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Performance of improved versions of Swarna introgressed with yield enhancing genes in multi-location trials

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ABSTRACT

Swarna (MTU7029), an Indian mega-variety of rice, is cultivated on an estimated 8Mha of land. There is a pressing need to address yield stagnation and wider adaptability to irrigated lowland and rainfed ecologies as a result of the negative consequences of climate change and population growth. The present study was aimed at improving Swarna for two yield-related traits through marker-assisted backcross breeding strategy by introgression of OsSPL14 (panicle branching) and SCM2 (stronger culm). Foreground and background selection was carried out at each generation. Homozygous BC_2F_2 plants harbouring both yield-enhancing genes were identified and advanced through pedigree selection till BC_2F_5 and evaluated in station trials. Three promising lines possessing higher yield over recurrent parent were identified, and a single line, IET 27661 exhibited superior yield in multi-location trials of the All India Coordinated Rice Improvement Programme (AICRIP) and was found to be promising.

Key words: *Swarna, high yield, SCM2, OsSPL14, marker assisted breeding*

INTRODUCTION

Rice, a cereal crop belonging to Gramineae family feeds millions of people throughout the world (Maclean et al., 2002). It plays a pivotal role in India's food security and more than 60% of the population relies on it, making it 'Rice is life'. However, with the escalating global population (at a breakneck pace of 1.05 percent per year), it is indeed a challenge to meet the food demand in the future (Roser et al., 2013).

In recent decades, rapid increase in rice production especially in India is due to introduction of early maturing and high-yielding varieties based on the green revolution's successes. In 1960s, the semi-dwarf trait was used to boost rice lodging resistance, fertilizer responsiveness along with harvest index that witnessed significant increase in rice production during green revolution period (Khush et al., 1999). Rice productivity, on the other hand, has been plateauing in recent years, with no major yield growth. This is a serious concern because India's population is rapidly rising, and without a corresponding growth in cereal production, the country's food security will be a challenge in the future

(Jena and Kim, 2020). One strategy to revive the stagnant yield levels in rice is to introduce a novel approach for boosting panicle branching and lodging resistance by increasing culm strength (Ookawa et al., 2010a). Panicle morphology, which is mostly governed by panicle branching trait, influences the grain number per panicle (Li et al., 2021). The gene, *OsSPL14* located on chromosome 8, increases the number of primary branches in the panicle and consequently the grain number (Jiao et al., 2010). Higher *OsSPL14* expression enhances panicle branching and grain yield in rice during the reproductive stage. Similarly, the thickness and breaking strength of the basal culm determine the varietal variation in lodging resistance, which defines the physical strength of the culm (Ookawa and Ishihara et al., 1992). Ookawa et al. (2010b) discovered a new major QTL and the underlying candidate gene on Chromosome 6 that regulates culm strength and named the gene as strong culm2 (*SCM2*). *SCM2* was transferred from Habataki (high-yielding *Indica* variety) and introgressed into Koshihikari (Japanese elite variety with very high yield), resulting in a near-isogenic line (NIL-*SCM2*) with enhanced culm strength, superior lodging resistance and higher grain yield.

An Indian mega-rice variety Swarna, which is cultivated across more than 6 million hectares (Singh et al., 2009), predominantly in eastern India, where farmers grow rice as rainfed crop, which is severely affected by recent climate change, and is prone to lodging under rains and strong winds, limiting its productivity. The present study aimed to improve Swarna for panicle branching and lodging resistance by targeted introgression of the genes, *OsSPL14* and *SCM2* through marker-assisted breeding.

MATERIALS AND METHODS

A breeding line from IRRI, The Philippines, IR121050-1-8-5, possessing the favourable alleles of the yield enhancing genes, *SCM2* and *OsSPL14* (containing the ST12 allele) was used as a donor parent for introgression into the mega variety Swarna.

Marker assisted backcross breeding strategy

Crossing was initiated between IR121050-1-8-5 and Swarna during *kharif* 2014 (Fig. 1). Hybridity of the F_1s was confirmed using newly designed gene-specific markers for yield enhancing genes *viz*., *SCM2*8F/8R (specific for *SCM2*) and *OsSPL14* Indel 2F/2R (specific for *OsSPL14*). The details of the primers are given in Table 1. The true F_1s were then backcrossed with the recurrent parent, Swarna, to generate BC_1F_1s. Simultaneously, a parental polymorphism study using 200 SSRs was carried out, and a set of 75 markers polymorphic between IR121050-1-8-5 and Swarna were found and used for background selection subsequently (Supplementary Table 1).The BC_1F_1 plants were subjected to foreground selection with the gene-specific markers as mentioned earlier and background selection was done using the parental polymorphic SSRs and backcrossing was continued till BC_2F_1s. A single heterozygous BC_2F_1 plant possessing target yield enhancing genes besides maximum recurrent parent genome recovery was selfed to develop BC_2F_2s. Homozygous positive BC_2F_2 plants possessing yield enhancing genes (i.e. homozygous for *SCM2* and *OsSPL14*) and advanced further till BC_2F_5 generation by selfing through morphology-based selection for plant characters similar to Swarna. Genomic DNA isolation was done by using mini-prep method (Zheng et al., 1991). PCR conditions for *SCM2*8F/8R (specific for *SCM2*) were as follows: Initial denaturation at 94°C for 5 minutes, Denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, Extension at 72°C for 1 minute followed by 35 cycles and final extension at 72°C for 7 minutes. While for *OsSPL14* Indel 2F/2R (specific for *OsSPL14*)- Initial denaturation at 94°C for 5 minutes, Denaturation at 94°C for 30 seconds, annealing at 55°C for 30 seconds, Extension at 72°C for 1 minute followed by 35 cycles and final extension at 72°C for 7 minutes. PCR amplicons were resolved

Table 1. Molecular markers used for yield enhancing genes in the backcross derived lines.

S. no.	Trait/Gene/QTL	Chromosome number	Marker name	Forward primer (5'- 3')	Expected size
1	Strong culm/*SCM2*	6	RMS *SCM2*-8F	GCATCCTCCGTCCAGATAGA	P-270bp
			RMS *SCM2*-8R	CTGGCTTAAAAAGGCCCCTA	N-300bp
2	Panicle branching /*OsSPL14*	8	RMS-*OsSPL14* Indel 2F	ATCCCCAAATCCTCAAATCC	P-400bp
			RMS-*OsSPL14* Indel 2R	TCCCGGTTCAAAAGGTTAGA	N-440bp

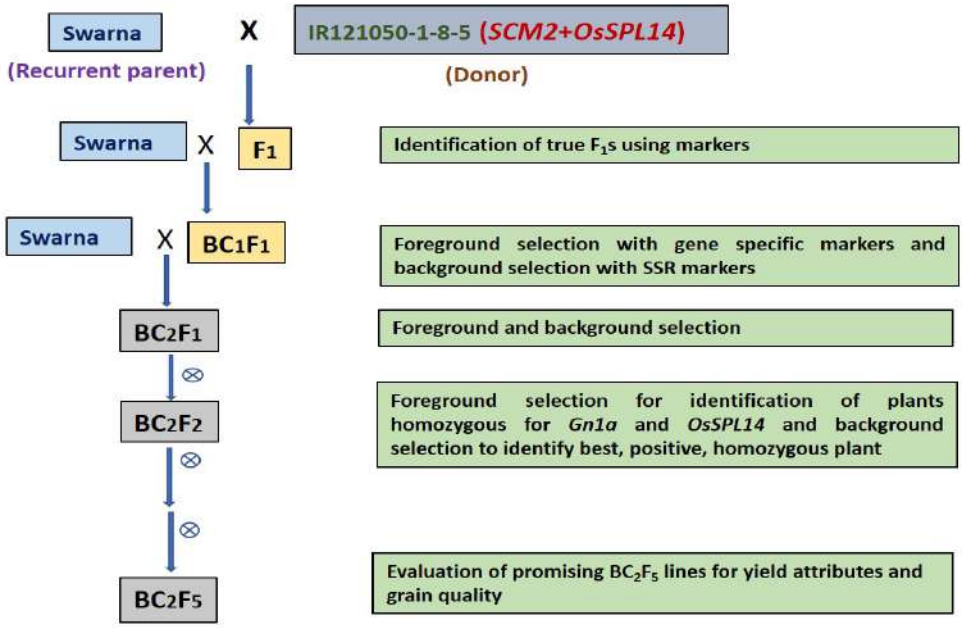

Fig. 1. Schematic representation of marker-assisted backcross breeding strategy to introgress yield enhancing genes into the genetic background of Swarna.

on 3% agarose gel.

Evaluation for agro-morphological characteristics and its yield related traits for the improved lines

During *kharif* 2017, three promising two-gene pyramided lines similar to Swarna at BC_2F_5 generation were transplanted together with parents on $2m^2$ plots in three replications with a spacing of 15 X 20 cm at experimental research farm, ICAR-IIRR, Hyderabad. Data was recorded for different agro-morphological traits: days to 50% flowering (DFF), plant height (PH; cm), number of productive tillers/plant (NPT), number of grains/panicle (NGPP), panicle length (PL; cm), flag leaf length (FLL), flag leaf width (FLW), culm diameter (CD), number of primary branches/panicle (PBP), number of secondary branches/panicle (SBP), panicle exertion, 1000 grain weight (TGW; g) and single plant yield (SPY) according to Rekha et al. (2018).

Statistical analysis

The data was statistically analyzed following the procedures described by Freeman et al. (1978). The statistical analysis was performed using SAS Version 9.2 (SAS Institute Inc., Cary, NC, USA). PROCGLM procedure of SAS was utilized for analysis of variance (ANOVA) for determination of significant variation; Least Significance Difference (LSD) values at 5 percent level of significance and Coefficient of variation (CV), Genetic advance and its mean percentage along with broad sense heritability of each trait were calculated among the improved breeding lines of

Table 2. Number of plants screened through foreground and background selection in each backcross generation.

S. no.	Cross combination	Generation	Number of plants screened	Number of positive (heterozygous/ homozygous) plants	Recurrent parent genome (%)[$]	Gene combination in the confirmed plants
1	Swarna X IR121050-1-8-5	F_1	70	33	-	*SCM2 + OsSPL14*
2	Swarna X F_1	BC_1F_1	185	18	75.5 %	*SCM2 + OsSPL14*
3	Swarna X BC_1F_1	BC_2F_1	224	101	87.25 %	*SCM2 + OsSPL14*
4	Selfed progeny of BC_2F_1	BC_2F_2	372	21	93.0 %	*SCM2 + OsSPL14*
		BC_2F_5		10	-	*SCM2 + OsSPL14*

Swarna.

RESULTS

Marker assisted introgression of yield genes (*SCM2* and *OsSPL14*) into Swarna

A total of 185 BC_1F_1 plants were generated from the cross between IR121050-1-8-5 and Swarna. We used DNA markers specific for *SCM2* and *OsSPL14*, a total of 18 heterozygous plants were identified. Background analysis was performed on these positive plants, and a single plant (#RP6294-1096) with the maximum recurrent parent genome recovery (75.5%) was identified and backcrossed with the recurrent parent, Swarna, and generated 224 BC_2F_1s, of which 101 BC_2F_1 positive plants were identified through foreground selection. Simultaneous background selection of these positive plants resulted in the identification of a single plant (#RP6294-1096-1101) with 87.25% recurrent parent genome recovery, which was subsequently selfed to produce 372 BC_2F_2 plants. Using gene specific markers, 21 homozygous two-gene positive plants were identified among the BC_2F_2 plants and a solitary plant (#RP6294-1096-1101-22) with 93.0 percent recurrent parent genome recovery was identified and advanced to the BC_2F_5 generation through pedigree breeding (Table 2). Based on their morphological similarities to Swarna, a total of ten homozygous positive BC_2F_5 lines were selected (Fig. 2 depicts analysis of these 10 lines with markers specific for the target genes). These lines were then evaluated for yield attributes and other key agro-morphological traits. Based on the yield attributes, ten best entries performing well in terms of yield traits were selected. Three promising entries *viz*., RP6294-1096-1101-22-3, RP6294-1088-1106-41-14 and RP6294-1092-1107-45-7 outperforming the recurrent parent in terms of culm strength, panicle branching, grain number and grain type equivalent or better than that of the the cultivar, Swarna were selected and advanced further.

