 20 and 25 days old seedlings. Higher number of tillers was observed with 15 days old seedlings in all the rice landraces *viz*., Chandikar, Kuliyadichan, Kuruvaikalanjiyam, Norungan, Nootripathu, Black Kavuni, Red Kavuni, Njavara and CO(R)50 rice variety. This might be due to the production of more tillers per phyllochron in young seedlings. This result is in conformity with the findings of Devi and Singh (2000) and Uphoff (2002).

Transplanting of young (14 to 15 days old) rice seedlings generally favours quick recovery, rapid growth and tillering compared to 25 and 30 day old seedlings. Older seedlings usually recover more slowly (Rajendran et al., 2004). Transplanting very young seedlings usually 8-10 days old not more than 15 days old had better tillering and rooting and it was reduced if the transplanting was done after 4th phyllochron usually about 15 days after emergence (Stoop et al., 2002). Rajendren (2009) reported fourteen days old seedlings produced more number of tillers and the per cent increase over 22 days old seedlings was 21.13 in 2007 and 23.66 in 2008, which was due to production of more tillers per phyllochron at the seedling stage with three to four leaves.

In rice landraces, at initial tillering, CO(R)50 rice variety produced more tillers m^{-2}, later on, Red Kavuni produced significantly more tillers m^{-2}. It produced 469 tillers m^{-2} at panicle initiation, 638 tillers m^{-2} at flowering and 536 tillers m^{-2} at maturity stage followed by Nootripathu. The lower number of tillers m^{-2} was recorded with Njavara (V8) 138 tillers m^{-2} at initial tillering, later on, CO(R)50 rice variety recorded lower number of tillers m^{-2}. It produced 339 tillers m^{-2} at panicle initiation, 430 tillers m^{-2} at flowering and 403 tillers m^{-2} at maturity stage .

Effect on dry matter production

The dry matter production in rice plants increased steadily with advancing growth stages and reaches the maximum during the maturity stage. In the present study there was a significant difference in DMP with age of seedlings and rice landraces. DMP was found to be more in 15 days old seedlings in all the rice landraces *viz*., Chandikar, Kuliyadichan, Kuruvaikalanjiyam, Norungan, Nootripathu, Black Kavuni, Red Kavuni, Njavara and CO(R)50 rice variety at flowering and maturity stages. It recorded higher DMP of 9.89 t ha^{-1} at flowering and 12.10 t ha^{-1} at maturity stages respectively. This result is in concurrence with Ancy Francis (2007). The increased dry matter production in 15 days old seedlings was mainly due to increased plant height, more number of tillers and better development of leaves, higher nutrient uptake, which ultimately resulted in higher plant dry matter production which is attributed to more tiller production and number of leaves, increased LAI and improved root characteristics $hill^{-1}$.

In the presence of adequate nutrient availability and larger photosynthesizing surface, the dry matter accumulation proceeded at a rapid rate leading to its greater accumulation. This result is in conformity with the findings of Rajesh and Thanunathan, (2003). Rajendren (2009) reported DMP was found to be more in fourteen days old seedlings.

Among the rice landraces, Red Kavuni recorded more DMP with 8.88 and 11.91 t ha^{-1} at

Table 2. Effect of seedling age in rice landraces on plant height (cm), tillers m^{-2} and dry matter production (t ha^{-1}) (maturity stage).

Seedling age	Plant height (cm)				Tillers m^{-2}				Dry matter production (t ha^{-1})			
Landraces	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	114.6	113.4	106.1	111.4	466	397	370	411	10.78	10.47	11.06	10.78
Kuliyadichan	122.5	119.7	114.5	118.9	443	433	406	427	10.19	9.73	10.25	10.19
Kuruvaikalanjiyam	101.3	100.4	98.2	100.0	477	469	443	463	9.69	9.20	9.66	9.69
Norungan	118.1	117.4	113.9	116.5	520	448	439	469	11.24	10.75	11.30	11.24
Nootripathu	134.0	120.7	115.5	123.4	513	490	464	489	9.36	9.00	9.71	9.36
Blackkavuni	136.9	127.3	123.4	129.2	481	436	410	442	11.38	10.58	11.45	11.38
Redkavuni	147.5	135.4	129.6	137.5	567	537	503	536	11.49	11.65	11.91	11.49
Njavara	128.7	121.9	118.5	123.0	482	469	442	464	8.23	8.04	8.42	8.23
CO(R)50	110.3	105.9	104.7	107.0	434	403	371	403	10.10	10.03	10.34	10.10
Mean	123.8	118.0	113.8		487	454	428		10.49	10.11		10.49
	A	V	A at V	V at A	A	V	A at V	V at A	V	A at V	V at A	V
SEd	0.5	1.3	2.2	2.3	8.75	18.12	30.86	31.39	0.52	0.88	0.90	0.52
CD(P=0.05)	1.5	2.7	4.6	4.6	24.31	36.44	NS	NS	1.05	NS	NS	1.05

flowering and maturity stages, but was on par with Black Kavuni, Norungan, Chandikar. Njavara recorded lower DMP with 5.76 and 8.42 t ha^{-1} at flowering and maturity stages, respectively (Table 2).

Effect on yield attributes

Since the analysis of yield components helps for better understanding of the physiological basis, the variation caused by the treatments on the productive tillers m^{-2}, panicle length, panicle weight, total number of grains panicle^{-1}, filled grains panicle^{-1} and sterility per cent was studied and discussed. The accumulation of carbohydrates, nitrogen and dry matter in vegetative part of rice reached maximum at full heading stage.

The 15 days old seedlings produced more number of productive tillers m^{-2} (383), panicle weight (2.28) and filled grains panicle^{-1} (86) than 25 days old seedlings. Lower sterility per cent of 23.12 was recorded in 15 days old seedlings.

The productive tillers m^{-2} and spikelet number per unit area at heading stage of 15 days old seedling exhibited an increasing trend and sink demand for source assimilate was enhanced, which might be the fundamental reason for higher assimilation and translocation percentage (Senthilkumar, 2002).

The 15 days old seedlings registered lower spikelet sterility which may be due to the availability of better light, optimum spacing and efficient translocation of photosynthates of spikelets. Rajendren (2009) reported that fourteen days old seedlings produced more number of productive tillers m^{-2} and filled grains panicle^{-1} and the present study confirms with 383 productive tillers m^{-2} and 86 filled grains panicle^{-1} in 15 days old seedling compared to 20 days old seedlings.

Regarding the rice landraces Red Kavuni recorded maximum number of productive tillers m^{-2}, lengthier panicles and higher thousand grain weight. CO(R)50 recorded maximum number of total spikelets panicle^{-1}, filled grains panicle^{-1} and lower sterility per cent. In present study, Red Kavuni recorded maximum number of productive tillers m^{-2} (418), but was on par with Norungan and Nootripathu. Njavara and Kuliyadichan recorded lower number of productive tillers m^{-2} (337 and 322).The reason for the difference in effective tillers hill^{-1}, is the genetic makeup of the variety, which is primarily influenced by heredity factors. This result was supported by Chowdhury et al. (1993) who stated that effective tillers hill^{-1} varied with the variety.

Red Kavuni recorded lengthier panicles of 26.67 cm and was on par with Black Kavuni. The minimum length of panicles was observed with landraces *viz*., Chandikar, Norungan, Nootripathu, Kuliyadichan, Njavara and CO(R)50 rice variety. The results indicated the differences in length of panicles might be due of the varieties, which coincides with the observations made by Irfan et al. (2005) (Table 4).

Among rice landraces, Kuruvaikalanjiyam (26.9g) recorded higher thousand grain weight followed by Chandikar (25.5g), Norungan (25.9 g) and Red

Table 3. Effect of seedling age in rice landraces on total spikelet panicle^{-1} and filled grains panicle^{-1} (maturity stage).

Seedling age	Total spikelet panicle^{-1}				Filled grains panicle^{-1}				Unfilled grains panicle^{-1}			
Landraces	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	104	108	26	26	26	26	104	108	104	2.19	2.23	2.43
Kuliyadichan	99	102	25	25	25	25	99	102	99	1.85	1.92	2.09
Kuruvaikalanjiyam	97	98	27	27	27	27	97	98	97	2.33	2.06	2.52
Norungan	112	115	28	28	28	28	112	115	112	2.14	2.30	2.34
Nootripathu	100	104	26	26	26	26	100	104	100	3.26	2.40	3.21
Blackkavuni	116	119	27	27	27	27	116	119	116	3.39	3.03	3.36
Redkavuni	121	126	27	27	27	27	121	126	121	4.11	2.97	4.46
Njavara	89	92	23	23	23	23	89	92	89	3.15	1.98	3.12
CO(R)50	136	139	18	18	18	18	136	139	136	2.61	2.26	2.75
Mean	108	111	25	25	25	25	108	111	108	2.78	2.35	
	A	V	A at V	A	V	A at V	A	V	A at V	A	V	A at V
SEd	5.44	2.63	0.61	0.61	0.61	0.61	5.44	2.63	5.44	0.13	0.24	0.22
CD(P=0.05)	10.94	NS	1.70	1.70	1.70	1.70	10.94	NS	10.94	0.25	0.53	0.44

Table 4. Effect of seedling age in rice landraces on panicle length (cm), panicle weight (g) and thousand grain weight (g) (maturity stage).

Seedling age	Panicle length				Panicle weight				Thousand grain weight			
Landraces	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	22.67	20.27	19.99	22.67	20.27	19.99	22.67	20.27	19.99	22.67	20.27	19.99
Kuliyadichan	23.37	22.87	22.03	23.37	22.87	22.03	23.37	22.87	22.03	23.37	22.87	22.03
Kuruvaikalanjiyam	21.90	20.83	20.33	21.90	20.83	20.33	21.90	20.83	20.33	21.90	20.83	20.33
Norungan	23.00	21.97	21.13	23.00	21.97	21.13	23.00	21.97	21.13	23.00	21.97	21.13
Nootripathu	22.93	21.80	20.80	22.93	21.80	20.80	22.93	21.80	20.80	22.93	21.80	20.80
Blackkavuni	25.37	24.93	24.30	25.37	24.93	24.30	25.37	24.93	24.30	25.37	24.93	24.30
Redkavuni	27.60	26.73	25.67	27.60	26.73	25.67	27.60	26.73	25.67	27.60	26.73	25.67
Njavara	23.50	22.13	21.73	23.50	22.13	21.73	23.50	22.13	21.73	23.50	22.13	21.73
CO(R)50	22.96	22.34	22.01	22.96	22.34	22.01	22.96	22.34	22.01	22.96	22.34	22.01
Mean	23.70	22.65	22.00	23.70	22.65	22.00	23.70	22.65	22.00	23.70	22.65	22.00
	A	V	A at V	A	V	A at V	A	V	A at V	A	V	A at V
SEd	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97
CD(P=0.05)	NS	2.33	NS	NS	2.33	NS	NS	2.33	NS	NS	2.33	NS

kavuni (24.9g). Njavara (17.0g) and CO(R) 50 (19.3g) recorded lower thousand grain weight during the period of study. Many native varieties have greater grain weight than most HYVs. The rice variety CO(R) 50 registered higher panicle weight of (3.21g) followed by Red Kavuni. The lower value of panicle weight was observed with Kuliyadichan (1.19g). Maximum number of total spikelets panicle-1 (136) was recorded in CO(R) 50 rice variety followed by Norungan, Black Kavuni and Red Kavuni. The total spikelets panicle^{-1} was minimum in Njavara, Kuliyadichan, and Kuruvaikalanjiyam. A positive and significant correlation exists between rice yield and number of panicles per plant. The findings indicated that plants with heavy panicles tend to have high number of fertile grains thereby increasing rice yield.