Assessment of agro-morphological traits of the introgression lines of Swarna

In comparison to the recurrent parent, Swarna (126 ± 1.2), all the improved lines were significantly recorded 10-15 days early 50% flowering. The mean values of improved lines in terms of plant height ranged from 95.7±1.8 to 99.0±1.2, with all the lines approximately 10-12cm taller than the recurrent parent Swarna (82.7 ± 1.5). The improved lines had mean values of 17±0.9 to 19±0.9 for the number of productive tillers per plant, whereas the recurrent parent, Swarna, had 16±0.9. The improved lines had a significant difference in panicle

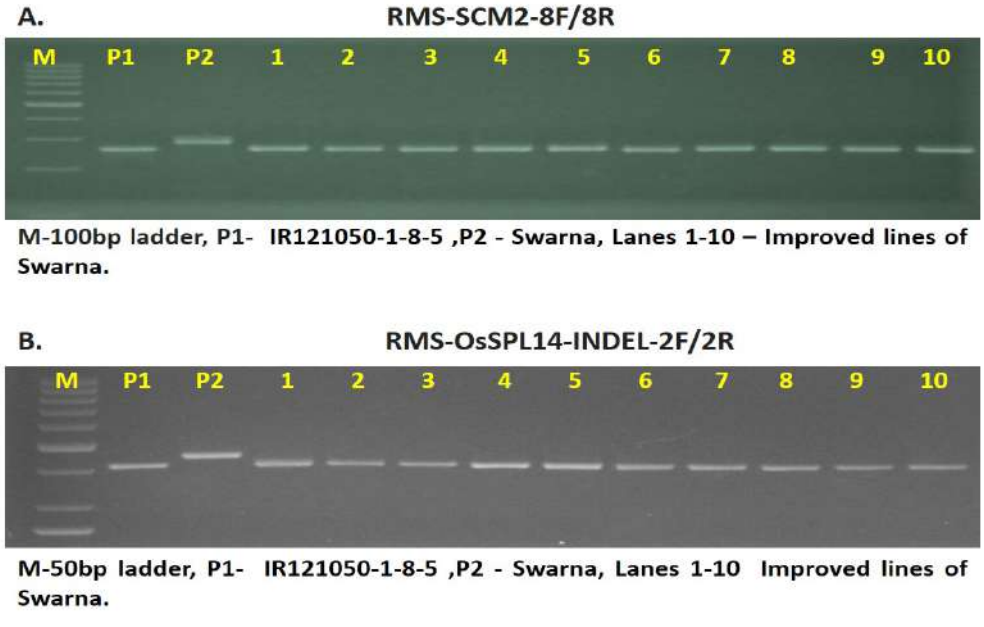

Fig. 2. Foreground selection of selected backcrossderived lines of Swarna for targeted genes, *SCM2* and *OsSPL14* at BC_2F_5 generation.

Table3: Evaluation of agro-morphological characters in the improved lines of Swarna along with parents under field conditions during *kharif* 2017.

S.No	Plant identity	DFF	PH(cm)	NPT	PL(cm)	TGW	GNPP	FLL	FLW	PBP	SBP	CD	SPY	Panicle exsertion
1	**Swarna**	126 ± 1.2	82.7 ± 1.5	16 ± 0.9	19.7 ± 0.9	18.1 ± 1.0	172.0 ± 1.2	35.0 ± 1.7	2.2 ± 0.2	10.7 ± 0.9	31.7 ± 1.5	23.6 ± 1.6	17.0 ± 1.0	PE
2	**IR121050-1-8-5**	115 ± 1.8	108.3 ± 1.5	20± 0.9	24.0 ± 1.7	17.2 ± 0.9	316.0 ± 1.0	41.3 ± 0.9	2.4 ± 0.2	14.7 ± 1.2	39.3 ± 0.9	28.6 ± 1.0	23.0 ± 1.0	PE
3	RP6294-1087-1105-28-24	103 ± 1.3	96.7 ± 1.2	17 ± 1.2	22.3 ± 1.5	17.6 ± 1.0	280.7 ± 1.2	38.0 ± 1.2	2.1 ± 0.2	12.0 ± 1.2	37.0 ± 1.2	27.4 ± 0.9	17.3 ± 0.9	PE
4	RP6294-1095-1102-32-29	105 ± 1.2	99.0 ± 1.2	18± 1.2	22.7 ± 1.8	17.2 ± 0.7	277.7 ± 1.2	38.0 ± 1.2	2.2 ± 0.2	11.3 ± 1.2	37.0 ± 1.0	27.7 ± 1.1	17.5 ± 1.0	FE
5	RP6294-1091-1103-48-21	106 ± 1.2	95.7 ± 1.8	17± 1.2	22.7 ± 1.5	17.8 ± 1.2	276.0 ± 1.5	37.3 ± 1.7	2.1± 0.1	12.0 ± 1.2	37.3 ± 1.2	27.3 ± 1.3	17.2 ± 0.8	PE
6	RP6294-1089-1104-38-20	106 ± 1.2	98.7 ± 1.5	17 ± 0.9	22.7 ± 1.5	17.6 ± 1.1	275.3 ± 1.5	38.7 ± 1.5	2.2 ± 0.2	12.3 ± 0.9	38.0 ± 1.2	27.4 ± 1.5	17.8 ± 1.0	FE
7	RP6294-1088-1106-41-14	106 ± 0.9	97.3 ± 1.5	18± 1.2	23.3 ± 0.9	17.8± 1.0	285.0 ± 1.5	38.7 ± 1.5	2.2 ± 0.2	13.0 ± 1.2	39.3 ± 0.9	28.2 ± 1.0	19.1 ± 1.6	FE
8	RP6294-1090-1108-29-19	106 ± 1.2	96.7 ± 1.8	17 ± 1.5	22.3 ± 0.9	17.7 ± 1.0	283.3 ± 1.5	38.3 ± 0.9	2.2 ± 0.2	11.3 ± 1.5	37.7 ± 0.9	27.5 ± 1.3	18.4 ± 1.6	FE
9	RP6294-1092-1107-45-7	106 ± 1.5	98.3 ± 1.2	18± 0.9	23.7 ± 0.9	17.9 ± 0.9	285.7 ± 0.9	38.3 ± 1.2	2.2 ± 0.1	13.3 ± 1.3	39.7 ± 1.9	28.2 ± 1.0	20.2 ± 0.9	PE
10	RP6294-1093-1109-8-35	105 ± 1.2	98.0 ± 1.2	17± 1.2	22.7 ± 0.9	17.5 ± 1.1	275.7 ± 1.8	38.5 ± 0.7	2.2 ± 0.2	11.7 ± 0.9	36.7± 1.5	27.7 ± 1.1	17.6 ± 0.2	FE
11	RP6294-1096-1101-22-3	106 ± 1.2	99.0 ± 1.2	19± 0.9	22.7 ± 1.2	17.9 ± 1.4	285.7 ± 1.9	39.0 ± 1.6	2.2 ± 0.2	12.3 ± 1.5	39.0 ± 1.7	28.2 ± 1.2	20.5 ± 0.9	FE
12	RP6294-1094-1110-23-6	106 ± 1.2	97.3 ± 1.2	17 ± 1.2	21.7 ± 0.9	17.8 ± 1.0	278.3 ± 1.8	37.0 ± 0.9	2.1 ± 0.1	11.7 ± 1.2	37.3 ± 0.9	27.7 ± 1.0	17.3 ± 0.2	FE
	Mean ± SE	107 ± 1.5	99.0 ± 2.3	12 ± 1.1	23.2 ± 0.8	17.8 ± 0.4	281.5 ± 5.8	38.6 ± 1.1	2.2 ± 0.1	12.5 ± 0.7	38.4 ± 1.3	27.4 ± 1.1	19.4 ± 0.7	
	CV%	2.49	4.05	15.63	5.96	4.1	3.58	5.28	8.52	10.45	6.07	7.33	7.07	
	CD	4.53	6.79	3.26	2.35	1.24	17.09	3.45	0.33	2.22	3.95	3.4	2.32	
	F value	16.49	4.4	1.33	2.18	1.95	36.75	3.14	2.07	2	3.68	1.18	6.49	
	P value	<.0001***	0.0011***	0.2702^{+}	0.0513*	0.0809	<.0001***	0.0087**	0.064	0.0727	0.0035**	0.3532^{+}	<.0001***	
	GCV	6.12	4.57	3.75	3.9	1.9	12.99	3.26	5.48	6.68	6.08	1.93	8.21	
	PCV	6.61	6.1	16.08	7.13	4.52	13.47	6.21	10.13	12.41	8.59	7.58	10.84	
	h^2 b	85.73	56.03	5.45	30.03	17.65	92.92	27.57	29.24	29	50.01	6.5	57.4	
	GA%	12.52	6.98	0.22	1.02	0.29	72.6	1.3	0.13	0.93	3.4	0.27	2.4	
	GAM	11.6	7.05	1.8	4.41	1.64	25.8	3.52	6.1	7.41	8.85	1.01	12.82	

Note: p d" .001*, p d" .01**, p d" .05*, p e" 0.1^{+}**

DFF- Days to 50% flowering, PH- Plant height, NPT-No. of productive tillers, PL- Panicle length, TGW – Thousand Grain Weight, GNPP- Grain number per panicle, FLL- Flag leaf length, FLW- Flag leaf width, PBP- Primary branches per panicle, SBP- Secondary branches per panicle, CD- Culm diameter, SPY- Single plant yield, PE – Partially Exserted, FE – Fully Exserted; Mean- Standard error mean; CV – Coefficient of Variation, CD-Critical difference; PCV – Phenotypic Coefficient of Variance, GCV – Genotypic Coefficient of Variance, h2b - Broad sense of heritability,; GA- Genetic advancement.; GAM – Genetic advance in % of mean.

length, which ranged from 21.7±0.9 to 23.7±0.9, compared to Swarna 19.7±0.9. The flag leaf length and width were similar to that of Swarna. Grain number per panicle in the backcross derived lines was observed in the range of 275.3 ± 1.5 to 285.7 ± 1.9 as compared to 172.0 ± 1.2 recorded for Swarna. The improved lines showed an increased primary and secondary panicle branching, with values ranging from 11.3 ± 1.2 to 13.3 ± 1.3 and 36.7 ± 1.5 to 39.7 ± 1.9, respectively as compared to 10.7 ± 0.9 and 31.7 ± 1.5, respectively with respect to the recurrent parent, Swarna. The culm diameter in the improved lines ranged from 27.3 ± 1.3 to 28.2 ± 1.2, while Swarna recorded 23.6 ± 1.6. The mean values for thousand grain weight of the improved lines varied from 17.2 ± 0.7 to 17.9 ± 1.4, while the recurrent parent, Swarna recorded 18.1 ± 1.0. Single plant yield of the improved lines ranged from 17.3 ± 0.2 g to 20.5 ± 0.9 g, whereas Swarna recorded 17.0 ± 1.0 g (Table 3; Fig. 3).

Evaluation of variability and genetic factors among the improved lines of Swarna

Highly significant genotypic and phenotypic variations were observed for all the traits studied. The results revealed that highest PCV and GCV were observed for grain number per panicle (13.47 and 12.99%, respectively) and the lowest PCV and GCV were recorded for thousand grain weight (4.52 and 1.9% respectively). Narrow differences between PCV and GCV were observed for days to 50% flowering and grain number per panicle. However, highest heritability 92.92% was observed for grain number per panicle accompanied with high genetic advance of 72.6% while moderate heritability was observed for the days to 50% flowering (85.73%) and plant height (56.03%) accompanied with 12.52% and 6.98% genetic advance respectively. However, Productive tillers per plant (5.45%) and culm strength (6.5%) traits recorded relatively low heritability values (Table 3).

Phenotypic correlation among agro morphological parameters

A highly significant positive correlation was observed between DFF and FLW (0.514) and highly significant negative correlation was observed between DFF and PH (-0.496), GNPP (-0.582), SBP (-0.459) and CD (-0.482) and Positive non-significant correlation was observed between DFF and NPT (0.083), TGW (0.103) whereas negative non-significant correlation was observed between DFF and PL (-0.302), FLL (-0.236), PBP (-0.007) and SPY (-0.073). Plant height showed highly significant positive association with PL (0.49), GNPP (0.737), FLL (0.438), PBP (0.427), SBP (0.432), CD (0.437) and SPY (0.418). GNPP (0.538) and FLL (0.498) displayed highly significant positive association with PL. Significant positive association was observed between GNPP and FLL (0.575), PBP (0.423), SBP (0.679), CD (0.595) and SPY (0.553). PBP has non-significant positive correlation with SBP (0.156) and CD (0.028). SBP has highly positive significant correlation with CD (0.553) and SPY (0.467). FLL has strong association between SBP (0.606), CD (0.369) and SPY (0.63) (Table 4).