CO(R) 50 rice variety recorded maximum number of filled grains panicle^{-1} (116). In rice landraces, the sterility per cent was higher (29.68) in Kuruvaikalanjiyam and lower (14.98) in CO(R) 50 rice variety. Moncada et al. (2001) observed a negative correlation between yield and percentage sterility of rice genotype. Hence, to increase grain yield, it is important to reduce spikelet sterility or increase spikelet fertility (Table 3).

Effect on grain and straw yield

In the present investigation, 15 days old seedlings resulted in higher increase in grain yield (11.55%) and

Table 5. Effect of seedling age in rice landraces on grain yield (kg ha^{-1}), straw yield (kg ha^{-1}) and harvest index.

Seedling age	Grain yield				Straw yield				*Harvest index			
Landraces	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	2,266	2,020	1,930	2,072	7,312	7,145	6,800	7,086	0.24	0.22	0.22	0.23
Kuliyadichan	2,190	1,976	1,879	2,015	6,919	6,417	6,056	6,464	0.24	0.24	0.24	0.24
Kuruvaikalanjiyam	1,807	1,723	1,675	1,735	5,999	5,645	5,455	5,700	0.23	0.23	0.23	0.23
Norungan	2,325	2,043	1,960	2,109	7,417	7,183	6,834	7,145	0.24	0.22	0.22	0.23
Nootripathu	2,136	1,948	1,898	1,994	5,998	5,767	5,599	5,788	0.26	0.25	0.25	0.26
Blackkavuni	2,338	2,269	2,030	2,212	7,487	7,267	6,932	7,229	0.24	0.24	0.23	0.23
Redkavuni	2,546	2,398	2,229	2,391	7,849	7,514	7,045	7,469	0.24	0.24	0.24	0.24
Njavara	1,425	1,325	1,275	1,342	4,924	4,867	4,735	4,842	0.22	0.21	0.21	0.22
CO(R)50	5,098	4,812	4,695	4,868	6,956	6,579	6,322	6,619	0.43	0.43	0.43	0.43
Mean	2,459	2,279	2,175		6,762	6,487	6,198		0.26	0.25	0.25	
	A	V	A at V	V at A	A	V	A at V	V at A	A	V	A at V	V at A
SEd	61	129	219	223	144	326	551	564	-	-	-	-
CD(P=0.05)	169	259	NS	NS	400	655	NS	NS	-	-	-	-

Table 6. Effect of seedling age in rice landraces on kernel length (mm), kernel breadth (mm), length and breadth ratio.

Seedling age	Kernel length (mm)				Kernel breadth (mm)				Length and breadth ratio			
Landraces	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	6.3	6.3	6.2	6.3	2.8	2.7	2.7	2.7	2.3	2.3	2.3	2.3
Kuliyadichan	5.9	5.8	5.8	5.8	2.6	2.6	2.5	2.6	2.3	2.2	2.3	2.3
Kuruvaikalanjiyam	5.8	5.7	5.7	5.7	2.7	2.7	2.6	2.7	2.1	2.1	2.2	2.2
Norungan	5.8	5.8	5.7	5.8	2.5	2.5	2.4	2.5	2.3	2.3	2.4	2.3
Nootripathu	6.4	6.3	6.3	6.3	2.4	2.3	2.4	2.4	2.7	2.7	2.6	2.7
Blackkavuni	6.1	6.0	6.0	6.0	2.1	2.1	2.0	2.1	2.9	2.9	3.0	2.9
Redkavuni	6.3	6.2	6.2	6.2	2.2	2.2	2.1	2.2	2.9	2.8	3.0	2.9
Njavara	5.8	5.8	5.7	5.8	2.0	2.0	2.0	2.0	2.9	2.9	2.9	2.9
CO(R)50	5.9	5.9	5.8	5.9	2.1	2.1	2.0	2.1	2.8	2.8	2.9	2.8
Mean	6.0	6.0	5.9		2.4	2.4	2.3		2.6	2.6	2.6	
	A	V	A at V	V at A	A	V	A at V	V at A	A	V	A at V	V at A
SEd	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97
CD(P=0.05)	NS	2.33	NS	NS	NS	2.24	NS	NS	NS	2.01	NS	NS

straw yield (8.34 %) increase over 25 days old seedlings. The older seedlings (20 and 25 day) remained for longer period in nursery affecting the phyllocron, which enable rice tillering resulting in lesser number of tillers in main field and reduced grain yield (Singh and Singh, 1999).

Transplanting of young seedlings provided sufficient nutrients for vegetative growth and reproductive phase by better root growth. This might be due to efficient utilization of resources that ultimately lead to increased plant height and yield attributes thereby increased grain and straw yields. Similar results were reported by Vijayakumar et al. (2005) and Ancy Francis (2007). More et al. (2007) reported proportion of grain yield to straw yield was higher with 15 days old seedlings compared to normal seedlings of 20 and 28 days age suggesting efficient translocation of photosynthates from source to sink in former case. Similar observations were seen in present study and also reported by Wang Shaao-Hua et al. (2002).

The rice variety CO(R)50 recorded higher grain yield of 4,868 kg ha^{-1}, followed by Red Kavuni and Black Kavuni which recorded yield of 2,391 and 2,212 kg ha^{-1}. Red Kavuni recorded higher straw yield of 7,469 kg ha^{-1}, but was on par with Black Kavuni 7,229 kg ha^{-1}, Norungan 7,145 kg ha^{-1} and Chandikar 7,086 kg ha^{-1}. Njavara recorded lower grain yield and straw yield of 1,342 kg ha^{-1} and 4,842 kg ha^{-1} respectively during the crop growth period (Table 5).

Table 7. Effect of seedling age in rice landraces on hulling percentage, milling percentage and head rice recovery (percentage).

Seedling age	Hulling percentage				Milling percentage				Head rice recovery (percentage)			
Landraces	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	73.9	72.4	72.6	73.0	70.4	70.1	70.2	70.2	35.4	34.5	34.2	34.7
Kuliyadichan	70.6	70.4	69.4	70.1	65.6	65.3	65.9	65.6	31.7	31.8	30.2	31.2
Kuruvaikalanjiyam	72.5	71.7	71.2	71.8	69.4	68.4	68.1	68.6	30.9	30.5	30.2	30.5
Norungan	71.3	70.2	69.4	70.3	68.3	68.4	68.8	68.5	30.3	30.4	29.9	30.2
Nootripathu	73.5	72.4	72.5	72.8	70.7	70.3	69.3	70.1	36.6	36.7	35.3	36.2
Blackkavuni	76.4	76.3	74.2	75.6	71.3	71.4	70.3	71.0	52.4	51.3	51.2	51.6
Redkavuni	77.8	77.4	76.2	77.1	72.3	72.3	71.5	72.0	53.6	52.5	52.9	53.0
Njavara	78.8	78.6	77.2	78.2	73.3	73.6	72.3	73.1	30.4	30.3	30.3	30.3
CO(R)50	82.5	81.2	80.5	81.4	72.5	72.3	71.9	72.2	60.1	60.8	59.3	60.1
Mean	75.3	74.5	73.7		70.4	70.2	69.8		40.2	39.9	39.3	
	A at V	V at A	A at V	V at A	A	V	A at V	V at A	A	V	A at V	V at A
SEd	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97
CD(P=0.05)	NS	2.33	NS	NS	NS	2.24	NS	NS	NS	2.01	NS	NS

Table 8. Effect of seedling age in rice landraces on kernel length after cooking (mm) and kernel breadth after cooking (mm).

Seedling age	Kernel length (mm)								Kernel breadth (mm)							
	Before cooking				After cooking				Before cooking				After cooking			
Landraces	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	6.3	6.3	6.2	6.3	9.7	9.6	9.6	9.6	2.8	2.7	2.7	2.7	3.2	3.2	3.1	3.2
Kuliyadichan	5.9	5.8	5.8	5.8	8.2	8.2	8.1	8.2	2.6	2.6	2.5	2.6	2.8	2.7	2.7	2.7
Kuruvaikalanjiyam	5.8	5.7	5.7	5.7	8.6	8.5	8.6	8.6	2.7	2.7	2.6	2.7	3.4	3.4	3.3	3.4
Norungan	5.8	5.8	5.7	5.8	8.4	8.4	8.3	8.4	2.5	2.5	2.4	2.5	3.2	3.2	3.1	3.2
Nootripathu	6.4	6.3	6.3	6.3	9.9	9.8	9.8	9.8	2.4	2.3	2.4	2.4	3.5	3.4	3.4	3.4
Blackkavuni	6.1	6.0	6.0	6.0	7.7	7.7	7.6	7.7	2.1	2.1	2.0	2.1	2.6	2.6	2.5	2.6
Redkavuni	6.3	6.2	6.2	6.2	8.2	8.1	8.0	8.1	2.2	2.2	2.1	2.2	2.8	2.7	2.6	2.7
Njavara	5.8	5.8	5.7	5.8	6.8	6.5	6.5	6.6	2.0	2.0	2.0	2.0	2.5	2.5	2.4	2.5
CO(R)50	5.9	5.9	5.8	5.9	9.3	9.2	9.2	9.2	2.1	2.1	2.0	2.1	3.3	3.2	3.2	3.2
Mean	6.0	6.0	5.9		8.5	8.4	8.4		2.4	2.4	2.3		3.0	3.0	2.9	
	A	V	A at V	V at A	A	V	A at V	V at A	A	V	A at V	V at A	A	V	A at V	V at A
SEd	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97	0.53
CD(P=0.05)	NS	2.33	NS	NS	NS	2.24	NS	NS	NS	2.01	NS	NS	NS	2.33	NS	NS

Quality parameters of rice landraces

Kernel length, kernel breadth and L/B ratio did not show higher variation in rice landraces, they were classified as medium in grain shape and size. This could be due to genetic constitution. The grain size and shape of rice varieties in general is short to medium slender with translucent appearance (Banu et al., 1992) (Table 6).

The dehulling of rice is one of the important postharvest processes. In the rice landraces, CO(R)50 (81.4) obtained higher hulling percentage followed by Njavara (78.2), Red Kavuni (77.1), Black Kavuni (75.6) Chandikar (73), Kuliyadichan (70.1), Kuruvaikalanjiyam (71.8), Norungan (70.3) and Nootripathu (72.8) (Table 7).

During cooking, rice kernels absorb water and increase in volume through increase in length or breadth (Hogan and Plank, 1958). Breadth wise increase is not desirable, whereas, length wise increase without increase in girth is desirable characteristics in high quality premium rice (Hossain et al., 2009). In the rice landraces, Nootripathu (9.8 mm), Chandikar (9.6 mm) and CO(R)50 (9.2 mm)registered higher KLAC values. Njavara obtained lower KLAC (6.6 mm). Some varieties elongate more than others upon hydration and starch gelatinization without increase in girth; considered to be a desirable cooking quality traits in most high quality rice of the world (Table 8 and Table 9).

Rice with soft to medium gel consistency, intermediate amylose content and intermediate gelatinization temperature is a preferred level for the consumers (Khush et al., 1979). The alkali spreading value was calculated as low, intermediate and high. In rice landraces, Kuliyadichan, Kuruvaikalanjiyam and Norungan recorded lower alkali digestion described as kernel not affected/swollen and comes under rating 2. Chandikar, Nootripathu and Black Kavuni are grouped in the rating 3 and 4 described as kernel swollen, collar complete/incomplete or wide based on the alkali digestion value. Red Kavuni and CO(R)50 grouped in the rating 5 described as kernel split or segmented collar complete and wide. Njavara has high alkali digestion value and grouped under 6th category described as kernel dispersed, merged with collar. The different range of drying also affects the GT (Cagampang et al., 1973). The intermediate GT is suitable for cooking purpose.

Table 9. Effect of seedling age in rice landraces on linear elongation ratio, breadth wise elongation ratio and volume expansion ratio.

Seedling age / Landraces	Linear elongation ratio				Breadth wise elongation ratio				Volume expansion ratio			
	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	1.54	1.52	1.55	1.54	1.14	1.19	1.15	1.16	3.0	2.9	2.9	2.9
Kuliyadichan	1.39	1.41	1.40	1.40	1.08	1.04	1.08	1.07	2.4	2.4	2.3	2.4
Kuruvaikalanjiyam	1.48	1.49	1.51	1.49	1.26	1.26	1.27	1.26	2.8	2.8	2.7	2.8
Norungan	1.45	1.45	1.46	1.45	1.28	1.28	1.29	1.28	2.6	2.5	2.6	2.6
Nootripathu	1.55	1.56	1.56	1.55	1.46	1.48	1.42	1.45	3.1	3.0	3.0	3.0
Blackkavuni	1.26	1.28	1.27	1.27	1.24	1.24	1.25	1.24	2.4	2.4	2.3	2.4
Redkavuni	1.30	1.31	1.29	1.30	1.27	1.23	1.24	1.25	2.6	2.5	2.6	2.6
Njavara	1.17	1.12	1.14	1.14	1.25	1.25	1.20	1.23	2.3	2.1	2.1	2.2
CO(R)50	1.58	1.56	1.59	1.57	1.57	1.52	1.60	1.57	3.5	3.4	3.4	3.4
Mean	1.41	1.41	1.42		1.28	1.28	1.28		2.8	2.7	2.7	
	A	V	A at V	V at A	A	V	A at V	V at A	A	V	A at V	V at A
SEd	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97
CD(P=0.05)	NS	2.33	NS	NS	NS	2.24	NS	NS	NS	2.01	NS	NS

Rice with soft to medium gel consistency, intermediate amylose content and intermediate gelatinization temperature is a preferred level for the consumers (Khush et al., 1979). Amylose content can play a significant role in determining the overall cooking, eating and pasting properties of a rice variety (Adu-Kwarteng et al., 2003). Rice with intermediate amylose content has been reported to cook moist and remain soft (when cool) and is widely preferred than rice with high or low amylose contents (Juliano, 1972).

Amylose content of rice determines the hardness and stickiness of cooked rice. Amylose content higher than 25 per cent gives non sticky soft or hard cooked rice. Rice having 20-25 per cent amylose gives soft and relatively sticky cooked rice.In rice landraces, amylose content in percentage of Kuliyadichan (26.3), Kuruvaikalanjiyam (24.5), Norungan (24.9) respectively were grouped under high amylose content category. The amylose content in percentage of Chandikar (23.5), Nootripathu (23.4), Black Kavuni (20.3), Red Kavuni (21.1), Njavara (18.5) and CO(R)50 (21.6) were grouped under intermediate amylose content category (Table 10).

Iron and zinc content of rice landraces

Among the rice landraces, Kuruvaikalanjiyam (29.3 mg kg^{-1}) recorded higher zinc content. The lower zinc content obtained in CO(R)50 (21.2 mg kg^{-1}) and Black Kavuni (21.6 mg kg^{-1}). The higher iron content was recorded in Nootripathu (18.3 mg kg^{-1}). CO(R)50 (10.4

Table 10. Effect of seedling age in rice landraces on gel consistency and amylose content (%).

Seedling age / Landraces	Length of gel (mm)				Amylose content (%)			
	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	56	56	56	56	23.6	23.5	23.5	23.5
Kuliyadichan	49	49	48	49	26.5	26.2	26.2	26.3
Kuruvaikalanjiyam	52	50	51	51	24.6	24.6	24.6	24.5
Norungan	55	55	54	55	24.9	24.8	24.8	24.9
Nootripathu	57	57	56	57	23.5	23.4	23.4	23.4
Blackkavuni	56	56	56	56	20.6	20.2	20.2	20.3
Redkavuni	58	58	57	58	21.4	21.8	21.8	21.1
Njavara	62	62	60	61	18.5	18.5	18.5	18.5
CO(R)50	83	83	82	83	21.7	21.5	21.5	21.6
Mean	59	58	58		22.8	22.7	22.7	
	A	V	A at V	V at A	A	V	A at V	V at A
SEd	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97
CD(P=0.05)	NS	2.24	NS	NS	NS	2.01	NS	NS

Table 11. Effect of seedling age in rice landraces on zinc and iron content (mg kg^{-1}).

Seedling age / Landraces	Zinc content				Iron content			
	15 days	20 days	25 days	Mean	15 days	20 days	25 days	Mean
Chandikar	23.8	23.8	23.5	23.7	14.7	14.6	14.3	14.5
Kuliyadichan	24.7	24.5	24.5	24.6	11.6	11.8	11.4	11.6
Kuruvaikalanjiyam	29.2	29.4	29.2	29.3	13.9	13.8	13.6	13.8
Norungan	26.6	26.9	26.3	26.6	16.5	16.6	16.4	16.5
Nootripathu	23.7	23.2	23.4	23.4	18.5	18.2	18.1	18.3
Blackkavuni	21.4	21.9	21.6	21.6	19.1	19.2	19.2	19.2
Redkavuni	23.4	23.3	23.5	23.4	19.9	19.6	19.9	19.8
Njavara	22.9	22.9	22.8	22.9	11.4	11.3	11.6	11.4
CO(R)50	21.1	21.2	21.3	21.2	10.6	10.4	10.3	10.4
Mean	24.1	24.1	24.0		12.9	12.8	12.8	
	A	V	A at V	V at A	A	V	A at V	V at A
SEd	1.16	1.97	0.53	1.16	1.97	0.53	1.16	1.97
CD(P=0.05)	NS	2.24	NS	NS	NS	2.01	NS	NS

mg kg^{-1}) and Black Kavuni (19.2 mg kg^{-1}) registered lower iron content. (Maganti et al., 2020) reported Iron concentration varied from 6.9 to 22.3 mg kg^{-1}, whereas zinc concentration ranged from 14.5 to 35.3 mg kg^{-1} in unpolished, brown rice (Table 11).

CONCLUSION

From the experimental results, it concluded that 15 days old seedlings in all the eight rice landraces and one high yielding variety, recorded higher growth and yield attributes. In rice landraces, Red Kavuni recorded higher grain, straw yield and B:C ratio followed by Black Kavuni. Hence, these two varieties can be suggested for cultivation in Tamil Nadu and it would be more profitable for small and marginal farmers.

REFERENCES

Adu-Kwarteng E, Ellis WO, OduroIand Manful JT (2003). Rice grain quality: A comparison of local varieties with new varieties under study in Ghana. Food Control 14: 507-514

Ancy Francis, (2007) Evaluation of different crop establishment methods for increasing yield in transplanted hybrid rice. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu

Azzez MA, Shafi M. (1966) Quality in rice. Department of Agriculture West Pakistan Technical Bulletin No 3. pp. 13-50

Banu B, Kabir KA, Begum F and Choudhury NH (1992). Physicochemical properties of modern and local rice varieties of Bangladesh. Bangladesh Rice Journal 3: 128-131

Cagampang RK, Chang TT, Sapra RL and Paroda RS (1973). Evaluation studies in indigenous rice (*Oryza sativa* L.) germplasm at IRRI. Philippines. Published in NBPGR Manual pp. 1-3

Chowdhury MJU, Sarkar MAR and Kashem MA (1993). Effect of variety and number of seedlings $hill^{-1}$ on the yield and yield components on late transplanted Aman rice. Bangladesh Journal of Agricultural Research 20(2): 311-316

CPG (2012). Crop Production Guide.Published by Directorate of Agriculture, Chennai and Tamil Nadu Agricultural University, Coimbatore

Das B, Mannan MA and Das PK (2012). Performance study of three high yielding varieties of Bangladesh and one exotic rice variety. B.Sc. (Ag.) Thesis, Khulna University, Khulna

Devi KN and Singh AR (2000). Influence of seedling age and planting density on the performance of rice. Oryza 37(1): 99-100

Hogan JT and Plank RW (1958). Hydration characteristics of rice as influenced by variety and drying method. Cereal Chemistry 35: 469-482

Hossain M S, Singh AK and Fasihuz Zaman (2009). Cooking and eating characteristics of some newly identified inter sub specific (*indica*) rice hybrids. Science Asia 35: 320-325

Irfan M, Muhammad T, Amin M and Jabbar A (2005). Performance of yield and other agronomic characters of four wheat (*Triticum aestivum* L.) genotypes under natural heat stress. International Journal of

Botany 1(2): 124-127

Juliano BO (1972). In: Physicochemical properties of starch and protein in relation to grain quality and nutritional value of rice. IRRI, Publications pp. 389-405

Juliano, BO and Perez CM (1984). Results of a collaborative test on the measurement of grain elongation of milled rice during cooking. Journal of Cereal Science 2: 281-292

Kaul AK (1970). Early generation testing for quality characteristics. II. Rice. Indian Journal of Genetics and Plant Breeding 30: 237-243

Khatun R (2001). Effect of variety and nitrogen on the performance of fine rice. M.Sc. (Ag).Thesis, Bangladesh Agricultural University, Bangladesh

Khush, GS, Paule CM and. Delaa Cruz NM (1979). In: Proc. Workshop on chemical aspects of rice grain quality. IRRI, Philippines pp. 22-31

Maganti S, Swaminathan R and Parida A (2020).Variation in Iron and Zinc Content in Traditional Rice Genotypes. Agricultural research 9: 316-328

Li X, Qian Q, Fu Z, Wang Y, Xiong G (2003). Control of tillering in rice. Nature 422(6932): 618-621

Little RR, Hilder GB, Dawson EH and Elsie H (1958). Differential effect of dilute alkali on 25 varieties of milled white rice. Cereal Chemistry 35: 111-126

Mohammed Ashraf A and Subbalakshmi Lokanadan (2017). A Review of Rice Landraces in India and its Inherent Medicinal Values - The Nutritive food values for Future. International Journal of Current Microbiology and Applied Sciences 6(12): 348-354

Mohammed Ashraf A, Subbalakshmi Lokanadan and Rajeswari S (2017). Studies on the Effect of Seedling age on Growth and Physiological Parameters in Rice Landraces. International Journal of Agriculture Sciences 9(10): 3984-3988

Moncada P, Martinez CP, Borrero J, Chatel M, Gauch HJr, Guimaraes E, Tohme J, McCouch SR (2001). Quantitative trait loci for yield and yield components in an *Oryza sativa* x *Oryza rufipogon* BC2F2 population evaluated in an upland environment. Theoretical Applied Genetics 102: 41 - 52

More MR, Pawar LG, Chavan SA and Misal RM (2007). Effect of methods of raising seedlings, seedling age and transplanting on growth and yield of rice. In: Second National Symposium on System of Rice Intensification (SRI). Oct 3-5, Agartala, Tripura, India

Rajesh V and Thanunathan K (2003). Effect of seedling age, number and spacing on yield and nutrient uptake of traditional Kambam chamba rice. Madras Agricultural Journal 90(1-3): 47-49

Ramaiah K and Rao MVBN (1953). Rice breeding and genetics. ICAR Science Monograph 19.Indian Council of Agricultural Research, New Delhi, India

Rajendran R, Ravi V, Nadanasabapathy T, Valliappan K, Ramanathan S, Jayaraj T and Balasubramanian V (2004). Modified Mat Nursery for producing robust young seedlings in 15 days for early transplanting and enhanced productivity under transformed rice cultivation. Indian Journal of Agronomy 49(2): 155-157

Rajendran K (2009). Evaluation of crop establishment techniques and weed management practices under System of Rice Intensification. Ph.D. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore

Rhoades A (2008).Basmati rice- the quality grain. (www.allcreatures.org)