DISCUSSION

Swarna is a mega-variety of rice in India that is cultivated in more than 6 Mha across the country due to its high yield, good plant type, high tillering ability, better nitrogen response and good grain quality (Rambabu et al., 2016). Further, a study carried out by Mahadevaswamy et al. (2020) has revealed that Swarna has the favourable allele with respect to the major QTLs associated with tolerance to low soil phosphorus (P), called *Pup1*, which could be responsible for wider adaptability of the variety across the country. Despite these beneficial attributes, the grain number in Swarna is significantly lower and the variety is prone to moderate to severe lodging in coastal areas of the country, where recurring cyclones are witnessed (Girija et al., 2019). Among the critical parameters, lodging has most negative impact on crop yield and it is caused due to heavy winds and rainfall plummeting the crop to the ground (Zhao et al., 2019). Keeping these points in view, we incorporated two major genes *viz*., *SCM2* and *OsSPL14*, contributing to increase in culm strength and panicle branching respectively in the genetic background of Swarna. Habataki, an improved high-yielding *Indica* type rice variety with robust and thick culms, has been found having the favourable allele, *SCM2* (Ookawa et al., 2010). Similarly, *OsSPL14* enhances panicle branching that result in higher grain number (Jiao et al., 2010). In this study, marker-assisted backcross breeding (MABB) approach was employed to introduce these two yield-enhancing genes *SCM2* and *OsSPL14* into Swarna, which resulted in increased yield by effective panicle branching, increased grain

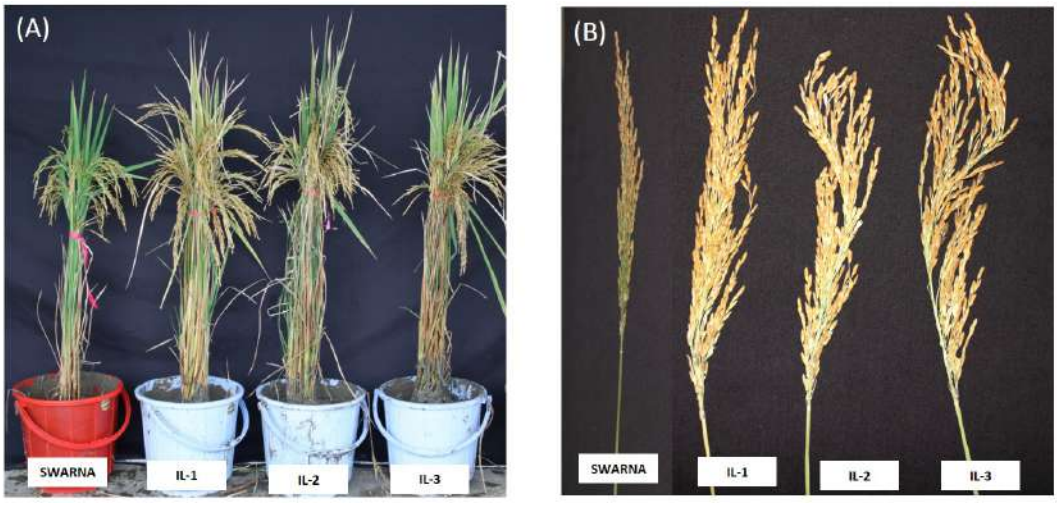

Fig. 3. (A) Improved backcross derived plants **(B)** panicle features of the selected backcross derived lines of Swarna possessing the gene combination , *SCM2* + *OsSPL14* along with recurrent parent, Swarna IL-1-RP6294-1096-1101-22-3; IL-2-RP6294-1088-1106-41-14; IL-3-RP6294-1092-1107-45-7.

number, and conferred lodging resistance through improved culm strength (Fig. 3).

Marker-assisted backcross breeding (MABB) is an ideal strategy that entails introducing one or more target genes/traits into an elite crop variety or a breeding line (Wen and GAO et al., 2012). It has been reported from the earlier studies that the background recovery of the recurrent parent was quicker via MABB in comparison to the traditional backcrossing strategy, thus saving time and resources (Sundaram et al., 2008; Pradhan et al., 2019). In addition to lodging, Swarna is also highly prone to submergence and is susceptible to many pests and diseases. Neeraja et al. (2007) developed a submerge tolerant version of Swarna, named Sub1-Swarna through MABB approach, Rambabu et al., (2016), used marker-assisted backcross breeding to introduce a major blast resistance gene, Pi54, into Swarna and an improved version of Swarna named DRR Dhan 51 was developed and released for cultivation in a few states of India recently. Pradhan et al. (2019) and Mohapatra et al. (2020) also developed improved versions of Swarna possessing tolerance against submergence and resistance against bacterial blight. However, to the best of our knowledge, there is no report about targeted improvement of Swarna for yield related traits and lodging tolerance. This is the first report to indicate that backcross derived Swarna lines not only have higher yield potential, but also have a robust culm which helps to protect the higher-yielding lines from lodging. Earlier, through MABB approach, *SCM2* and *OsSPL14* were introduced into the popular bacterial blight resistant rice variety, Improved Samba Mahsuri and increased culm strength and yield was noticed in the backcross progenies (Jayamma et al., 2019).

Foreground selection was carried out for an indirect selection of targeted genes, *i.e*., *SCM2* and *OsSPL14*. Despite the fact that a set of molecular markers have been earlier designed for selection of these two genes (Kim et al., 2016) and these markers were observed to show very low resolution/ polymorphism or no polymorphism in distinguishing the parents used for crossing in our study (data not shown). As a result, foreground selection was carried out using a new set of gene-specific markers, namely *SCM2* 8F/ 8R and *OsSPL14* Indel 2F/2R, which exhibited clear polymorphism between the donor parent IR121050-1-8-5 and Swarna (Fig. 2). Simultaneously recurrent parent genome contribution was determined at each backcross generation through marker-assisted background selection involving a set of 75 parental polymorphic SSR markers. The number of backcrosses was limited to just two due to the use of marker-based background selection and the fact that the donor

genotype employed in this study, IR121050-1-8-5, is an elite breeding line. Based on the identification of plants homozygous for the two-target yield-enhancing genes with maximum recurrent parent genome recovery in BC_2F_2 generation (Plant # RP6294-1096-1101-22; 93 percent recurrent parent genome recovery; Table 2), we adopted a pedigree breeding approach for further advancement through selfing (Rekha et al., 2018; Mahadevaswamy et al., 2020).

Among the backcross derived lines, a set of 10 homozygous positive BC_2F_5 lines were selected (RP6294-1087-1105-28-24, RP6294-1095-1102-32-29, RP6294-1091-1103-48-21, RP6294-1089-1104-38-20, RP6294-1088-1106-41-14, RP6294-1090-1108-29-19, RP6294-1092-1107-45-7, RP6294-1093-1109-8-35, RP6294-1096-1101-22-3, RP6294-1094-1110-23-6) and evaluated in replicated station trials for yield related traits and other important agro-morphological traits. Three promising entries *viz*., RP6294-1096-1101-22-3, RP6294-1088-1106-41-14 and RP6294-1092-1107-45-7 were shown to be superior to Swarna in terms of key agronomic traits such as stronger culm, higher panicle branching and grain number (Table 3; Fig. 3) as well as grain type. The strong culm is due to presence of the gene *SCM2* which is a gain-of-function mutant of APO1 (ABERRANT PANICLE ORGANIZATION 1), an another gene identified through positional cloning which is associated to control panicle structure, but it doesn't show any negative effects that are reported for APO1 over expression mutants such as decreased panicle number and abnormal spikelet morphology (Ookawa et al., 2010) in our study. Among these three, the line RP6294-1096-1101-22, exhibited a significant improvement in yield, culm strength along with grain type similar to Swarna, was nominated under the category Initial Variety Trial-Irrigated Medium (IVT-M) of the All India Coordinated Rice Improvement Project (AICRIP) trials.

Based on correlation analysis between yield and its contributing character, it was established in the present study that the genotypic correlation coefficients were higher in most cases than the phenotypic correlation coefficients, indicating that the association is primarily due to genetic factors (Yusuff Oladosu et al., 2018). In a study conducted by Rashid et al. (2017) phenotypic correlation coefficients were shown to be stronger than genotypic correlation coefficients for features whose expression is considerably impacted by the environment. In a study aimed at analysis of correlation between grain yield and its component traits showed the significance of traits which are directly associated with grain yield in rice breeding programmes (Saleh et al., 2020). In the present study, positive correlation was observed between culm diameter (CD), primary and secondary branches per panicle (PBP and SBP) with single plant yield (SPY). PBP showed non-significant positive correlation with SBP and CD. SBP displayed highly positive significant correlation with CD and SPY (Table 4) indicating the positive association between the targeted traits with respect to yield related traits in this study.

Under the All India Coordinated Rice Improvement Project (AICRIP), multidisciplinary and multi-location evaluation of elite cultures, crop management, and crop protection technologies in various agro-climatic zones of the country is being conducted every year. RP 6294-1096-1101-22-3, an improved Swarna breeding line possessing *SCM2* and *OsSPL14* genes that showed promise in terms of agro-morphological traits and yield under station trial evaluation, was nominated for AICRIP trials in the Initial Variety Trial - Irrigated Medium (IVT-IM) in the wet season of 2020 under the name IET 27661. The entry displayed a yield advantage of 34% over National check (NC), 15% over Zonal check (ZC) and 7% over Local check (LC). It has also displayed 16% yield superiority over the best varietal check (BVC) in Zone II (consisting of the states of Punjab, Haryana, Delhi, Uttar Pradesh and Rajasthan). Overall, IET 27661 yielded 5788 kg/ha and outperformed over the local check, which yielded 5425 kg/ha. Based on its overall performance, it has been identified as promising for the Zone II.

CONCLUSION

Farmers, particularly in coastal areas prone to cyclonic weather conditions with strong winds, will benefit greatly from the improved version of Swarna developed through this study, which has increased yield potential with sturdy culm. The *SCM2* gene transferred into Swarna genetic background, enhanced culm diameter, resulting in a robust stem, thus minimizing the impact of lodging and retaining the grain yield contributed by *OsSPL14*. Improved lines of Swarna with *SCM2* and

Table 4. Phenotypic correlation among agro-morphological traits in backcross derived lines of Swarna.

	DFF	PH	NPT	PL	TGW	GNPP	FLL	FLW	PBP	SBP	CD	SP
DFF	1											
PH	-0.496**	1										
NPT	0.083	0.269	1									
PL	-0.302	0.49**	0.359*	1								
TGW	0.103	-0.356*	-0.064	-0.185	1							
GNPP	-0.582**	0.737**	0.392*	0.538**	-0.314	1						
FLL	-0.236	0.438**	0.285	0.498**	-0.221	0.575**	1					
FLW	0.514**	-0.179	0.012	-0.072	0.306	-0.273	-0.048	1				
PBP	-0.007	0.427**	0.401*	0.297	0.005	0.423*	0.221	0.409*	1			
SBP	-0.459**	0.432**	0.226	0.277	-0.434**	0.679**	0.606**	-0.333*	0.156	1		
CD	-0.482**	0.437**	0.279	0.274	-0.222	0.595**	0.369*	-0.34*	0.028	0.553**	1	
SPY	-0.073	0.418*	0.22	0.504**	-0.338*	0.553**	0.63**	0.16	0.396*	0.467**	0.199	1

Note: ** Significant at 1% level of significance, *significant at 1% level of significance.

OsSPL14 can also be used as potent donors for these traits in breeding programmes targeted at improving yield and lodging resistance in elite rice varieties and hybrids.

AUTHORS' CONTRIBUTION

Conceptualization of research (SRM); Designing of the experiments (SRM, NCN and KMBVN); Contribution of experimental materials (KKJ); Execution of field/ lab experiments and data collection (PE, RG, CK, DT, HSK, SRK, LPB, MSK, AM, AD, KRR,SP,VG); Analysis of data and interpretation (FRA, SP); Preparation of the manuscript (KMBVN, RG, PE, SRM, FRA, SP).

DECLARATION

The authors should declare that they do not have any conflict of interest.