Sathya A, Rajamanickam V and Ramasamy K (2007). Evaluation of biodiversity in traditional paddy varieties of Tamil Nadu. Ph.D Thesis, Tamil University. Thanjavur. Tamil Nadu

Senthilkumar K (2002). Productivity of hybrid rice under water saving irrigation and insitu weed incorporation. M.Sc. (Ag.) Thesis, Tamil Nadu Agricultural University, Coimbatore

Singh, RS and Singh SB. (1999) Effect of age of seedlings, N levels and time of application on growth and yield of rice under irrigated conditions. Oryza 36(4): 351-354

Stoop WA, Uphoff N and Kansam A (2002). A review of agricultural research issue raised by the System of Rice Intensification (SRI) from Madagascar: Opportunities for improving farming systems for resource poor farmers, Agricultural Systems 71(3): 249-274

Uphoff N (2002). System of Rice Intensification (SRI) for enhancing the productivity of land, labour and water. Journal of Agricultural Extension Management 1(1): 43-49

Vijayakumar M, Sundar Singh SD, Prabhakaran NK and Thiyagarajan TM (2005). Effect of SRI (System of Rice Intensification) practices on the yield attributes yield and water productivity of rice (*Oryza sativa* L.). Acta Agronomica 52(4): 399-408

Wang Shao-Hua, Cao Weixing, Jiang Dong, Dai Tingbo and Zhu Yan (2002). Physiological characteristics and High-yield Techniques with SRI Rice.(Eds), Assessments of the System of Rice Intensification. In: Proceedings of an International Conference, Sanya, China. Ithaca: NY: Cornell International institute for Food, Agriculture and Development. pp. 116-124

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Standardisation of soaking and germination time for preparation of germinated brown rice

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ABSTRACT

A study was undertaken to standardise the soaking and germination time for preparation of germinated brown rice (GBR). Freshly harvested paddy of Prativa variety was dehusked (dehusked or dehulled) in rubber roll sheller to get brown rice which was soaked in demineralised water followed by germination at 27±1°C temperature and 85-90 % relative humidity for different time period. The soaking and germination time were standardised based on moisture content, germination percentage, Gamma-Aminobutyric acid (GABA) content and total microbial load for preparation of germinated brown rice. Germination percentage and GABA content increased significantly with germination time (p<0.05). Soaking of brown rice for 12 h followed by 24 h germination was standardised for preparation of germinated brown rice to get maximum GABA content of 70.3 mg/100 g d.m.

Key words: *Germinated brown rice, GABA content, soaking, germination, microbial load*

Brown rice is less desirable due to its poor cooking and eating qualities in spite of high nutritional value (Patil and Khan, 2011). Now this problem is overcome by germinating it, which improves its texture, palatability and the amount of the bioactive molecules. Germination of brown rice increases the rate of water absorption and softens the cooked brown rice kernels, improving eating quality. Moreover, it activates residual enzymes in brown rice, thus inducing the formation of various metabolic components having bioactive functions. Therefore, germinated brown rice (GBR) has gained significant attention during the last decade as a tool of enhancing eating quality and potential health-promoting functions.

Germinated brown rice, also known as 'sprouted brown rice', is another popular form of brown rice attributing to its high nutritional value. The high nourishing content of the germinated brown rice is due to the presence of gamma-aminobutyric acid (GABA), which increased 8.31 times upon 24 h germination (Imam et al., 2012). The germinated form can be obtained by soaking brown rice in water and sprouting for specified periods. The nutrients which have increased significantly after germination include GABA, lysine, vitamin E, dietary fibre, niacin, magnesium, vitamin B1, and vitamin B6 .These nutrients aid in better absorption during digestion and prevent intestinal irritations, inflammations, and allergies (Patil and Khan, 2011).

Patil and Khan, 2011 showed that GBR contains considerable phytic acid with a powerful anticancer activity and a prolyl endopeptidase activity inhibitor related to the metabolism of peptide. GABA is having high biological activity including pharmacological functions and neurotransmitters in brain and spinal cord of mammals (Liu et al., 2013).

Soaking and germination can make brown rice soften and activate bioactive compounds such as phenolics, flavonoids and GABA. Increase in GABA content in GBR was reported to be correlated with soaking condition and germination conditions (Maisont and Narkrugsa, 2010).Soaking is the first step in water penetration which transforms the inactivate tissue into living tissues. Hydrolytic enzymes are activated and these decompose large molecular substances such as starch, non- starch polysaccharides and proteins to small molecular compounds. These processes result in an increase of simple sugars, peptides and the amino acids of germinated seeds (Moongngarm and Saetung, 2010). Several researchers attempted to find out the optimum soaking condition, incubation condition and drying temperature to obtain GBR with high content of nutrients and bioactive compounds (Singh et al., 2017; Mohan et al., 2010). But there are still problems and disputes on soaking and germination conditions to obtain germinated brown rice from different varieties. So, the study was undertaken to standardise the soaking and germination time for preparation of germinated brown rice.

Collection of sample

Freshly harvested paddy of Prativa variety was collected from the Central Farm of Odisha University of Agriculture and Technology, Bhubaneswar. Paddy was first cleaned and graded in a cleaner-cum-grader to remove all foreign matters and immature grains. Paddy samples were dehusked using rubber roll sheller (MG make) followed by aspiration to obtain brown rice.

Preparation of germinated brown rice

About 500 g of brown rice was taken and rinsed twice using clean tap water until the water is clear. The sample was sterilised in 0.1 % sodium hypochlorite solution for 30 min and then washed twice with distilled water. The rice was soaked in demineralized water with sample to water ratio of 1:5 (w/v) at 30±2°C for different time periods of 6, 12, 18, 24 and 30 hours to saturate the kernel. The steeping water was replaced with fresh demineralised water after every 4 h to avoid any possible fermentation and contamination. Samples were drawn after regular interval of 6 h and moisture content of the soaked samples were determined using hot air oven method. Soaking time was standardised based on achievement of saturation moisture content level.

Standardisation of germination time

After soaking for the desired time period, the water was drained out and subsequently the soaked brown rice sample was spread in a single layer on germination paper in plastic trays. Water was sprinkled to keep the paper moist during germination. The trays were placed in an incubator maintained at 27±1^{o}C temperature and 85-90 % relative humidity up to 30 h for germination. Samples were drawn at regular interval of 6 h to determine the percentage of germination, total microbial load and GABA content.

Germination percentage

The percentage of germination was calculated by taking 20 g of representative sample and counting the number of germinated and ungerminated seed. This experiment was replicated for three times and average value of germination percentage was taken.

Drying of germinated brown rice

Germinated brown rice obtained after the desired period of soaking and germination was dried to 8±0.5% moisture content at 50°C temperature in a tray dryer. The dried GBR was cooled before packing in zipped pouches and stored at 4^{o}C. The GABA content of the germinated brown rice was determined using standard procedure

Determination of GABA content

About 0.5 g of germinated brown rice sample was soaked for 30 minutes and cooked for 15 minutes in 3 ml of water. Amino acids were extracted from the cooked rice paste by 2.5 ml of ethanol:deionized water (7:3). Standard GABA solution and sample solutions were applied to the 10 cm x 10 cm high-performance standard silica gel plate in HPTLC. The scanned areas of the rice samples were matched with the scanned area of the standard GABA solution and GABA content was calculated based on concentration of the standard solution.

Microbiological test

The rice samples were analysed for total microbial load using Nutrient Agar. Media was sterilised by autoclaving at 15 lbs pressure (121^{o}C) for 15 minutes and poured into the petri-plates and kept in laminar air flow chamber for 20-25 min under the surveillance of UV light. The samples were diluted in sterile 0.85 g/100 ml saline solution and spread into the plates. Plates were incubated at 37^{o}C for 24 h to obtain bacterial colonies and every colony on the plate were counted. Results were expressed as colony forming units per gram.

CFU/ml = (No.of colonies ×dilution factor)/(volume of sample plated)

Statistical analysis

The experiments were planned with completely randomized design (CRD). Data obtained from the experiments in triplicate were analysed using MS EXCEL software. The effects of soaking and germination period were analysed statistically through analysis of variance (ANOVA) at 5 % level of significance.

Effect of soaking time on moisture content of brown rice

Brown rice was soaked in water prior to germination to increase its moisture content favourable for sprouting. The change in moisture content of brown rice with soaking time is shown in Fig. 1. The moisture content increased rapidly up to 6 h and rate of water absorption decreased after that. There was no significant difference in moisture content of soaked brown rice above 12 hours of steeping. The moisture content of brown rice increased from an initial value of 14.5 to 47.9 % (d.b.) after soaking for 12 h at 30^{o}C. Our results are in agreement with Maisont and Narkrugsa (2010) who reported that water absorption rate increased rapidly at early stages of soaking up to 15 h from 12.68 to 28.31 % (w.b.). Singh et al. (2017) reported that moisture content of Phitsanulok2 rice variety reached 8.74 % after 5 h of soaking at 30oC and Cao et al. (2015) reported that moisture content of Dongnong 419 rice variety reached 29 % after 8 h of soaking at 30^{o}C. It indicating that hydration characteristics varied with variety. Soaking helps water absorption for activating the germination process and enzymatic activities (Patil and Khan, 2011).

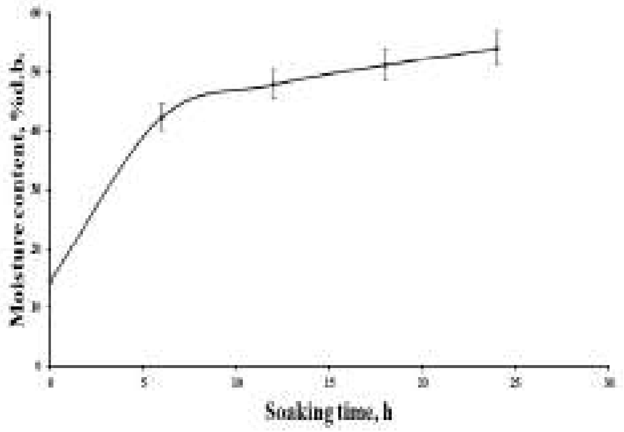

Fig. 1. Change in moisture content of brown rice with soaking time.

Effect of soaking and germination time on germination percentage of brown rice

The germination percentage of brown rice for different soaking and germination period is shown in Fig. 2. Germination percentage varied significantly with soaking and germination time at 5% level (p<0.05). Germination percentage was found to be more than 90 % above 12 h of soaking and 12 h of germination. Soaking for long time resulted in off flavour development and loss of solubles, whereas mould growth was noticed with prolonged germination time. Moongngarm and Saetung (2010) reported germination percentage of 84.3 % in brown rice soaked for 12 h and incubated for 24 h.

Effect of soaking and germination time on GABA content of GBR

The effect of soaking and germination time on GABA (Gamma-Aminobutyric Acid) content of germinated brown rice is shown in Fig. 3. GABA content increased significantly with soaking and germination time (p<0.05). Soaking and germination time above 12 h resulted in GABA content in the range of 60-80 mg/kg d.m. Moongngarm and Saetung (2010) also reported that GABA content in germinated grains was higher than in non-germinated brown rice after soaking and

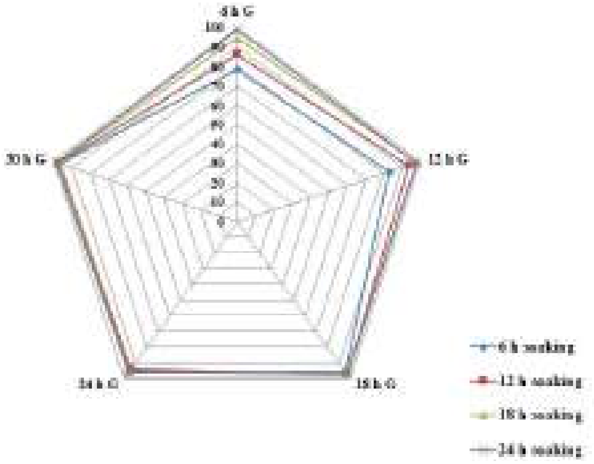

Fig. 2. Effect of soaking and germination time on germination percentage.

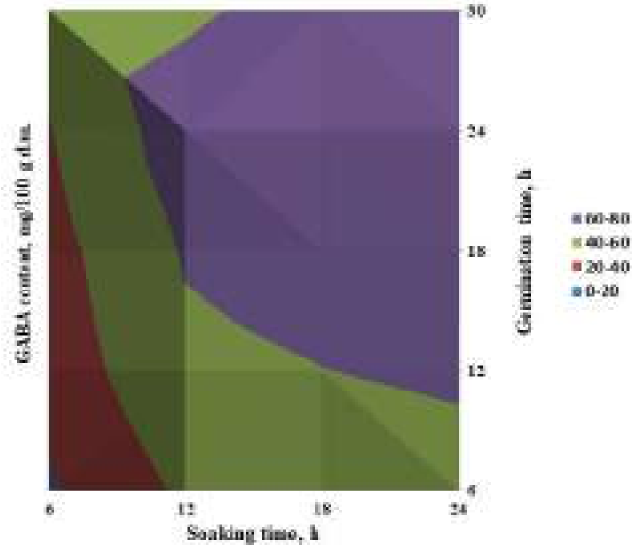

Fig. 3. Effect of soaking and germination time on GABA content of GBR.

incubation.

The effect of germination time on GABA content of GBR obtained from 12 h soaked sample is shown in Fig. 4. GABA content increased with germination time and maximum value of 70.3 mg/100 gd.m. was obtained in 24 h germinated sample which was seven times more than brown rice. The GABA content decreased after 24 h of germination which might be due to conversion to other compounds. So, brown rice soaked for 12 h followed by 24 h germination was standardised to obtain GBR with higher GABA content. Our results were consistent with Moongngarm and Saetung (2010) who reported that GABA content increased from 23.8±1.74 in ungerminated rice to 68.4±4.43 mg/100 g d.w. in GBR after 12 h steeping and 24 h germination at 28-30°C.

Different values of GABA contents of GBR were reported by various researchers, which might be due to varietal effect and variation in steeping and germination conditions studied (Zhang et al., 2014). Increase in GABA content ranging from 80 to 220 mg/100 g embryo fresh weight for 12 to 60 h germination time was reported by Maisont and Narkrugsa (2010). However, lower value of GABA content of 2.74 ± 0.23 mg/100 g for BR and 16.9±0.72 mg/100 g for GBR was reported by Srisang et al. (2011). Singh et al. (2017) reported that GABA content of brown rice increased gradually with increase in steeping time and highest GABA content was recorded to be 17.66 mg/100g when brown rice was steeped at 33°C for 5 h. Liu et al. (2013) reported increase in GABA content from 4.68 mg/100g in brown rice to maximum value of 24.36 mg/100 g in GBR and it increased significantly after germination and over time. Singh et al. (2017) also reported that GABA content increased dramatically with pre-germination time during soaking process. GABA content ranging from 1 to 10 mg/100g d.w. was found in different varieties of brown rice which increased to a level ranging from 8 to 94 mg/100g after 72 h of pre-germination soaking. GABA content of GBR was reported to be 17 mg/100g after 12h soaking at 35°C by Sirisoontaralak et al. (2015).

During germination, sprout activated dormant enzymes in the brown rice to supply the best nutrition required for the sprout growth increasing the existing nutrients and releasing new components such as digestible vitamins, minerals, amino acids including GABA (Sirisoontaralak et al., 2015). Glutamate decarboxylase (GAD) enzyme converted glutamic acid to accumulate GABA. Germination should stop at a certain point, unless GABA would be converted to succinicisemi aldehyde and succinate at last.

Effect of germination period on total microbial load on surface of brown rice

Total microbial count on surface of GBR after different germination period is shown in Fig. 4. The total microbial load of germinated brown rice increased with increase in germination period. The total microbial load increased from 4.04 log cfu/g after 6 h of germination to 7.45 log cfu/g after 30 h of germination. Liu et al. (2013) reported that number of microbes on the surface of BR soaked in tap water for 8 h was 6.53 log cfu/g which reduced by using effective washing system. Lu et al. (2010) reported that aerobic plate count of GBR increased above 6.52 log cfu/g after germination at 37°C for 36 h, which decreased from 4.82 to 5.38 log CFU/g after using Electrolyzed Oxidizing Water for soaking. The decrease in GABA content above 24 h of germination may be due to higher microbial load leading to fermentation and decomposition of nutrients. Washing, cooking and subsequent drying could decrease the number of aerobic counts to the level safe according to food safety standards.

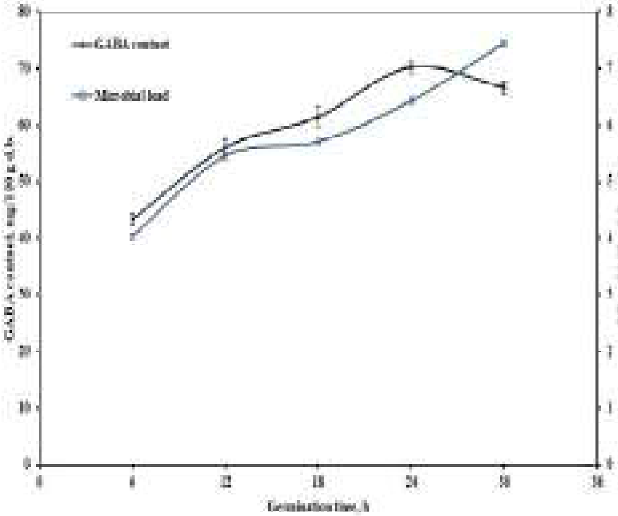

Fig. 4. Effect of germination time on GABA content and microbial load of GBR.

CONCLUSION

The moisture content of brown rice increased from an initial value of 14.5±0.72 to 47.9±2.39% (d.b.) after soaking for 12 h at 30oC. Germination percentage varied significantly with soaking and germination time ($p < 0.05$) and found to be more than 90 % above 12 h of soaking and 12 h of germination. GABA content increased with germination time, maximum value of 70.3 mg/100 g d.m. was obtained in 12 h soaked, and 24 h germinated sample. The total microbial load increased from 4.04 log cfu/g after 6 h of germination to 7.45 log cfu/g after 30 h of germination. As GABA content decreased after 24 h of germination and microbial load continued to increase, soaking of brown rice for 12 h followed by 24 h germination was standardised for preparation of germinated brown rice.

REFERENCES

Cao Y, Jia F, Han Y, Liu Y and Zhang Q (2015). Study on the optimal moisture-adding rate of brown rice during germination by using segmented moisture conditioning method. Journal of Food Science Technology 52(10): 6599-6606

Imam MU, Musa SA, Azmi NH and Ismail M (2012). Effects of white rice, brown rice and germinated brown rice on antioxidant status of type 2 diabetic rats. International Journal of Molecular Sciences (13): 12952-12969

Liu LL, Zhai HQ and Wan JM (2013). Accumulation of y-aminobutyric acid in giant-embryo rice grain in relation to glutamate decarboxylase activity and its gene expression during water soaking.Cereal Chemistry 82(2): 191-196

Lu ZH, Zhang Y, Li LT, Curtis R B, Kong XL, Fulcher G, Zhang G and Cao W (2010). Inhibition of microbial growth and enrichment of c-aminobutyric acid during germination of brown rice by electrolyzed oxidizing water. Journal of Food Protection 73(3): 483-487

Maisont S and Narkrugsa W (2010).Effects of salt, moisture content and microwave power on puffing qualities of puffed rice.Journal - Natural Science 44(2): 136-145

Mhoan BH, Malleshi MG and Koseki T (2010). Physico-chemical characteristics and non-starchpolysaccharide contents of Indica and Japonica brown rice and their malts, LWT- Food Science and Technology 43(5):784-791 https://doi.org/10.1016/j.lwt.2010.01.002

Moongngarm A and Saetung (2010). Comparison of chemical compositions and bioactive compounds of germinated rough rice and brown rice. Food Chemistry 122(3): 782-788

Patil SB and Khan MK (2011). Germinated brown rice as value added rice product: A review. Journal of Food Science and Technology-Mysore 48: 661-667

Singh K, Simapisan P, Decharatanangkoon S and Ang N U (2017). Effect of soaking temperature and time on GABA and total phenolic content of germinated brown rice (Phitsanulok 2). Current Applied Science and Technology (17): 222-227

Srisang N, Prachayawarakorn S, Varanyanond W and Soponronnarit S (2011). Germinated brown rice drying by hot air fluidization technique. Drying Technology 29(1): 55-63

Sirisoontaralak P, Nakornpanom N, Koakietdumrongkul K and Panumaswiwath C (2015). Development of Quick cooking germinated brown rice with convenient preparation and containing health benefits. Food Science and Technology 61: 138-144

Zhang ZH, Jhaveri DJ, Marshall VM, Bauer CD, Edson J, Narayan RK, Robinson GJ, Lundberg AE, Bartlett PF, Wray NR and Zhao QY (2014). A comparative study of techniques for differential Expression Analysis on RNA-Seq Data. Journal of Food Engineering 8(76): 256-260

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Heterosis for yield and quality traits in traditional and evolved Basmati

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ABSTRACT

A study was undertaken on 36 F_1s obtained from diallel (without reciprocals) mating design to explicate the genetic behavior of traditional and evolved basmati varieties for 14 traits. F_1s were divided into traditional x traditional (T x T); evolved x evolved (E x E) and traditional x evolved (T x E) basmati types; amidst which E x E and T x E type of crosses exhibited high positive average heterosis in comparison to T x T type of crosses for yield per plant trait. Assessment of quality traits for average heterosis indicated that, all the three types of crosses were equally effective for KL, KB and LBR. Manifestation of average heterosis for KLAC was better in TxT type as none of the ExE type of crosses showed significant positive heterosis; likewise, among 20 T x E type of crosses only 3 crosses showed significant positive heterosis. Heritability in narrow sense was high for: MPL, 100-GW, NGP, YPP, KL, LBR, and KLAC.

Key words: *Traditional Basmati, evolved Basmati, average heterosis, heritability in narrow sense*

Basmati is a type of aromatic rice which is famous for its sweet aroma and excellent organoleptic properties *viz*., long slender kernel, elongation ratio of cooked rice texture, taste (Siddiq et al., 2012). Among the long list of aromatic rice cultivated in India, basmati has its own popularity among its consumers. Basmati is an aromatic rice but all aromatic rice is not basmati. There are set of rules devised by government of India under "Export of Basmati Rice (Quality Control and Inspection) Rules, 2003", to define any variety of rice to be called as Basmati. These rules encompass the different prime quality traits of Basmati rice *viz*., kernel dimension, aroma, cooking quality, etc. Aromatic rice varieties which qualify these rules are called Basmati thereafter. Basmati earns highest foreign exchange from export of agricultural commodities in India. The pleasant aroma, kernel dimension (slender long kernel), excellent cooking quality (cooked kernel length, texture) are quality traits of Basmati which are accountable to its everlasting demand both in domestic and international market. Given, the importance of crop in terms of demand and as a valuable agricultural export product (Sharma and Jaiswal, 2020b), basmati is a very important crop for India.