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Principal component and cluster analyses for assessing agro-morphological diversity in rice

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ABSTRACT

Forty-five rice genotypes were evaluated for determining the pattern of variation and relationship among 14 yield contributing traits. Four principal components (PCs) exhibited eigen values >1.0 and explained about 79.5 % of the total phenotypic variability. From rotated component matrix it has been observed that the highest positive eigen vector was taken by secondary branches (0.945), followed by total spikelet number (0.945), fertile spikelet number (0.889), primary branches (0.676) and harvest index (0.632) in PC1, indicating the major effects in the overall variation among the genotypes. Seven groups were formed after cluster analysis. Cluster I had lowest average for days to 50% flowering, Cluster II had highest mean value for harvest index, Cluster III had highest mean for flag leaf area, test weight, and straw and grain yield per plant, and Cluster V had highest mean value for primary branches, total spikelet number, fertile spikelet number and fertility %. So, desirable genotypes fromdifferent cluster can be selected and hybridization programme may be initiated to utilize heterosis in F1 generation and wide spectrum of recombinants in segregating generations for selection of promising segregants.

Key words: *Rice, correlation, cluster analysis, PCA*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important major food staple and more than three billion people depend on rice as their major source of calories. Asian continent occupies about 90% of the global rice area, where 90% of the world's rice is produced and consumed (Kang and Priyadarshan, 2008). The human population is increasing at an exponential rate, and it is expected to reach 9 billion by 2042, and it has been projected that yield of major food staple must be increased by at least 70% to feed entire population (Singh and Singh, 2015). On the other hand, the environment is abruptly changing in an unpredictable way and impacting crop production including rice productivity. Hence, increase in grain yield is very necessary for developing countries, but grain yield is polygenic trait, determined by cumulative effects of numerous genes with small additive effects. Hence direct selection may not be beneficial for yield improvement. Thus by indirect selection *i.e*., by giving weightage to other characters those having strong association and high heritability with grain yield may be beneficial for crop improvement.

Substantial amount of genetic variability along with greater genetic diversity is essential for any crop improvement programme. Genetic diversity *i.e*., heritable variation within and between populations, distances between genotypes, and the correlation between genetic distances plays a pivotal role to determine the breeding strategies. These also help in determining heterotic groups and estimation of hybrid performance (Acquaah, 2012). Population grouping by genetic distance can be reckoned by various methods. Understanding the usable variability existing in the population under study is very essential (Nachimuthu et al., 2014). Multivariate analysis is a fundamental approach. Principal component analysis (PCA), cluster analysis, and discriminate function analysis come under multivariate analysis (Oyelola, 2012). PCA can be

utilised to identify patterns and wipe out redundancy in data sets as variations for yield occur frequently in various crops (Maji and Shaibu, 2012). Principal components are often determined from a covariance or correlation matrix. Scale effects can have an impact on the components when the variables are evaluated in different units. Standardizing the variables at this phase becomes crucial.In this experiment, the principal components were extracted using a correlation matrix. It accomplishes this reduction by determining directions (positive and negative), called principal components, along which the variation in the data is maximal (Singh and Narayanan, 1993). Therefore, the major advantage of PCA is that it quantifies the importance of each dimension to describe the variability in a data set. Given the significance of PCA, this investigation was conducted in a population comprising 45 diverse rice germplasm with an objective to dissect yield and yield components and to determine and classify maximum variability into total variability for the purpose of grouping the accessions while considering various traits and their relationships with one another.

MATERIALS AND METHODS

Forty-five diverse genotypes of rice (Table 1) were grown during warm wet (*kharif*) season in 2020 at the Agriculture Farm, Institute of Agriculture, Visva-Bharati, Sriniketan (23º19' N, 87º42' E, 58.9 m above msl), in a randomized complete block design (RCBD) with 3 replications. Twenty-seven-day old seedlings were transplanted with a plant to plant spacing of 15 cm and row to row spacing of 20 cm in a five-row plot of 5-meter length. Standard cultural practices were followed with a fertilizer dose @ N: P_2O_5: K_2O= 60:30:30 kg ha^{-1} for raising a healthy crop. Randomly five plants were selected from the middle rows of each replication for recording data on 14 quantitative characters *viz*., plant height (PH), flag leaf area (FLA), tiller number (TN), main panicle length (MPL), primary branches $panicle^{-1}$ (PB), secondary branches $panicle^{-1}$ (SB), total spikelet number $panicle^{-1}$ (TSN), fertile spikelet number $panicle^{-1}$ (FSN), fertility % (FP), test weight (TW), straw yield per plant (SYPP), harvest index (HI), and grain yield per plant (GYPP). Data on days to 50% flowering (DFF) were recorded during flowering period on a whole plot basis.

Statistical analysis

Principal component analysis (PCA) was used to find the patterns of variance. The PCs having Eigen values above one were chosen as proposed by Jeffers (1967). Correlations between various traits and the respective PCs were calculated. The agglomerative clustering (average linkage) method was used to conduct the cluster analysis adopting Euclidean distance measure. The SPSS (version 25), STAR 2.0, and R-studio were used for statistical analysis.

RESULTS AND DISCUSSION

All the fourteen quantitative traits under study *viz*., DFF,

Table 1. List of rice genotypes used in under this study.

Sl. no.	Name of Genotypes	Sl. no.	Name of Genotypes	Sl. no.	Name of Genotypes
1	MTU-1010	16	Gangajali	31	Manipur Rice
2	MTU-1075	17	Gheush	32	MBR(Manipur Black Rice)
3	MTU-7029	18	Gotra Bidhan-3	33	Nilanjana
4	Annada	19	Gotra Bidhan-1	34	Protiksha
5	Bahadur	20	Green Rice	35	Rajendra Masuri
6	Bango Bandho II	21	Joha	36	Ranjeet
7	Baskamini	22	Kajoli	37	Red Husk Rice
8	Benajhuri	23	Kalonunia	38	RH-1442
9	Biharisal	24	Kartikkhas	39	RH-15
10	Black Rice	25	Kerela Sundari	40	Santibhog
11	Chinakamoni	26	Khajurkata	41	Shilkhi
12	Danaguri	27	Lalat	42	Simulphool
13	Dangapatnai	28	Lalmarich	43	Sitabhog
14	Dudheshwar	29	Latisal	44	Super Shamali
15	Falguni	30	Malsiraj	45	Tulaipanji

*Source: All the 45 germplasm are maintained in Agriculture Farm, Visva-Bharati.

Table 2. Descriptive statistics to summarize the variability in the germplasm.

Variable	Min	Max	Mean	StdDev	SE_Mean	CV	Skewness
Days to 50% flowering (DFF)	83.12	126.95	114.08	9.2	1.37	8.07	-1.78
Plant height (PH) (cm)	98.54	169.33	137.35	24.65	3.67	17.94	-0.33
Flag leaf area (FLA) (cm^2)	23.01	68.78	47.27	10.2	1.52	21.58	0.28
Tiller number (TN)	7.43	19.97	10.44	2.13	0.32	20.38	1.95
Main panicle length (MPL) (cm)	23.4	33.3	29.24	2.59	0.39	8.86	-0.64
Primary branches $panicle^{-1}$ (PB)	8.75	13.96	11.39	1.23	0.18	10.8	-0.08
Secondary branches $panicle^{-1}$ (SB)	23.58	58.28	40.94	7.67	1.14	18.74	0.02
Total spikelet number $panicle^{-1}$ (TSN)	83.7	259.64	168.15	40.56	6.05	24.12	0.25
Fertile spikelet number $panicle^{-1}$ (FSN)	56.48	219.32	124.49	35	5.22	28.11	0.65
Fertility % (FP)	44.51	68.34	59.56	6.17	0.92	10.36	-0.76
Test weight (TW) (g)	8.01	28.45	17.43	4.73	0.7	27.12	-0.3
Straw yield per plant (SYPP) (g)	20.66	59.54	40.57	10.46	1.56	25.79	0.18
Harvest index (HI)	19.52	47.44	34.81	6.31	0.94	18.12	-0.33
Grain yield per plant (GYPP) (g)	10.25	36.81	21.33	5.57	0.83	26.1	0.29

PH, FLA, TN, MPL, PB, SB, TSN, FSN, FP, TW, SYPP, HI and GYPP showed wide variation (*i.e.*, CV ranging from 8.07 to 28.11). The mean, maximum, minimum, standard deviations, standard error mean, coefficient of variation, and skewness of the 14 morphological variables are given in Table 2. Lower value of the skewness (*i.e.*, measure of the asymmetry) indicates the normal distribution of the traits under study.

All the genotypes under study showed substantial amount of genetic variations for all the studied traits. For any crop improvement programme, the extent of variability for any character is very essential. Significant variation in traits under study indicated the presence of high genetic diversity among all the genotypes of rice. Pearson correlation coefficients between 14 morphological characters of the rice accessions are given in Fig. 1 which showed FLA, PB, SB, TSN, FSN, TW, SYPP, and HI exhibited significant positive correlation with grain yield per plant.

In modern data analysis Principal Component Analysis (PCA) is a powerful toolsince it is a popular multivariate statistical techniquefor dimension reduction which is used to find out the minimum number of components, explaining maximum variability out of the total variability (Anderson, 1972 and Morrison, 1978) and also ordered the genotypes according to their PC scores. PCs are usually estimated from a covariance matrix or a correlation matrix.Scale effects can alter the make-up of derived components when the variables are measured in different units. It becomes crucial to standardize the variables under such circumstances.

Utilizing yield and yield components on rice germplasm, PCA was carried out. The genetic divergence among the rice accessions based on their phenological traits that participated in the assessment of the genotypes has been suggested for the further improvement programme (Shahidullah et al., 2009). Out of fourteen, only six principal components (PCs) possessed > 0.5 eigen values and showed about 89.98% of total variability among the characters studied. Here only those PCs with eigen value more than one (>1) were selected, which shows 79.5% of the total

Table 3. Rotated component matrix for 14 variables in rice germplasm.

Characters	PC1	PC2	PC3	PC4
Days to 50% flowering (DFF)	0.30	**0.60**	-0.04	0.22
Plant height (PH) (cm)	0.13	**0.85**	0.14	-0.07
Flag leaf area (FLA) (cm^2)	0.10	0.38	**0.78**	0.11
Tiller number (TN)	-0.30	0.23	-0.59	0.48
Main panicle length (MPL) (cm)	0.28	**0.79**	-0.10	-0.26
Primary branches $panicle^{-1}$ (PB)	**0.68**	0.39	0.35	0.26
Secondary branches $panicle^{-1}$ (SB)	**0.95**	0.10	0.05	-0.07
Total spikelet number $panicle^{-1}$ (TSN)	**0.95**	0.19	-0.05	-0.09
Fertile spikelet number $panicle^{-1}$ (FSN)	**0.89**	0.13	0.01	0.37
Fertility % (FP)	0.17	-0.06	0.15	**0.84**
Test weight (TW) (g)	-0.17	-0.13	**0.88**	0.03
Straw yield per plant (SYPP) (g)	-0.21	**0.71**	0.39	0.37
Harvest index (HI)	**0.63**	-0.59	0.31	-0.07
Grain yield per plant (GYPP) (g)	0.44	0.12	**0.67**	0.31
Eigen value	4.80	2.69	2.30	1.35
Percentage variance	34.30	19.20	16.40	9.61
Cumulative variance	34.30	53.50	69.89	79.50

#In rotated component matrix bold values are indicated for the traits having values more than 0.5 in each PCs.

Table 4. Distribution of genotypes into different clusters.

CLUSTER	SIZE	GENOTYPES
I	4	MTU-1010, ANNADA, FALGUNI, LALAT
II	13	MTU-1075, MTU-7029, BAHADUR, BANGO BANDHO-II, GOTRA BIDHAN-3, GOTRA BIDHAN-1, LATISAL, NILANJANA, PROTIKHA, RAJENDRA MASURI, RED HUSK RICE, SHILKHI, SUPER SHAMALI
III	12	BASKAMINI, BENAJHURI, BIHARISAL, DANGAPATNAI, GANGAJALI, KAJOLI, KERELA SUNDARI, MALSIRAJ, MBR, RANJIT, SANTIBHOG, SIMULPHOOL
IV	4	BLACK RICE, DANARGURI, KARTIKKHAS, MANIPUR RICE
V	4	CHINAKAMONI, GREEN RICE, KHAJURKATA, RH-15
VI	1	DUDHESHWAR
VII	7	GHEUSH, JOHA, KALONUNIA, LALMARICH, RH-1442, SITABHOG, TULAIPANJI

COPHENETIC CORRELATION COEFFICIENT = 0.754

variability. Akhtar et al. (2022) found four components contributed 82.58 % of the total variation when PCA with eigenvalues >1 has been taken. So, these PCs were given more importance. The PC1 showed 34.29%, while PC2, PC3, and PC4 exhibited 19.196%, 16.396% and 9.607% of variability, respectively among the germplasm for the traits under study. Scree plot (Fig. 2) explained the percentage of variance associated with each PCs and the maximum variation was observed in PC1 in comparison to other 13 PCs. So, selection of genotypes based on this PC will be effective.

Rotated component matrix

The principal component scores (PC scores) of all the 14 characters under study were estimated and presented in Table 3. These scores can be used to construct precise selection indices, the magnitude of which can be decided by variability explained by each of the PCs. High PC score in a particular component denotes high values for the variables. Rotated component matrix revealed that PC1 accounts for PB, SB, TSN, FSN, and HI. PC2 included DFF, PH, MPL, and SYPP. PC3 included FLA, TW, and GYPP and at

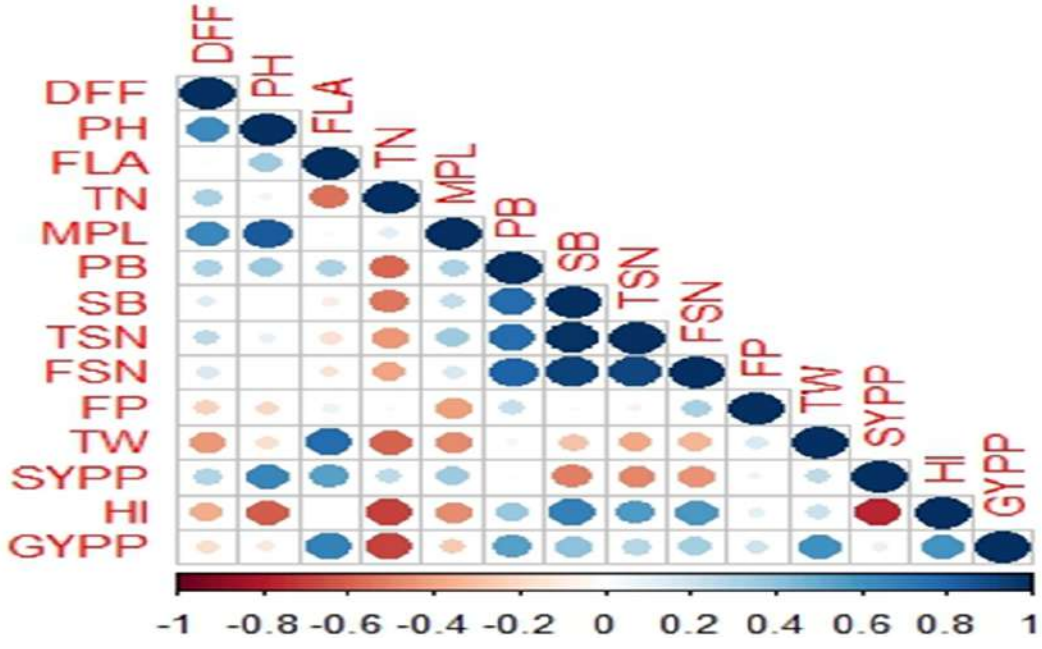

Fig. 1. Pearson correlation coefficients between 14 morphological traits

Table 5. Cluster means of different traits.

CLUSTER	DFF	PH (cm)	FLA (cm^2)	TN	MPL (cm)	PB	SB	TSN	FSN	FP	TW (g)	SYPP (g)	HI	GYPP (g)
I	91.34	111.98	41.38	9.38	27.56	9.8	34.6	120.01	82.53	68.55	20.22	37.75	32.55	18.06
II	113.74	112.42	44.42	9.69	27.52	11.31	41.96	172.36	128.96	75.28	18.44	31.8	40.95	21.82
III	118.59	155.46	59.6	10.28	29.86	12.14	41.59	168.66	130.88	77.61	21.03	52.82	33.6	26.57
IV	113.58	155.28	43.82	8.39	31.44	11.06	45.47	198.98	107.79	54.77	14.49	29.79	34.78	15.66
V	117.66	161.28	42.5	11.41	30.96	13.22	54	239.02	202.99	84.92	12.39	38.95	37.73	23.88
VI	116.32	99.98	23.01	19.97	24.16	8.75	23.58	83.7	56.48	65.12	10.93	38.24	20.72	10.25
VII	117.89	148.49	42.94	11.94	30.8	10.71	34	140.92	103.6	73.54	13.25	44.92	27.15	16.69

last PC4 included FP.

So based on these, a precise selection strategies can be designed for improving dependent traits i.e. grain yield. Agro-morphological diversity and variation among the rice genotypes plays an important role for the crop improvement (Seetharam et al., 2009).

As first two PCs contributes most of the variation, so, to investigate the relationship among the 45 rice genotypes based on the observed yield and yield components, the first two PCs (PC1 and PC2) were plotted in a biplot in the current study (Fig. 3). Genotypes *viz*., MBR, Gangajali, Biharisal, Santibhog, Malsiraj, Benajhuri, Simulphool, Dangapatnai, Kajoli, and Ranjeet established a group in the biplot's right upper corner and displayed positive values for both PCs and the character primary branches, flag leaf area, days to 50% flowering, main panicle length, plant height, and straw yield took place in the same quadrant influencing the grain yield.

Agglomerative cluster analysis

In the present study, as illustrated in the diagram (Fig. 4), the average linkage distance approach of hierarchical clustering was used for grouping the 45 rice genotypes into seven clusters. Seven clusters (Table 4) were formed considering euclidean distance, and overall phenotypic appearance and apparent phenotypic similarity of the genotypes. Clusters I to VII consisted of 4, 13, 12, 4, 4, 1, and 7 genotypes, respectively. Cluster means of different traits of 45 rice genotypes are given in Table 5.

Cluster I had the lowest mean value for days to 50% flowering and moderate values for plant height, main panicle length, and test weight. Cluster II had highest mean value for harvest index and moderates value for plant height, main panicle length, secondary

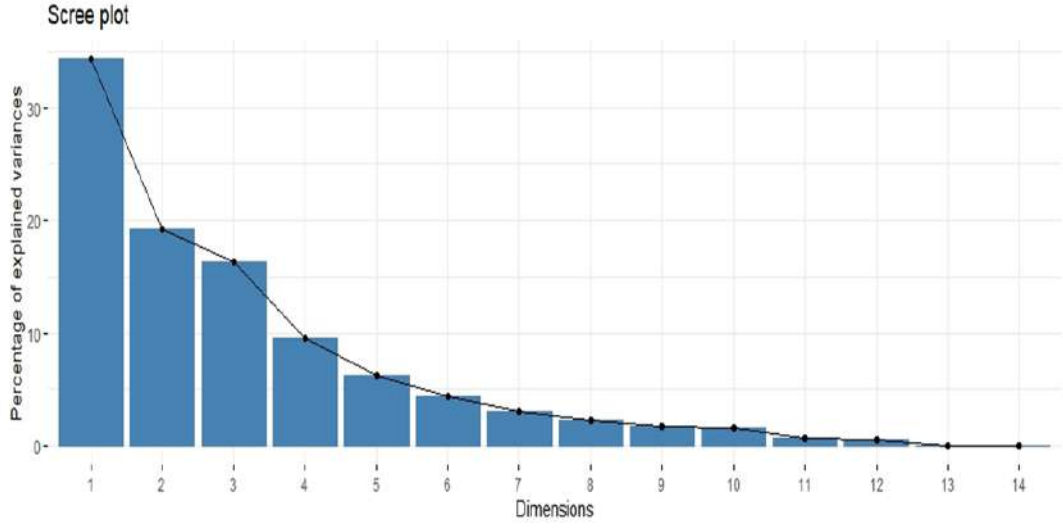

Fig. 2. Scree plot of PCA of rice germplasm between eigen value and PCs.

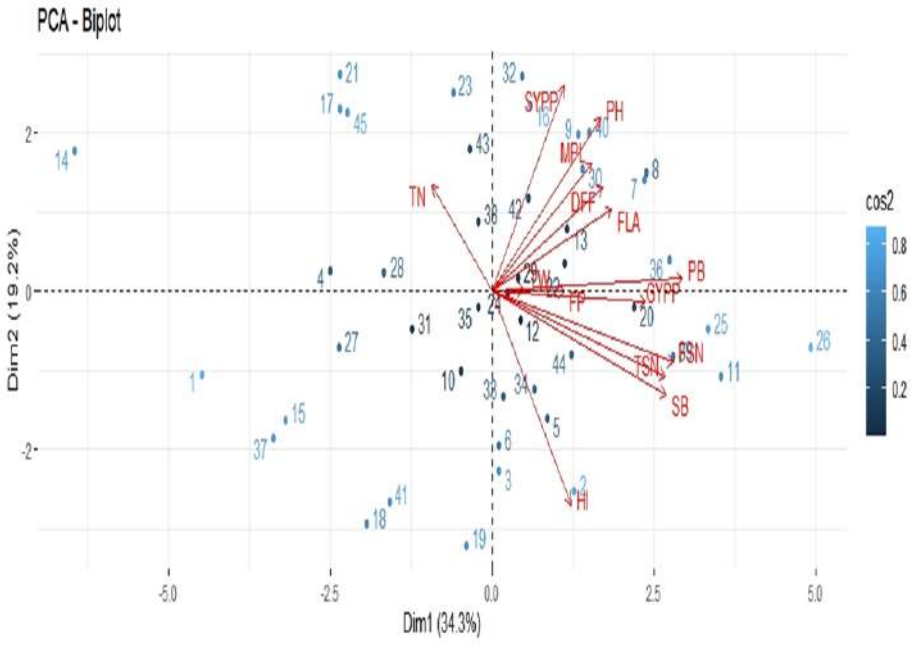

Fig. 3. The PC1 VS PC2 biplot of rice accessions.
** (Note: DFF - days to 50% flowering, TN - number of productive tillers per plant, PH - plant height (cm), MPL - main panicle length (cm), PB-primary branches per panicle, SB - secondary branches per panicle, FLA-flag leaf area (cm^2), HI - harvest index, TSN-Number of spikelet per panicle, FSN - number of filled grain per panicle, FP-fertility percentage TW- test weight(g), SYPP - straw yield per plant (g), and GYPP - grain yield per plant (g). 1 to 45-rice genotypes under study).

branches, total spiklet number, fertility %, and grain yield per plant. Cluster III represented highest mean values for flag leaf area, test weight, and straw and grain yield per plant. Cluster showed highest mean values for main panicle length, and secondary branches. Cluster V recorded highest mean value for primary branches, total spiklet number, fertile spikelet number and fertility %. Cluster VI had highest mean value for tiller number and lowest mean value for plant height, while Cluster VII had moderate mean value for main panicle length, fertility%. Hence, the genotypes belonging to these clusters can be utilized for improvement of the yield and yield component traits.

The cophenetic correlation coefficient has been widely utilized in various studies as a criterion for assessing the efficiency of various clustering approaches as well as a measure of how well a categorization fits a collection of data (Farris et al., 1969). For a high-quality solution, the magnitude of this value should be very close to 1. In this investigation, cophenetic correlation coefficient was 0.754 which indicated the greater efficiency of the clustering pattern.

The traits investigated in this study are important, as they have significant association with yield. FLA, PB, SB, TSN, FSN, TW, SYPP, and HI are important traits of rice for higher yield. Several experiments have been carried out in rice (Tiruneh, 2019; Sinha and Mishra, 2013) as well as different species (Morris, 2007, 2008, 2009) which suggested that principal components and cluster analysis provide useful information about the variability present in germplasm collections and to characterize genetic resources these are successfully used. To examine the variation and to estimate the relative contribution of various traits for total variability, PCA was used. The PC1 showed 34.29%,while, PC2, PC3, and PC4 exhibited 19.196%, 16.396% and 9.607% variability, respectively. It can be concluded that PCA highlights the characters with maximum variability.