Basmati varieties are divided into traditional and evolved varieties depending upon how they were developed. As per "Export of Basmati Rice (Quality Control and Inspection) Rules, 2003" - traditional Basmati varieties are selected from land races and identified under the Seeds Act 1966 (54 of 1966); while evolved Basmati varieties are developed using molecular or traditional breeding methods in such a way that they have a traditional basmati variety in their lineage. Traditional basmati shows poor general combining ability (Ahuja et al., 1995 and Sharma et al., 2021); respond less to yield improvement due to lack of genetic variability (Siddiq et al., 2012). Sharma and Jaiswal (2020a); Kour et al. (2019) and Kumar et al. (2015) have also recorded low general combining ability in traditional Basmati varieties for yield per plant trait. In cognizance of aforementioned information, in this paper we have evaluated the performance of traditional and evolved Basmati varieties for yield and quality traits by estimating average heterosis, genetic variance, and heritability.

Experimental materials

Study of the present manuscript was based on nine parental material of Basmati. Among the nine varieties used, four varieties were traditional basmati *viz*., Type-3 (T-3), Basmati-370 (B-370), Taraori Basmati (TB), and Ranbir Basmati (RB); and five varieties Pusa Basmati-1, (PB-1), CSR-30, Pusa Basmati-1121 (PB-1121), HUBR10-9, and Pusa Basmati-1509 (PB-1509) were evolved basmati. The experiment was conducted at the Agricultural Research Farm, Institute of Agricultural Sciences, BHU, Varanasi, Uttar Pradesh under irrigated conditions in two seasons (*Kharif* 2016 and 2017).

Crossing program

In *kharif* 2016, nine parental varieties were sown at three different dates in crossing blocks. 36 F_1s were developed following 9 x 9 diallel (without reciprocals) mating design. The F_1s seeds from each of the 36 crosses were harvested separately in a paper bag with proper labeling and kept in cool and dry place. In *kharif* 2017, the 36 F_1s along with their nine parents were sown in three replications in Randomized Block Design.

Data collection

Observations were recorded in field on eight yield traits *viz*., days to 50% flowering (DTF), days to maturity (DTM), plant height (PH), main panicle length (MPL), number of panicles per plant (NPP), total number of grains per panicle (NGP), 100-grain weight(100-GW), yield per plant (YPP) and on 6 quality traits *viz*., kernel length (KL), kernel breadth (KB), kernel length/breadth ratio (LBR), kernel length after cooking (KLAC), kernel breadth after cooking (KBAC), and aroma, in laboratory. To take aforementioned observations, 10 plants were randomly selected and tagged from each of the 45 lines (36 F_1s and 9 parents) of three replications. Data for yield and quality traits were recorded according to Standard Rice Evaluation System, IRRI, 2013.

Statistical analysis

Data was analyzed using Windostat services (9.3 version) following Method II (Griffing, 1956) and Model I (Eisenhart, 1947). Analysis of variance of Randomized Block Design was done using Panse and Sukhatme (1967). Genetic variance and heritability were studied from the analyzed data obtained from Griffing numerical approach of Method II (F_1s without reciprocals and parents) and Model I (fixed model). Mid-parent heterosis was calculated by using formula given below:

$$\text{MPH} = \frac{F_1 - \text{MP}}{\text{MP}} \times 100$$

Where MPH=mid-parent heterosis; F_1 = mean value of cross between P_1 and P_2; MP = mid-parent; which is mean value of two parents (P_1 and P_2).

Standard error of Difference (S.Ed.) and Critical Difference (CD) for heterosis over mid parent was calculated using formula:

$$\text{S.E.}\left(\text{MPH}\right) = \frac{\sqrt{3\text{Me}}}{2}$$

Where, Me= mean sum of square of error; Critical difference = S. Ed x t (at 5 and 1% level for error d.f.),

$h^2ns = V_A/V_P$; h^2ns =heritability in narrow sense; V_A= additive variance; V_P =phenotypic variance

Mean performance of hybrids and varieties

Aromatic landraces give poor yield (Ahuja et al., 2008; Prodhan et al., 2017; Prasad et al., 2020) and so is traditional Basmati varieties (Singh et al., 2018). In the present finding we observed that yield per plant of traditional basmati varieties were significantly less than evolved basmati varieties. Among seven yield attributing traits examined in the paper, all types of crosses performed better or equivalent to parents. For yield per plant ExE type of crosses were better than T x E. For pre-cooked kernel dimension related traits (KL, more than 6.61 mm; KB, less than 2 mm; and L/B ratio, more than 3.5) all the 36 crosses from three different types measured equivalent or better than the minimum acceptable standards of Basmati milled rice according to export of Basmati rice (Quality Control and Inspection) Rules, 2003. We even observed KL of more than 8 mm; but in ExE or TxE type of crosses only *viz*., CSR30 X PB-1509, PB-1121 X PB1509, T3 X PB-1121, T3 X PB-1509, TB X PB-1121 and TB X PB1509 (Table 1). Minimum KLAC of more than 12 mm and

Table 1. Mean Performance of parents, crosses and checks for yield and quality traits of Basmati.

TxT	DTF	DTM	PH	MPL	NPP	GYP	100-GW	YPP	KL	KB	LBR	KLAC	ER	KBAC	Aroma
T-3xB-370	97	138	127.4	30.3	10.6	76.5	2.2	19.3	6.8	1.6	4.2	12.9	1.9	2.9	2.5
T-3xTB	98	139	136.9	30.4	7.7	91.4	2.4	21.3	7.2	1.7	4.3	11.7	1.6	2.8	1.4
T-3xRB	98	131.7	124.6	30	8.4	82.8	2.2	18.9	7	1.6	4.3	10.6	1.5	2.8	2.6
B-370xTB	97	128	142.3	28.4	14.2	107.1	2.4	22.5	7.8	1.7	4.6	15.3	2	2.2	1.3
B-370XRB	97	131	152.9	33.3	14.2	144.8	2.3	38.4	7.2	1.7	4.1	13.9	1.9	2.2	1.6
TBxRB	99	132	112.5	31.5	11.9	95.6	2.3	25.4	7.4	1.7	4.4	10.9	1.5	2.8	1.5
ExE															
PB-1xCSR-30	94	129.7	151	28.7	15.6	85.2	2.4	39.5	7.7	1.7	4.4	10.9	1.4	2.2	1.5
PB-1xPB-1121	91	132	110.9	30.1	8.8	81	2.6	29.3	8	1.7	4.7	16	2	2.7	1
PB-1xHUBR 10-9	98	131	103.7	32.3	11.6	156.4	2.6	52.3	7.3	1.7	4.2	14.7	2	2.8	1.5
PB-1xPB-1509	97	129	107.6	32.7	15.7	108.6	2.7	49.3	7.9	1.7	4.6	13.4	1.7	2.8	1.6
CSR-30xPB-1121	97	134.3	107	27.8	12	62.5	2.9	18.7	7.9	1.7	4.7	15.1	1.9	2.1	1
CSR-30xHUBR 10-9	108	146	143.5	30.8	20.4	127.9	2.9	55	7.4	1.7	4.3	11.1	1.5	2.1	1.4
CSR-30xPB-1509	100	138	124.2	29.8	9.8	88.3	3.1	24.8	8.1	1.7	4.6	12.6	1.6	2.3	1.5
PB-1121xHUBR 10-9	96	129	122.9	32.3	13.5	120.6	2.9	52.3	8	1.7	4.7	14.4	1.8	2.8	1.4
PB-1121xPB-1509	98	130.3	134.9	27.3	10.4	59.4	3	22.4	8.4	1.7	4.9	15.9	1.9	2.1	1.5
HUBR10-9xPB-1509	98	128	124.6	32.4	15.9	136.3	2.9	52.1	7.6	1.7	4.5	15.1	2	2.6	1.6
TxE															
T-3xPB-1	99	131	160	33.3	11.2	113.1	2.4	25.6	7.2	1.8	4.2	11	1.5	2.8	2.4
T-3xCSR-30	98	134	126.5	27.2	10.6	86.1	2.3	20.2	7.2	1.6	4.4	11.4	1.6	2.6	2.5
T-3xPB-1121	99	132.7	134.8	30.2	12.5	84.4	2.9	31.1	8.1	1.8	4.4	17.6	2.2	2.8	1.8
T-3xHUBR 10-9	102	135	142.6	32.1	14.1	117.6	2.6	38.1	7	1.9	3.8	12.8	1.8	2.6	1.6
T-3xPB-1509	97	133	118.7	30	9.8	82.6	2.8	24.3	8.1	1.7	4.7	17.6	2.2	2.7	1.7
B-370xPB-1	98	135	99.3	32.9	12.2	140.6	2.4	33.6	7.2	1.7	4.2	13.3	1.9	2.7	1.8
B-370xCSR-30	99	133	107.3	30.1	16.3	92	2.4	28.3	7.5	1.7	4.5	11.4	1.5	2.4	1.5
B-370xPB-1121	102	136.7	134.9	25.2	13.5	90.9	2.7	30.5	7.3	1.7	4.4	13.9	1.9	2.5	1
B-370xHUBR 10-9	106	142	135.3	32.7	14.6	115.1	2.5	39.4	7	1.8	3.9	12.1	1.7	2.7	1.5
B-370xPB-1509	103	136	154.3	27	10.5	82.1	2.8	25.8	8.1	1.8	4.5	15.5	1.9	2.6	1.7
TBxPB-1	100	135	130.2	30.8	11	122.7	2.5	32.7	7.8	1.8	4.4	11.9	1.5	2.8	1.7
TBxCSR-30	98	134	96.9	28	16.2	81.2	2.4	25.1	7.7	1.7	4.6	11.1	1.4	2.6	1.7
TBxPB-1121	99	131	124.7	30.4	12.1	80.4	3.2	28.6	8.4	1.8	4.7	11.3	1.4	2.2	1.4
TBxHUBR 10-9	109	139	88.7	31.1	15.1	144.7	2.6	45.6	7.4	1.8	4	13.3	1.8	2.6	1.3
TBxPB-1509	95	126	157.4	29.6	12.2	110.1	3.1	25.1	8.2	1.7	4.7	17.6	2.1	2.5	1.7
RBxPB-1	96	127	96.9	33.6	10.7	118.6	2.5	27.2	7.4	1.7	4.3	11.9	1.6	2.7	1
RBxCSR-30	98.3	131	145.2	29.3	7.9	107.9	2.4	21	7.3	1.6	4.5	11.5	1.6	2.1	1.5
RBxPB-1121	98	133	127.5	30.2	13.1	83.8	2.9	26.9	7.9	1.8	4.5	14.3	1.8	2.1	1.4
RBxHUBR 10-9	108	142	135.1	33.9	14.1	149.3	2.6	45.9	7.1	1.8	3.8	13.8	2	2.8	1
RBxPB-1509	99	129	114.2	29.4	11.5	94.9	2.2	26.7	7.1	1.8	3.9	13.5	1.9	2.2	1.5

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PARENTS															
T-3	103.7	141	147.9	29.6	8.7	90.1	2.3	20.7	6.8	1.9	3.6	12	1.8	2.4	2.9
B-370	100.7	139	154.3	28.4	8.8	74.4	2.2	18.8	6.7	1.8	3.8	12.4	1.9	2.4	2.9
TB	110	145.7	152.7	25.6	9.2	61.1	2.5	21.3	7.1	1.8	4	13	1.8	2.3	2.9
RB	102	137	150	27.3	10.5	88.4	2.2	18.5	7.1	1.8	4	13.7	1.9	2.1	2.8
PB-1	100	134	97.2	29.8	9.3	105.7	2.1	21.1	6.8	1.7	3.9	12.9	1.9	2.2	1.5
CSR-30	113.7	140.3	153.5	27.4	11.2	108.8	2.8	26.9	7	1.9	3.6	12.8	1.8	2.5	1.5
PB1121	104.3	139.3	117.4	26.7	10.5	44.9	2.9	22.8	8.5	1.9	4.5	19.9	2.3	2.3	1.6
HUBR-10-9	101.7	131.7	113.1	27.9	10.6	146.3	2.5	34.4	7.3	1.8	4.1	13.1	1.8	2.5	1.6
PB-1509	107	137.3	98.3	26.9	14.3	64.8	2.9	27.6	8.1	1.8	4.4	17.4	2.2	2.3	1.5

For aroma, we have followed IRRI, SES, 2013; protocol which measured aroma on scale of 1=very mild to none; 2=mild to moderate; and 3=strong aroma; present in polished rice kernel powder/flour.