To feed the humankind, study of landraces is very important in crop improvement programme as they are the repositories of important genes evolved under different ecological niches and surviving lines qualified

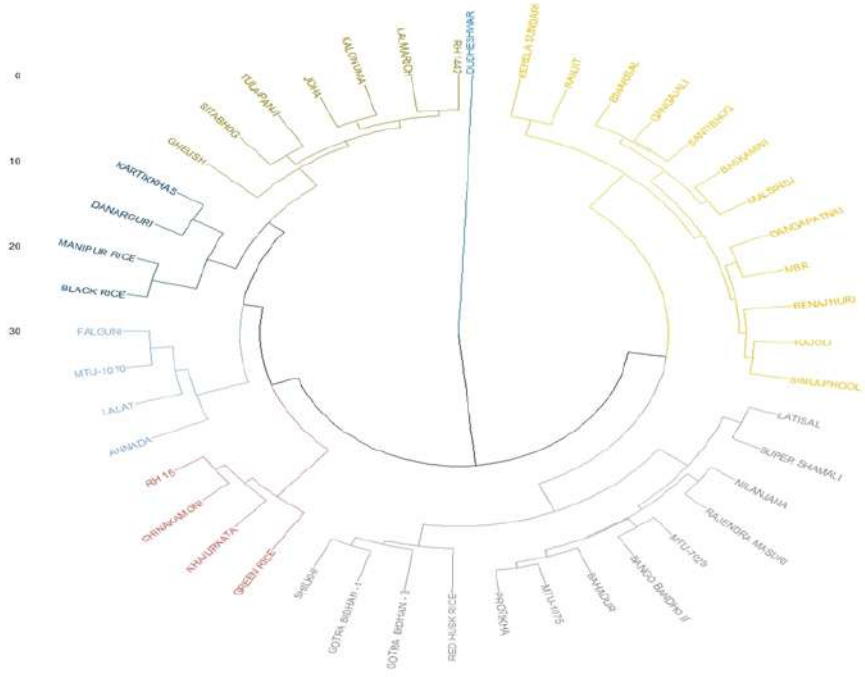

Fig. 4. Dendrogram using agglomerative clustering method in 45 rice genotypes.

the rigorous testing under growing drivers of climate changes over eon. So, identification of desirable agro-morphological characters is the major factors. Keeping this in mind, in this experiment attempts have been made to find out important charactersand clustering of genotypes which could be used in future crop breeding programme. Multivariate analysis also shows utility of different genotypes for different traits.

Considering the genetic divergence among the genotypes, relative importance of the yield components in this particular population and per se performance of the studied genotypes along with cluster means, it is suggested that for intra-cluster and inter-cluster hybridization programme may be attempted. Suchcrosses are most likely to provide a considerable amount of heterosis in first filial (F_1) generation and a widearray of recombinants in further segregating generations. This may help in utilizing heterosis through hybrid breeding in the F_1 generation and /or selection of promising segregates to utilize favourable accumulation of genes from the cross-parents in the advanced segregating generations.

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Estimation of genetic variability, correlation and path analysis in elite rice genotypes (*Oryza sativa* L.)

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ABSTRACT

A trial was carried out to explore the variability and correlation among thirty five rice (Oryza sativa L.) germplasm for fifteen characters, during kharif 2017-18 at Bihar Agricultural University, Sabour, (Bihar). Analysis of variance revealed significant differences among all the genotypes and for all fifteen characters, which reflects that considerable amount of variability, were present in the genotypes. Two genotypes LPD104-B-B-1-8-2-1-1and RP5124-11-6-2 were identified as the superior genotypes for yield. The highest magnitude of genotypic coefficient of variation was recorded for length breadth ratio of rice grain, however highest phenotypic coefficients of variation were recorded for grain yield per plant followed by kernel length. High heritability coupled with high genetic advance as per cent mean was observed for kernel length and length breadth ratio. In this experiment grain yield per plant had positive and significant association with biological yield per plant, days to 50 per cent flowering, days to maturity, while remaining characters had non-significant association with yield. Path coefficient analysis revealed that the biological yield per plant, harvest index and kernel length had high positive direct effects on grain yield per plant indicating true relationship of these characters with grain yield. Therefore, these traits could be considered in rice improvement programme.

Key words: *Biological yield, correlation, harvest index, variability*

INTRODUCTION

Rice is a major staple food of more than three billion people of the world and it is known as "Global Grain". It belongs to family *Poaceae* (2n = 24). There are 25 species of genus *Oryza*, and only two species, namely *Oryza sativa* and *Oryza glaberrima* are cultivated (Onwueme and Sinha, 1991). The *Oryza sativa* is divided into three sub-species, namely, *O. indica*, *O. japonica* and *O. javanica*. The global production of rice is nearly 476 million tones grown in an area of 160.9 million hectare with an average productivity of 2.95 tons per hectare (Food and Agriculture Organization). India is the second largest rice producing country in the world after China. In India rice is grown on an area of 43.78 million hectare with production 118.40 million tones and productivity 2.7 tons per hectare (Annual report 2020-21, www.agricoop.nic.in). In eastern states of India *viz*., Bihar, Odisha, Chhattisgarh, Jharkhand, Madhya Pradesh, Eastern Uttar Pradesh and West Bengal has the highest intensity of rice cultivation. These states receive heavy rainfall and rice is grown mainly under rain fed conditions. In Bihar, the cultivation of rice is nearly 3.30 million hectares with the production of 8.09 million tones and its productivity is 2.44 tons per hectare (Annual report 2020-21, www.agricoop.nic.in). Global demand of rice is rising everyday and to meet out the demand the global food production needs to be increase by over 40% by 2030 and 70% by 2050. In view of high demand and low productivity of paddy in our country, there is need and scope to improve the per hectare yield to fulfill the demand of paddy.

Crop improvement depends on magnitude of

genetic variability and the extent to which the desirable characters are heritable. Thus, a survey of genetic parameters such as genetic coefficient of variation, heritability estimates and genetic advance as well as correlation is essential for selection and effective rice improvement programme.

MATERIALS AND METHODS

The experiment was carried out in the year 2017-18 *kharif* at research farm of Bihar Agricultural University Sabour, Bihar, in randomized block design with three replications. Research farm is geographically situated between 25°15'40" N latitude to 87°2'42" E longitude at 46 m above mean sea level. The observations were recorded for fifteen characters; days to 50 percent flowering, days to maturity, plant height, number of effective tiller per hill ,panicle length ,number of spikelets per plant, flag leaf length, number of fertile grains per plant, grain yield per plant, biological yield per plant, harvest index ,1000 grain weight, kernel length , kernel width and length breadth ratio. Plot area was 4.8 m^2 with plant-to-plant distances was 15 cm and row to row distance was 30 cm. The data was recorded on five randomly selected plants in all three replications for fifteen quantitative characters.

The analysis of variance was worked out to test the differences among genotypes by F-test. It was carried out according to the procedure of Randomized Block Design for each character as per methodology advocated by Panse and Sukhatme (1967). ANOVA helps in partitioning the total variance into three component *viz*., replication, treatment and error. It is an effective measure of variability, which permits partition of variance into various components, like phenotypic, genotypic and environmental variances obtained .Heritability in broad sense is the ratio of genotypic variance to the total variance and is calculated by the formula given by Lush (1940). Genetic advance is the improvement in mean genotypic value of selected plants over the parental population. The estimates of genetic advance were obtained by the formula given by Lush (1949) and Johnson et al. (1955). Genotypic and phenotypic correlation between yield and its component traits were worked out as per the method suggested by Johnson et al. (1955) and Al - Jibouri et al. (1958). The significance of correlation coefficient was tested by referring to the standard table given by Fisher and Yates (1938). Path coefficient analysis was carried out as suggested by Dewey and Lu (1959). To test the significance of correlation coefficient, the estimated values were compared with table values of correlation coefficient prescribed by Fisher and Yates (1938) at (n -2) treatment degree of freedom and 5% and 1% level of significance. The path coefficient analysis is simply the standardized partial regression coefficient, which splits the correlation coefficient into the measures of direct and indirect effects of independent variables on the dependent variables. Path analysis was worked out by using the estimates of correlation coefficient in all possible combinations among the dependent variables

List of genotypes included in the study is presented in Table 1.

RESULTS AND DISCUSSION

The mean sum of square due to genotypes was significant for all the characters of thirty-five genotypes (Table 2) at 1 percent level of significance. This indicates that considerable amount of variability was present in the genotypes and results were similar to the findings of Sumnath et al. (2017), Devi et al. (2016), Rai et al. (2014), Seyoum et al. (2012). Hence, there is an ample scope for inclusion of promising genotypes in breeding program for yield and its component characters

Table 1. List of thirty-five rice genotypes including three checks.

Sl. no.	Genotypes	Sl. no.	Genotypes
1.	Badshahbhog-4	19.	PR35015-5-4
2.	IR 88968-2-1-1-2	20.	IR 09N142
3.	HHZ 14-SAL10-DT1-DT1	21.	LPD104-B-B-1-8-2-1-1
4.	HH15-DT7-SAL4-SAL1	22.	IR1576-1698-2-556-1
5.	Sabour Ardhjal	23.	IR 07A140SBR-1
6.	HHZ 11-Y6-Y2-SUB1	24.	MTU1001
7.	ZHONGHUA 1	25.	CR2713-179
8.	PRR 78	26.	IR 13K5134
9.	HHZ9-SAL9-Y3-SUB1	27.	PUSA 5001-4-2-2
10.	HHZ2-Y3-Y1-Y1	28.	PAU3130
11.	CR2994-5-3-2-1-1	29.	CR3825-2-1-2
12.	IR11A127	30.	NDR9718
13.	CRR547-20-1-1	31.	IR96321-327-300-B-1-1
14.	IR88964-24-2-1-4	32.	RP5124-11-6-2
15.	CN1646-1-9	33.	Prabhat (Check)
16.	IR 05A164	34.	MTU1010 (Check)
17.	NR-241	35.	Rajendra sweta (Check)
18.	HHZ 5-DT8-DT1-Y1		

Table 2. Analysis of variance and genetic parameters of fifteen quantitative characters for thirty-five genotypes of rice.

S. no.	Character	Mean sum of square			Range		Mean	GCV	PCV	h^2 (broad sense) (%)	Genetic advance	Genetic advance as % mean
		Replications d f = 2	Treatments d f =34	Error D f = 68	Lowest	Highest						
1.	Days to 50% flowering	0.866	186.86**	1122.93	75.00	114.66	99.19	7.60	8.63	77	13.66	13.77
2.	Days to maturity	9.409	148.94**	7.88	111.66	143.66	128.21	5.35	5.78	86	13.07	10.20
3.	Plant height (cm)	22.59	227.53**	21.42	93.46	125.66	107.67	7.70	8.82	76	14.91	13.85
4.	Effective tillers / hill	0.539	1.71**	0.42	5.80	8.96	7.57	8.68	12.20	51	0.96	12.72
5.	Panicle length (cm)	0.424	12.85**	1.62	20.80	28.93	25.12	7.70	9.23	70	3.33	13.25
6.	No. of spikelets / plant	9171.55	28776.84**	11542.41	1094.00	1587.33	1265.35	5.99	10.39	33	90.01	7.11
7.	No. of fertile grains/plant	7102.31	22004.16**	8986.74	969.33	1389.33	1144.86	5.75	10.08	33	77.43	6.76
8.	Flag leaf length (cm)	4.712	27.17**	5.66	29.60	39.86	34.45	7.77	10.40	56	4.12	11.97
9.	Grain yield /plant (g)	2.650	50.00**	7.28	15.36	29.72	22.06	17.10	21.03	66	6.32	28.66
10.	Biological yield/plant	24.52	171.37**	31.08	29.72	67.44	49.39	13.85	17.86	60	10.92	22.11
11.	Harvest index (%)	11.77	104.29**	20.05	34.00	60.18	44.89	11.80	15.46	58	8.34	18.57
12.	1000 Grain weight (g)	1.743	16.24**	1.99	19.33	27.73	22.60	9.64	11.49	70	3.77	16.68
13.	Kernel Length (mm)	0.00	2.61**	0.01	3.80	7.20	5.45	17.09	17.21	99	1.91	34.96
14.	Kernel Breadth (mm)	0.00	0.03**	0.00	1.26	1.66	1.46	6.44	9.05	51	0.14	9.43
15.	L/B Ratio	0.13	1.65**	0.05	2.42	5.63	3.78	19.37	20.28	91	1.44	38.11

Among all the fifteen characters, the widest range was found for number of spikelets per plant (1094-1587.33), followed by number of fertile grains per plant (969.33-1389.33), biological yield per plant (29.72-67.44), days to 50 percent flowering (75.00-114.66)Results of experiment revealed that all fifteen characters are individually significant and similar results were observed by Patil and Sarawgi (2005), Padmaja et al. (2008), Vange (2008), Seyoum et al. (2012), Rai et al. (2014) and Senapati and Kumar (2015). Singh et al. (2006).

The phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) for all the fifteen characters are presented in Table-3. The estimates of PCV were higher than their corresponding GCV with all characters. PCV ranged from 5.78 percent (days to maturity) to 21.03 percent (grain yield per plant), however GCV varied from 5.35 percent (days to maturity) to 19.37 percent (length/breadth ratio). Similar findings were exhibited by Bidhan et al. (2001), Singh et al. (2006), Lal and Chavan (2011) and Seyoum et al., (2012). Higher magnitude of PCV was recorded for grain yield per plant (21.03%), length breadth ratio (20.28%), whereas GCV had high magnitude for length-breadth ratio (19.37%), grain yield/plant (17.10%), kernel length (17.09).Relatively lower difference between the magnitude of GCV and PCV was observed for days to 50 percent flowering, plant height, panicle length, days to maturity, 1000 grain weight biological yield /plant, kernel length and harvest index indicates less environmental influence in the expression of these traitsand findings was similar to the result of Mathure et al. (2011) and Nayak et al. (2001).

The heritability (broad sense) was estimated for the fifteen quantitative characters, ranged from 33 percent (number of spikelets/plant and number of fertile grains/ plant) to 99 percent (kernel length). High heritability was observed for the traits like, kernel length (99%), length-breadth ratio (91%), days to maturity (86%), days to 50% flowering (77%), plant height(76%), panicle length(70%),1000-grain weight (70%), grain yield / plant (66%), whereas moderate heritability was noted for flag leaf length(56%), effective tillers per hill(51%), harvest index (58%). Panse and Sukhatme (1967) reported that characters showing high heritability were governed by additive gene action and could be improved through individual plant selection. High heritability was also reported earlier for days to flowering, plant height, grain yield, and panicle length by Akinwaler et al. (2011) and also reported high heritability by Patil et al. (2003) for test weight and grain yield.

Genetic advance of the fifteen characters was ranged from 0.14 (kernel breadth) to 90.01 (number of spikelets per plant). High genetic advance was observed for number of spikelets per plant (90.01), number of fertile grains per plant (77.43). High genetic advance

as percent of mean was observed for kernel length/ breadth ratio (38.11%), kernel length (34.96%), grain yield per plant (28.66%). High heritability with high genetic advance as per cent of mean for length-breadth ratio, kernel length and grain yield per plant character. These results are in accordance with the results of Patil et al. (2005), Seyoum et al.(2012) and Nayak et al. (2016). The results for these characters indicated that heritability is most likely due to additive gene effects and selection may be effective. This type of characters could be improved by mass selection and other breeding methods based on progeny testing.

The analysis of correlation coefficient measures the mutual relationship between fifteen quantitative characters to determine the valuable characters on which selection can be emphasized for yield improvement. The phenotypic coefficients are presented in Table 3 respectively. The magnitudes of genotypic correlation coefficients were higher than the respective phenotypic correlation coefficient in most of the cases. In present study, the highest positive significant value of days to maturity was correlated with days to 50 percent flowering (0.9075**), and length-breadth ratio was correlated with kernel length (0.8916**). Days to maturity showed positive and significant association with flag leaf length (0.5188**), biological yield per plant (0.5569**), 1000 grain weight (0.2249**), grain yield per plant (0.3397**) while significantly negatively correlated with kernel length (-0.3494**) and length-breadth ratio (-0.3188**)and finding as similar to Mathure et al. (2011) and Sanniet al. (2010). Plant height exhibited positive and significant association with panicle length (0.4747**), flag leaf length (0.2373*), biological yield/plant (0.2168*), 1000 grain weight (0.2191*), kernel breadth (0.2677**) and showed negative significant correlation with harvest index, kernel length and length-breadth ratio.

The high positive and significant correlation existed between biological yield per plant and flag leaf length with days to 50 per cent flowering and days to maturity. These results are similar to Chandra et al (2009) and Sandya et al. (2007). High positive significant value of panicle length correlated with plant height was also found by Madhavi lata et al. (2002). However, the characters which showed positive and significant correlation with the grain yield per plant are grain yield per plant (0.8957**), biological yield per plant

Table 3. Phenotypic correlation of fifteen quantitative parameters for thirty-five rice genotypes.

S. no.	Character	Days to 50% flowering	Days to maturity	Plant height (cm)	Effective tillers/ hill	Panicle length	No. of spikelets/ plant	No. of fertile grains/ plant	Flag leaf length (cm)	biological yield / plant(g)	Harvest index(%)	1000 grain weight(g)	Kernel length (mm)	Kernel breadth (mm)	L/B Ratio	Grain yield/ plant
1.	Days to 50% flowering	1	0.9075**	0.1228**	0.0719	0.0569	-0.0414	-0.0030	0.4601**	0.5732**	-0.1383	0.2295*	-0.3220**	-0.0042	-0.2662**	0.3762**
2.	Days to maturity		1	0.1274	0.0373	0.0597	-0.0958	-0.0761	0.5188**	0.5569**	-0.1776	0.2249*	-0.3494**	0.0528	-0.3188**	0.3397**
3.	Plant height (cm)			1	-0.2584	0.4747**	-0.0396	-0.0593	0.2373*	0.2168*	-0.2353*	0.2191*	-0.2581**	0.2677**	-0.3249**	0.0036
4.	Effective tillers / hill				1	-0.1794	-0.1025	-0.0727	-0.0547	-0.0115	0.0200	0.0628	-0.0827	-0.1510	-0.0314	-0.0326
5.	Panicle length (cm)					1	-0.0772	-0.1565	0.1423	0.1228	-0.2303*	0.2944**	0.0469	0.1471	0.0072	-0.0530
6.	No.of spikelets/plant						1	0.9157	0.0132	-0.1045	0.2067*	-0.0660	-0.1065	0.0433	-0.1067	0.0623
7.	No. of fertile grains/plant							1	-0.0088	-0.1220	0.2235*	-0.0988	-0.1298	-0.0216	-0.1103	0.0487
8.	Flag leaf length(cm)								1	0.3666	-0.1373	-0.0730	-0.3160**	-0.0724	-0.2354*	0.2154
9	Biological yield / plant(g)									1	-0.1769	0.4009**	-0.1835	0.2113*	-0.2549**	0.7290**
10	Harvest index (%)										1	-0.1877	0.1944*	-0.1059	0.2173*	0.5297**
11	1000 Grain weight(g)											1	0.1102	0.3288**	-0.0455	0.2118
12	Kernel length(mm)												1	-0.0611	0.8916**	0.0234
13	Kernel breadth (mm)													1	-0.4677	0.01283
14	L/B Ratio														1	-0.0360
15.	Grain yield/plant															1

Residual effect 0.1188 R SQUARE 0.9859

Table 4. Direct (diagonal) and indirect effects of component traits attributing to grain yield /plant in rice at genotypic level.

S.n.	Character	Days to 50% flowering	Days to maturity	Plant Height (cm)	Effective tillers / hill	Panicle length (cm)	No. of spikelets/plant	No. of fertile grains/ plant	Flag leaf length (cm)	Biological yield/ plant	Harvest index(%)	1000 grain weight(g)	Kernel length (mm)	Kernel breadth (mm)	L/B Ratio
1	Days to 50% flowering	0.0284	0.0282	0.0059	0.0010	0.0027	-0.0037	-0.0021	0.0144	0.0236	-0.0054	0.0095	-0.0098	0.0055	-0.0109
2	Days to maturity	0.2788	0.2808	0.0600	0.0017	0.0260	-0.0420	-0.0252	0.1798	0.2353	-0.0735	0.0791	-0.1043	0.0386	-0.1086
3	Plant Height (cm)	0.0188	0.0194	0.0911	-0.0340	0.0468	-0.0053	-0.0093	0.0305	0.0223	-0.0328	0.0283	-0.0276	0.0312	-0.0340
4	Effective tillers /hill	-0.0013	0.0002	0.0133	-0.0355	0.0111	0.0175	0.0150	0.0089	0.0037	-0.0030	-0.0033	0.0034	0.0062	0.0014
5	Panicle length (cm)	-0.0216	-0.0209	-0.1159	0.0702	-0.2255	0.0281	0.0199	-0.0555	-0.0359	0.0839	-0.0967	-0.0102	-0.0153	-0.0090
6	No. of Spikelets/plant	-0.0560	-0.0647	-0.0253	-0.2134	0.0540	0.4328	0.4217	-0.1018	-0.0718	0.2184	-0.0898	-0.0872	0.0400	-0.0806
7	No. of fertile grains/plant	0.0307	0.0368	0.0419	0.1730	0.0361	-0.3992	-0.4098	0.1351	0.0723	-0.2113	0.1237	0.1026	0.0127	0.0832
8	Flag leaf length(cm)	0.0135	0.0171	0.0090	-0.0067	0.0066	-0.0063	-0.0088	0.0268	0.0137	-0.0059	-0.0025	-0.0108	0.0031	-0.0105
9	Biological yield /plant	-0.1023	-0.1034	-0.0302	0.0127	-0.0196	0.0205	0.0218	-0.0633	-0.1233	0.0123	-0.0719	0.0278	-0.0552	0.0428
10	Harvest index(%)	-0.0707	-0.0978	-0.1346	0.0317	-0.1390	0.1884	0.1925	-0.0819	-0.0374	0.3735	-0.0998	0.0926	-0.1245	0.1256
11	1000 grain weight(g)	0.0683	0.0575	0.0634	0.0187	0.0875	-0.0424	-0.0616	-0.0189	0.1190	-0.0546	0.2042	0.0274	0.1168	-0.0120
12	Kernel length(mm)	0.1743	0.1875	0.1531	0.0488	-0.0228	0.1017	0.1265	0.2047	0.1139	-0.1252	-0.0678	-0.5050	0.0690	-0.4789
13	Kernel breadth(mm)	0.0111	0.0078	0.0194	-0.0099	0.0039	0.0052	-0.0018	0.0065	0.0254	-0.0189	0.0324	-0.0077	0.0567	-0.0258
14	L/B Ratio	-0.1898	-0.1908	-0.1843	-0.0201	0.0197	-0.0919	-0.1002	-0.1937	-0.1711	0.1660	-0.0290	0.4680	-0.2247	0.4935
15	Grain yield/plant(g)	0.5249	0.4838	-0.0221	-0.1113	-0.1102	0.1848	0.1723	0.2780	0.7692	0.5437	0.2983	0.0351	0.1950	-0.0273

(0.7290**), harvest index (0.5297**), days to 50% flowering (0.3762**) and days to maturity (0.3397**) . This result has been supported by Murthy et al (2004), Sanker et al. (2006) and Nandan et al. (2010). The character flag length was positively correlated with plant height and results are similar with the with the findings of Rathore et al. (2016). Panicle length exhibited positive significant association with 1000- grain weight (0.2944**) and plant height (0.4747*). Present observation was in accordance with the result of Nayak et al. (2001) and Mathure et al. (2011). Number of spikelets per plant showed positive significant association with harvest index (0.2067*). The direct and indirect effects of traits on grain yield at genotypic level are presented in Table 4 respectively. Biological yield per plant (0.7727) and harvest index (0.6183) had high positive direct effect on grain yield per plant. Days to 50 percent flowering had high indirect positive effect (0.3762) on grain yield (kg/plant) via days to maturity, fertile grains per plant, flag leaf length, biological yield per plant (0.4429). Findings were similar with the result of Naseem et al (2014) and Wattoo et al. (2010). Days to maturity had high indirect positive effect (0.3397) on grain yield per plant via fertile grains per plant, flag leaf length, biological yield per plant, kernel breadth . These results are similar to the results of Wattoo et al (2010). Biological yield per plant had a high indirect positive effect (0.7290) and harvest index had high indirect positive effect (0.5297) on grain yield per plant via days to maturity, effective tillers per hill, fertile grains per plant, flag leaf length and kernel breadth. Similar results was found by Ambili and Radhakrishnan (2011). Days to maturity, effective tillers per hill, fertile grains per plant, flag leaf length, biological yield per plant , kernel length, kernel breadth, length-breadth ratio showed positive indirect effect on grain yield per plant. Results were resemblance to Naseem et al (2014) and Wattoo et al. (2010), Shivani and Reddy (2000) and Madhavilata (2002).

CONCLUSION

On the basis of mean performance the genotypes *viz.*, LPD104-B-B-1-8-2-1-1and RP5124-11-6-2 were identified as the superior genotypes for yield and yield attributing traits. The higher magnitude of genotypic coefficient of variation was recorded for length breadth ratio while in phenotypic coefficient of variation was recorded high for grain yield per plant followed by kernel

length. High heritability coupled with high genetic advance as percent mean was observed for kernel length, length-breadth ratio. These characters can be considered for the rice improvement through adapting selection without progeny testing. Grain yield per plant showed positive and significant association with biological yield per plant, days to 50 percent flowering and days to maturity. In path coefficient analysis biological yield per plant, harvest index and kernel length had high positive direct effects on grain yield per plant. Therefore, the direct selection for these characters will be rewarding.