·The mean values of different trait given in Table1, has been round of to one digit after decimal; due to which, few crosses might show same numerical value for a particular trait. However, in actual data collection these digits varied two places after decimal.

ER of more than 1.7 are required for Basmati varieties; 13 F_1s for KLAC and 16 F_1s for ER did not qualify the minimum acceptable standards. Two parental varieties HUBR-10 and CSR-30 measured maximum KBAC (2.5mm each) in comparisons of other parental varieties. Among 36 F_1s studied, 22 F_1s showed more KBAC than HUBR-10 and CSR-30. In aroma estimation, only TxT crosses were highly aromatic while other type of crosses showed mild to moderate aroma (Table 1).

Average heterosis

Average heterosis was calculated to discern the performance of crosses on the basis of magnitude and direction of heterosis manifested by them. Heterosis is desirable in both directions depending on the traits, for example in the case of Basmati rice traits, negative heterosis is desirable for DTF, DTM, PH, KB, and KBAC. While for other traits *viz*., MPL, NPP, NGP, 100-GW, YPP, KL, KLAC, LBR, and aroma positive heterosis is preferable.

Traditional x Traditional Basmati types of crosses

Among yield attributing traits, B-370 x RB performed relatively better than other five TxT types of crosses as the estimate of average heterosis for cross was significant in desirable direction for traits *viz*., DTF, MPL, NGP, 100-GW, and YPP. For yield per plant trait cross B-370 x RB exhibited highest positive average heterosis of 106.37 percent followed by cross TB x RB which showed 27.92 percent of average heterosis (Table 2).

Cross B-370 x TB manifested high average heterosis in desirable direction for traits *viz*., KL, LBR KLAC and KBAC and hence came up as best cross among TxT types of crosses. For aroma, no crosses showed positive heterosis, indicating that F_1s showed weaker aroma intensity in comparison to average of their respective parents (Table 3).

Evolved x Evolved Basmati types of crosses

Manifestation of high average heterosis in desirable direction was observed for traits *viz*., DTF, NPP, GPP, 100-GW and YPP in the cross PB-1 x HUBR-10-9. Among 10 crosses of ExE types, 5 crosses displayed more than 50 % of average heterosis. Nevertheless, two crosses *viz*., PB-1121 x PB-1509 and CSR-30 x

Table 2. Estimates of average heterosis for yield traits in Basmati F$_1$s.

T X T	DTF	DTM	PH	MPL	NPP	NGP	100-GW	YPP
T-3 x B-370	-5.06**	-1.43*	-15.69**	4.41*	20.91*	-7.03	-1.57	-1.92
T-3 x TB	-8.27**	-3.02**	-8.96**	10.22**	-14.50	20.86*	-0.26	1.71
T-3 x RB	-4.70**	-5.28**	-16.40**	5.54*	-12.80	-7.30	-1.84	-3.56
B-370 x TB	-7.91**	-10.07**	-7.30**	5.04*	57.78**	58.03**	3.08	12.42
B-370 x RB	-4.28**	-5.07**	0.46	19.39**	46.55**	77.81**	4.64*	106.37**
TB x RB	-6.60**	-6.60**	-25.68**	19.13**	20.27**	27.85**	-0.82	27.98**
E X E								
PB-1 x CSR-30	-12.01**	-5.47**	20.44**	0.19	52.20**	-20.55**	-1.88	64.51**
PB-1 x PB-1121	-10.93**	-3.41**	3.32**	6.53**	-11.11	7.62	1.95	33.23**
PB-1 x HUBR 10-9	-2.81**	-1.38	-1.40	12.06**	16.97*	24.09**	11.39**	88.64**
PB-1 x PB-1509	-6.28**	-4.91**	10.11**	15.21**	32.96**	27.48**	8.92**	102.40**
CSR-30 x PB-1121	-11.01**	-3.93**	-21.00**	2.74	10.26	-18.70*	2.90	-24.87**
CSR-30 x HUBR 10-9	0.31	7.35**	7.68**	11.66**	87.16**	0.26	8.72**	79.31**
CSR-30 x PB-1509	-9.37**	-0.60	-1.35	9.74**	-23.24**	1.75	9.80**	-9.17
PB-1121 x HUBR 10-9	-6.80**	-4.80**	6.69**	18.36**	27.96**	26.15**	9.50**	82.99**
PB-1121 x PB-1509	-7.26**	-5.78**	25.07**	1.84	-15.97**	8.36	4.73**	-11.07*
HUBR10-9 x PB-1509	-6.07**	-4.83**	17.87**	18.41**	27.88**	29.10**	9.74**	68.24**
T X E								
T-3 x PB-1	-2.78**	-4.73**	30.52**	12.18**	24.44**	15.56*	8.30**	22.72**
T-3 x CSR-30	-9.82**	-4.74**	-16.06**	-4.34	6.18	-13.44*	-8.29**	-15.12**
T-3 x PB-1121	-4.81**	-5.35**	1.57	7.45**	29.76**	25.04**	11.20**	42.95**
T-3 x HUBR 10-9	-0.65	-0.98	9.25**	11.92**	46.46**	-0.51	11.04**	38.58**
T-3 x PB-1509	-7.91**	-4.43**	-3.55**	6.12**	-14.62*	6.61	7.11**	0.86
B-370 x PB-1	-2.33**	-1.10	-21.02**	13.03**	34.69**	56.14**	11.06**	68.73**
B-370 x CSR-30	-7.62**	-4.77**	-30.28**	7.86**	63.06**	0.38	-6.60**	24.00**
B-370 x PB-1121	-0.49	-1.80*	-0.75	-8.49**	40.00**	52.33**	3.66*	46.57**
B-370 x HUBR 10-9	4.78**	4.93**	1.19	16.09**	50.43**	4.30	6.12**	48.35**
B-370 x PB-1509	-0.80	-1.57*	22.16**	-2.41	-9.38	17.96	10.94**	11.20
TB x PB-1	-4.76**	-3.46**	4.22**	11.20**	18.77*	47.14**	9.52**	54.43**
TB x CSR-30	-12.37**	-6.29**	-36.71**	5.65*	58.56**	-4.43	-10.08**	4.35
TB x PB-1121	-7.62**	-8.07**	-7.67**	16.37**	22.64**	51.79**	17.71**	29.79**
TB x HUBR 10-9	2.99**	0.24	-33.28**	16.42**	52.45**	39.54**	3.06	63.92**
TB x PB-1509	-12.44**	-10.95**	25.42**	12.88**	4.11	74.92**	13.86**	2.70
RB x PB-1	-4.95**	-6.27**	-21.57**	17.44**	7.74	22.16**	13.53**	37.36**
RB x CSR-30	-8.81**	-5.53**	-4.36**	6.92**	-27.72**	9.46	-3.35	-7.61
RB x PB-1121	-5.01**	-3.74**	-4.65**	11.72**	24.05**	25.73**	12.62**	30.45**
RB x HUBR 10-9	6.06**	5.71**	2.68**	22.76**	33.65**	27.22**	13.23**	73.73**
RB x PB-1509	-5.26**	-5.95**	-8.04**	8.46**	-7.38	23.89**	-14.56**	15.71**
Standard error of difference	0.73	0.97	1.12	0.62	0.75	6.31	0.04	1.33

Significance levels * = <.05 and ** = <.01

PB-1121 showed negative heterosis (Table 2).

High heterosis for all the quality traits was not estimated in all the crosses; however, few crosses showed high average heterosis for two or more than two traits in desirable direction. Cross PB-1 x HUBR-10-9 showed highest positive heterosis for KLAC (13.51); and cross PB-1 x CSR-30 for KL (11.56) and LBR (18.02). Among ten ExE types of crosses, two crosses showed negative average heterosis for aroma *viz*., CSR-30 x PB-1121 and PB-1 x PB-1121. Eight crosses showed non-significant positive average heterosis. Like, TxT type of crosses, ExE type crosses also exhibited negative heterosis for all crosses (Table 3).

Traditional x Evolved types of crosses

For yield attributing traits, crosses B-370 x PB-1 (68.75), RB x HUBR-10-9 (73.73), TB x HUBR-10-9 (63.92) and TB x PB-1 (54.43) outperformed other crosses among 20 TxE types of crosses by exhibiting more than

Table 3. Estimates of average heterosis for quality traits in Basmati F$_1$s.

TXT	KL	KB	LBR	KLAC	KBAC	Aroma
T-3xB-370	0.96	-12.55 **	15.03 **	5.76*	19.97 **	-13.14**
T-3xTB	4.09 **	-9.91 **	15.40 **	-6.54 **	20.40 **	-51.6**
T-3xRB	0.72	-11.71 **	13.54 **	-17.86 **	26.77 **	-9.45*
B-370xTB	13.40 **	-6.36 **	19.74 **	20.68 **	-5.71 *	-53**
B-370xRB	4.44 **	-2.62 *	6.78 **	6.42 **	-3.71	-41.91**
TBxRB	4.33 **	-6.85 **	11.61 **	-18.62 **	27.36 **	-47.66**
EXE						
PB-1xCSR-30	11.56 **	-5.63 **	18.02 **	-15.08 **	-4.50	9.77
PB-1xPB-1121	4.49 **	-6.15 **	11.92 **	-2.04	22.02 **	-28.57**
PB-1xHUBR 10-9	4.00 **	-1.24	5.43 **	13.51 **	16.34 **	8.03
PB-1xPB-1509	6.22 **	-3.08 *	9.69 **	-11.60 **	26.14 **	6.04
CSR-30xPB-1121	2.23	-13.27 **	17.57 **	-7.40 **	-12.96 **	-22.98*
CSR-30xHUBR 10-9	3.84 **	-7.44 **	11.69 **	-13.73 **	-14.82 **	13.53
CSR-30xPB-1509	6.82 **	-8.02 **	15.45 **	-16.42 **	-3.16	8.33
PB-1121xHUBR 10-9	1.14	-7.08 **	9.29 **	-12.71 **	16.86 **	9.09
PB-1121xPB-1509	0.79	-9.27 **	11.13 **	-14.62 **	-7.78 **	4.84
HUBR10-9xPB-1509	-0.96	-5.35 **	5.43 **	-0.76	6.22 **	16.28
TXE						
T-3xPB-1	6.28 **	-0.83	12.04 **	-11.82 **	20.44 **	13.05*
T-3xCSR-30	4.85 **	-15.00 **	22.77 **	-7.65 **	8.85 **	20.1**
T-3xPB-1121	6.61 **	-3.51 **	8.54 **	10.64 **	18.62 **	-14.94**
T-3xHUBR 10-9	-0.02	1.81	-1.96	2.31	7.57 **	-20.93**
T-3xPB-1509	8.98 **	-7.49 **	17.25 **	19.36 **	14.78 **	-23.66**
B-370xPB-1	6.92 **	-2.96 *	10.02 **	4.77	19.13 **	-17.07**
B-370xCSR-30	9.96 **	-10.15 **	22.69 **	-9.35 **	0.90	-26.42**
B-370xPB-1121	-4.03 **	-8.55 **	5.63 **	-13.91 **	7.64 **	-52.57**
B-370xHUBR 10-9	-0.15	2.82 *	-2.05	-4.75	11.41 **	-29.11**
B-370xPB-1509	9.09 **	-1.48	10.87 **	4.07	10.24 **	-22.12**
TBxPB-1	11.28 **	-0.47	11.93 **	-7.89 **	22.52 **	-21.24**
TBxCSR-30	8.65 **	-11.13 **	21.76 **	-13.63 **	11.47 **	-19.28**
TBxPB-1121	7.51 **	-3.60 **	11.90 **	-31.41 **	-1.68	-31.43**
TBxHUBR 10-9	2.16	2.99 *	-0.64	2.23	9.71 **	-38.49**
TBxPB-1509	7.68 **	-4.32 **	12.73 **	16.01 **	6.83 **	-23.67**
RBxPB-1	6.35 **	-1.61	8.07 **	-10.74 **	27.36 **	-52.72**
RBxCSR-30	2.92 *	-13.87 **	19.17 **	-12.96 **	-9.45 **	-26.99**
RBxPB-1121	1.43	-3.96 **	6.22 **	-14.88 **	-2.05	-33.49**
RBxHUBR 10-9	-2.12	2.99 *	-4.98 **	2.95	19.05 **	-50.86**
RBxPB-1509	-7.44 **	-1.10	-6.33 **	-13.35**	-1.21	-29.94**
Standard error of difference	0.09	0.02	0.07	0.30	0.05	0.12

Significance levels * = <.05 and ** = <.01

50% of average heterosis. Cross RB x HUBR-10-9 showed highest positive average heterosis (73.73). Cross TB x PB-1121 exhibited high average heterosis in required direction for all yield attributing traits, *viz.*, DF (-7.62), DM (-8.07), PH (-7.67), PL (16.37), NPP (22.64), GP (51.79), HGW (17.71) and YPP (29.79).

Traits related to kernel dimension KL, KB, LBR and KLAC manifested high average heterosis in preferable direction in crosses T-3 x PB-1121, TB x PB-1509. While, B-370 x CSR-30, T-3 x PB-1509, T-3 x PB-1, TB x CSR-30, TB x PB-1121 and B-370 x PB-1 crosses showed high average heterosis in desirable direction for KL, KB and LBR. For the aroma trait, two out of 20 crosses did show heterosis, but magnitude was very less and hence it was non-significant (Table 3).

Assessing the performance of all types of crosses, we finally concluded that among all the three types of crosses; ExE types crosses performed better than the TxT and TxE types for yield per plant trait. This indicates that the traditional varieties lack genetic variability; such findings have been communicated by Siddiq et al. (2012). Nagaraju et al. (2002) studied genetic diversity on evolved and traditional basmati with the help of ISSR markers; reported low genetic diversity in traditional basmati in comparison to evolved Basmati varieties.

After estimating yield traits, we further evaluated quality traits of Basmati rice which distinguish them from other aromatic or non-aromatic rice. For KL, KB and LBR, all the three types of crosses showed nearly same magnitude of average heterosis in desirable direction. For KLAC, TxT type of cross T-3 x B-370, B-370 x TB, and B-370 x RB showed significant positive heterosis; while ExE or TxT types exhibited negative average heterosis. The slender long cooked kernel is one of the desirable features of basmati rice form customer point of view. So, kernel should increase more in length and less in breadth. Hence, KBAC was calculated, which showed that ExE type of crosses exhibited minimum increase in breadth after cooking as highest negative heterosis of magnitude -14.82 was recorded in cross CSR-30 x HUBR-10-9. One cross each of TxT type, B-370 x TB; and TxE type, RB xCSR-30; showed significant negative heterosis for KBAC. No cross showed significant positive average heterosis for aroma, although non-significant positive average heterosis was recorded in two crosses of TxE type.

Heritability in narrow sense

The amount of variation present in the population is expressed in terms of variance. The total variance of a population accounts for phenotypic variance which is sum of genetic variance and environmental variance. Additive genetic is variance of breeding values; principal cause of similarity between parent and their offspring, and affects the response of a population to selection (Falconer and Mackay, 1996). The ratio V_A/V_P also called as heritability in narrow sense, measures the degree of resemblance between parents and progeny; estimate the role of genes transmitted from parents to the progeny which affects phenotype expression (Falconer and Mackay, 1996). According to Robinson (1966), heritability in narrow sense (h^2ns) is categorized into three groups *viz*., high (> 30%), medium (10-30%) and low (<10%) (Katiyar et al., 2020). Evaluated values of additive genetic variance were more than the phenotypic variance; consequently, high heritability in narrow sense were recorded in traits *viz*., MPL, 100-GW, GPP, YPP, KL, LBR, and KLAC (Table 4). Amidst all the yield and quality traits studied, KB showed low heritability in narrow sense. Environmental variance is source of error which influences the genetic studies. The estimates of environmental variance were comparatively less than genetic and phenotypic variance for all traits in the present findings. High heritability in broad sense was reported earlier by Allam et al. (2015) for different yield and quality traits of Basmati.

Table 4. Estimates of genetic components for yield and quality traits of Basmati rice crop.

	s^2 g	s^2 e	s^2 a	s^2 D	s^2 p	h^2n (in percent)
DTF	2.31	0.35	4.61	19.16	24.12	19
DTM	2.20	0.63	4.40	22.22	27.24	16
PH	41.12	0.83	82.24	372.59	455.66	18
MPL	0.91	0.25	1.83	3.58	5.66	32
NPP	0.97	0.37	1.93	6.03	8.34	23
NGP	242.11	26.53	484.21	251.62	762.36	64
100-GW	0.03	0.00	0.06	0.03	0.09	66
YPP	30.89	1.18	61.78	57.37	120.33	51
KL	0.08	0.01	0.17	0.08	0.26	67
KB	0.0003	0.0003	0.0053	0.0065	0.0074	7
LBR	0.02	0.003	0.04	0.07	0.12	36
KLAC	1.39	0.06	2.78	2.34	5.19	54
KBAC	0.01	0.002	0.02	0.06	0.08	27
Aroma	0.06	0.01	0.12	0.17	0.30	41

s^2 g= genetic variance; s^2 e= environmental variance; s^2 a= additive variance; s^2 D= dominance variance; s^2 p= phenotypic variance; h^2n = Heritability in Narrow Sense(%)

CONCLUSION

TxT types of crosses performed poor for both quality and yield traits, with few exceptions; as traditional Basmati varieties possess narrow genetic variability. The ExE and TxE types of crosses showed significant improvement in yield per plant and KL, L/B ratio, ER, suggesting the potential use of these parents in future Basmati Breeding program. Heritability in narrow sense for of all traits except KB was high construing that there is high degree of resemblance between the parents and progeny used to study in this manuscript.

REFERENCES

Ahuja SC, Panwar DVS, Ahuja U and Gupta KR (1995). Basmati Rice: The scented pearl. Hisar, Haryana, India: Directorate of Publications, CCS Haryana Agricultural University pp. 1-61

Ahuja U, Ahuja SC Thakrar R and Rani NS (2008). Scented Rice of India. Asian Agri-History 12(4): 267-283

Allam CR, Jaiswal HK, Venkateshwarlu AQC and Reddy YS (2015). Variability, Heritability and Genetic advance studies in some indigenous genotypes of Basmati rice (*Oryza sativa* L.). Elec. J. Plt. Breed. 6(2): 506-511

Eisenhart C (1947). The Assumptions Underlying the Analysis of Variance. Intern Biomet Soc. 3(1): 1-21

Falconer DS and Mackay TFC (1996). Introduction to quantitative genetics Essex, England: Longman. pp. 125-141

Griffing B (1956). Concepts of general and specific combining ability in relation to diallel crossing systems. Aust. J. Bio. Sci. 9: 463- 495

Katiyar A, Sharma A, Singh S, Srivastava A and Vishwakarma SR (2020). A study on Genetic Variability and Heritability in Barley (*Hordeum vulgare* L.). Int. J. Curr. Microbiol. App. Sci. 9(10): 243-247

Kour A, Kumar B and Singh B (2019). Genetic evaluation of yield and yield attributing traits in rice (*Oryza sativa*). Elec. J. P. Breed. 10(1): 39-46

Kumar A, Singh and S, Magadum SK (2015). Combining ability analysis for yield and yield components in basmati rice. Oryza 52(4): 275-283

Nagaraju J, Kathirvel M, Kumar R, Siddiq EA, Hasnain SE (2002). Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. Proceedings National Academy of Sciences of the United States of America. pp. 5836-5841

Panse VG and Shukatme PV (1967). Statistical methods for agricultural Workers, ICAR, New Delhi, India

Prasad GSV, Padmavathi G, Suneetha K, Madhav MS and Muralidharan K (2020). Assessment of diversity of Indian aromatic rice germplasm collections for morphological, agronomical, quality traits and molecular characters to identify a core set for crop improvement. CABI Agric. Biosci. 1: 13

Prodhan ZH, Faruq G, Rashid KA and Taha RM (2017). Effects of temperature on volatile profile and aroma quality in rice. Intern. J. Agric. Bio. 19(5): 1065-1072

Sharma A and Jaiswal HK (2020a). Combining ability analysis for grain yield and quality traits in basmati rice (*Oryza sativa* L.) Plnt. Arch. 20(1): 2367-2373

Sharma A and Jaiswal HK (2020b). Heterosis for yield and grain quality parameters in basmati rice (*Oryza sativa* L.). Elec. J. Plt. Breed.11(4):1106-1115

Sharma A, Sandhya S, Srivastava A, Singh S, Mishra S, Mohan S, Chhavi C, Singh A, Singh AK, Jaiswal HK (2021). Aromatic rice of India: It's types and breeding strategies. In (Ed.), Integrative Advances in Rice Research. IntechOpen

Siddiq EA, Vemireddy LR and Nagaraju J (2012). Basmati Rices: Genetics, Breeding and Trade. Agric. Res. 1(1): 25-36

Singh V, Singh AK, Mohapatra T, Krishnan SG and Ellur RK (2018). Pusa Basmati 1121 - a rice variety with exceptional kernel elongation and volume expansion after cooking. Rice 11: 19

OBITUARY

Dr. Kshetra Mohan Das
June 17, 1950 to February 2, 2022

All the members of the Association of Rice Research Workers (ARRW) are deeply saddened by the sudden demise of Dr. K. M. Das, Retired Principal Scientist, Division of Plant Protection, ICAR - National Rice Research Institute, Cuttack, on 22nd February, 2022.

Dr. Kshetra Mohan Das was born on 17 June, 1950 at Airi, Tirtol in the district of Jagatsinghpur, Odisha. He completed his schooling in Sarada Academy, Kanakpur and his pre degree and post graduate degree in Ravenshaw College, Cuttack. He was awarded PhD in Botany during 1987 by Utkal University, Vanivihar, Bhubaneswar. Dr. Das joined Central Rice Research Institute in 1971 as senior field assistant and got inducted to Scientist in 1978 and was promoted to Senior scientist in January 1986. He worked in the discipline of Plant Pathology for more than 41 years. He worked on the epidemiology and management of major rice diseases like bacterial leaf blight, Sheath Blight and blast. He has more than 30 research papers in reputed journals, five bulletins and number of popular articles to his credit.He guided four MSc students and disseminated his knowledge through number of in campus and off campus farmers training programmes and through doordarshan and All India Radio. He was very popular among the farmers. He was actively involved in institute Building activities and remained officer in charge of NRRI KVK, Santhapur (2004-2007).

Dr. Das was a Life member of Association of Rice Research Workers. The Association of Rice Research Workers will remember him as a gentle, polite personality for rendering his services in the field of Plant Pathology. The members of the society pray to the almighty that his soul will rest in peace and to give strength to the bereaved family members to bear with the irreparable loss.

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