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Multivariate analysis for evaluation of indigenous rice (*Oryza sativa* L.) accessions collected from Madhya Pradesh

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ABSTRACT

Eighty indigenous rice genotypes were evaluated for twenty-nine quantitative and quality traits, planted in RCBD with three replications. The data on different characters were analyzed through Mahalanobis' generalized distance D^2 (1936) and Principal Component Analysis for estimating genetic diversity and identification of superior rice genotypes. On the basis of genetic distance the 80 genotypes were grouped into 18 clusters following Tocher's method. Among them, five clusters contain more than one genotype, while thirteen clusters contain single genotype. The genotypes of cluster VIII and IX showed a higher (19816.1) inter cluster distance followed by cluster IV and VIII (12414.7). Cluster V has been discovered to have the largest intra-cluster distance. In order to increase the genetic diversity of rice, genotypes from these clusters may be crossed. On the basis of PCA findings, among all genotypes contributing their presence in more than one PC with high PC score only five genotypes viz., Biranjphool Shivram, Jeera Phool, Basmati Purani, Kardhana Baldev and Kailari Ram Madan confine with favourable yield as well as quality associated PCs, and had excellent remark for both the traits. These genotypes might be utilized in hybridization programme for the transfer of good yield as well as good quality traits in the recipient rice genotypes for the development of promising rice cultivars.

Key words: *Divergence, indigenous rice, mahalanobis D^2, multivariate analysis, PCA*

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to the genus *Oryza* and has two cultivated and twenty-two wild species. In the wild spices, 9 species are tetraploid (2n=48) and the remaining 13 wild species are diploid and two cultivated species are diploid (2n=24). The cultivated species are *Oryza sativa* (Asian cultivated rice) and *Oryza glaberrima* (African cultivated rice). Asian cultivated rice is grown worldwide, while African cultivated rice *Oryza glaberrima* is grown on a limited scale in West Africa. Traditional varieties have large amount of diversity in grain shape, size, colour, and nutritive value, some varieties that have aroma also contains iron and zinc. Black colour rice contains antioxidant properties which are good for human health. Scientific studies suggested that these colour pigments have antioxidant properties that may be useful to human health. Diverse plant types are immediately valuable for shaping new varieties and this will possible only from the land races available in the tribal localities as well as in the genetic stock of the rice for the utilization in breeding programme.

Genetic diversity is a prerequisite for any crop improvement program and it helps in the identification of superior segregants and facilitates their use and management. Instead of the parental lines belonging to the same cluster or clusters with a small genetic distance, the divergent lines belonging to distinct and distant clusters are more likely to produce heterotic hybrids or superior offsprings (Rao, 1952). It is quite imperative to group or classify genotypes on the basis of an appropriate scale to understand the usable variability that exists among them. Principal component analysis was first proposed by Pearson (1901) and further

developed by Hotelling (1933). It is essentially a well-known data reduction technique (1933). The objective of principal component analysis is to determine the smallest number of components that may account for the greatest amount of variability (Anderson, 1972 and Morrison, 1982). Considering the importance of multivariate analysis, an investigation was carried out on eighty indigenous rice genotypes with the objective to dissecting yield and quality related inter-componential traits to obtain precise information about the contribution of characters to genetic divergence and to rank the genotypes on the basis of PC Score.

MATERIALS AND METHODS

The experimental material consisted of 80 indigenous rice accessions collected from different districts of Madhya Pradesh. The research material was obtained from the Rice Improvement Project, Department of Plant Breeding & Genetics, College of Agriculture, JNKVV, Jabalpur. These lines were planted in RCBD with three replications. One seedling per hill was maintained when twenty-one day old seedlings were transplanted at the experimental site with a distance of 15 cm X 20 cm between plants and rows. Gap filling was completed within a week in order to maintain an effective plant population. An application of 120 kg N, 60 kg P_2O_5, and 60 kg K_2O fertiliser was made. For normal crop growth, regular agronomic procedures were used. The observations were recorded from five randomly chosen plants for twenty-nine yield assessment and quality attribute traits as per standard descriptors of rice (IRRI-IBPGR Rice advisory committee, 1980) and statistical analysis was performed using the mean values.

Statistical analysis

The replicated mean values of various traits in each genotype were used in the statistical analysis. The data were analyzed through Mahalanobis' generalized distance D^2 (1936) and grouping of the populations into various clusters was done by using Tocher's method (Rao,1952). Principal components are generally estimated either from a correlation matrix or a covariance matrix. In the present investigation, the principal components were extracted using a correlation matrix and the methodology for PCA analysis was performed as described by Massy (1965) and Jolliffe

Table 1. Clustering pattern of eighty indigenous rice genotypes.

Cluster no.	Number of genotypes	Genotypes
I	21	Faram, Shera-2, Sellow dhan, Janki, Amagaur, Nadwal, Govind, Kailari Papra, Pisso, Culture, Lohandi-1, Ponga, Dhaurdhan, Khuddi,Dhaur Gaya Prasad,Tharibharijag, Shrijat, Safri, Kardhana Baldev, Tendhaniya dhan Kaushal, Janki-1
II	17	Doodh Newari Chopal, Kardhani, Sikia Vishwanath, Tin Pakhia, Sikia Kallu, Sikiya, Bagri Sarethi, Lohandi Rambhajan, Biranj phool Shivram, Dhooth, Thooth dhan Kaushal, Kailari Ram Madan, Butnagar, Karan Phool, Methi Choor, Jhuri, Dhammu Dhani
III	1	Soorag
IV	1	Chapti
V	18	Jamun Surkhi, Nawari-2, Rameshwar, Bhaisan, Nawari-1, Laldhan, Chhindphool, Safed Jeera Shankar, Ratna, Bedhaar Papra, Luchai-1,Jeera Phool, Luchai-2, Hanskanak Karwahi, Luchai-3, Kariya Parvat, Kshtriya, Mahuan
VI	1	Sonkharchi Mojya
VII	1	Geeta Shivram
VIII	1	Usha
IX	8	Harau Luchai, Karonda Budh, Lal Laichi Rosar, Vishnu Bhog, Kadanbhog, Lohandi Lalitpur, Jharga Raghuraj,Mula Pradhan
X	1	Gurmatiya
XI	1	Mohanbhog
XII	1	Soniya
XIII	3	Dihula Ramesh, Basmati Purani, Dubrajlallu
XIV	1	Jeera Phool,
XV	1	Sinduri Mado
XVI	1	Bragbhog Jagdish
XVII	1	Bharaphoo lKarwahi
XVIII	1	Kardhana

(1986).

RESULTS AND DISCUSSION

Genetic divergence

The percentage contribution of all the characters towards genetic divergence is graphically presented in Fig. 1. The character stem length (48.51%) contributed the most to the genetic divergence followed by thousand grain weight, panicle weight per plant, days of 50% flowering, sterile spikelets per panicle, biological yield per plant, flag leaf length, number of spikelets per plant, number of tillers per plant, stem thickness, fertile spikelets per panicle, panicle index and the rest of the traits under study had no contribution towards genetic divergence. These results were supported by the findings of Kumari et al. (2015), Chandramohan et al. (2016), Ashok et al. (2017), Shivani et al. (2018), Prakash et al. (2019) and Rahangdale (2019).

On the basis of genetic distance the 80 genotypes were grouped into 18 clusters following Tocher's method (Table 1). The clusters I, II, V, IX and XIII were poly genotypic, while clusters III, IV, VI, VII, VIII, X, XI, XII, XIV, XV, XVI, XVII and XVIII were mono genotypic (1 genotype in each). Based on the procedure given by Singh and Choudhary (1979), the average intra and inter-cluster D^2 values and the cluster mean values were estimated and presented in Table 2 and Table 3. The values of average intra and inter-cluster distance (D^2) revealed that the maximum intra cluster distance was found for Cluster XIII (D^2 =256.66) followed by Cluster IX (D^2 = 231.98), Cluster V (D^2 =211.9), Cluster II (D^2 = 142.44) and Cluster I (D^2 = 132.43). The maximum inter cluster distance was noted between cluster XVII and XVIII (D^2 = 2522.33) indicating wide diversity between them and the hybridization between genotypes belonging to cluster XVII (Bharaphool Karwahi) and cluster XVIII (Kardhana) may lead to the formation of superior recombinants. The highest cluster mean values were recorded for number of tillers / plant (10.01) and stem thickness (6.21) while, lowest cluster mean values were recorded for total spikelets / panicle (132.78) and panicle index (124.45). Some traits *viz*., panicle length, number of tillers / plant, number of productive tillers per plant, plant height, hulling percentage, plant weight, harvest index, flag leaf width, grain length, grain breath, decorticated grain length, decorticated grain breadth,

Table 2. Average intra and inter cluster distance (D2) among eighteen clusters in rice

Cluster	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
I	132.43	271.22	195.14	190.10	438.62	276.34	381.06	192.08	274.88	467.64	217.93	453.78	358.52	771.89	500.12	606.48	1107.76	429.61
II		142.44	570.02	540.43	757.13	271.41	303.37	327.61	300.44	848.94	529.30	236.91	604.13	789.59	483.04	424.30	1571.84	350.49
III			0.00	48.02	213.30	383.12	576.48	358.29	350.38	231.52	159.98	854.76	301.92	701.69	715.47	1069.07	670.44	789.46
IV				0.00	218.78	366.81	499.05	309.00	344.90	153.76	111.92	837.72	270.59	664.33	798.73	933.23	680.05	782.27
V					211.90	409.46	559.24	651.56	401.36	290.61	394.88	1022.93	411.17	426.42	938.15	1270.69	456.18	1256.48
VI						0.00	62.07	347.59	192.42	651.45	437.51	252.08	321.49	282.61	386.04	422.41	771.71	851.94
VII							0.00	346.94	293.57	735.24	494.91	301.91	384.65	356.05	584.28	340.88	940.15	954.76
VIII								0.00	440.19	695.00	156.93	414.92	325.21	1057.35	516.81	348.67	1345.11	474.31
IX									231.98	550.64	464.02	431.71	433.08	415.53	528.25	703.99	916.69	715.43
X										0.00	351.98	1388.86	475.88	713.29	1469.28	1525.09	640.70	1204.23
XI											0.00	821.85	328.31	945.12	817.70	787.48	1030.02	676.60
XII												0.00	705.12	856.04	173.84	174.56	1698.51	570.36
XIII													265.66	636.09	698.34	758.04	611.20	1055.37
XIV														0.00	968.72	1182.63	452.38	1729.47
XV															0.00	461.85	1490.20	715.17
XVI																0.00	1971.43	750.46
XVII																	0.00	2522.33
XVIII																		0.00

Table 3. Cluster mean values showing importance of different traits.

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII
DTF	95.68	75.92	111.67	105.33	103.48	86.67	72.00	87.00	89.00	94.33	94.00	84.33	99.44	86.67	112.67	77.00	118.00	84.33
FLL	30.76	28.32	36.03	32.13	35.14	30.20	27.40	35.10	31.80	31.87	35.20	27.97	41.56	29.20	31.60	28.90	35.93	30.33
SL	83.47	74.38	101.67	103.07	118.73	91.93	92.07	72.80	95.01	124.33	96.20	57.00	93.99	124.27	56.27	53.47	135.40	53.13
PL	20.48	18.90	23.73	21.50	22.55	21.60	21.33	22.20	19.86	20.80	22.63	16.33	22.33	24.07	18.50	16.83	24.13	16.30
TTPP	10.01	10.33	9.20	9.40	9.51	12.53	12.47	8.87	9.42	10.93	10.33	10.87	8.82	10.67	11.00	12.27	9.60	11.60
PTPP	8.67	9.06	7.75	8.71	8.32	11.17	10.94	7.53	8.11	9.93	9.30	10.07	7.83	9.57	9.72	10.53	8.47	10.47
PWPP	17.18	12.74	18.33	19.27	16.98	18.73	22.40	26.40	11.98	17.17	26.67	15.20	23.78	12.13	16.20	24.73	22.13	10.47
ST	6.21	5.26	6.13	6.00	6.61	4.67	6.00	6.73	6.11	8.20	3.53	5.47	8.07	6.33	5.07	5.67	8.80	4.30
NSPP	132.78	139.86	110.20	172.47	158.80	152.80	172.40	163.20	157.34	165.04	127.47	190.87	226.67	224.00	181.27	226.07	254.40	119.11
FSPP	112.84	114.37	96.33	152.67	127.10	131.27	144.67	130.60	126.34	127.71	103.40	162.67	167.04	184.80	146.73	209.67	187.67	100.93
SSPP	19.94	25.49	13.87	19.80	31.01	21.53	27.73	32.60	31.00	37.33	24.07	28.20	59.62	39.20	34.53	16.40	66.73	18.17
TGW	25.07	22.01	25.87	26.97	21.65	14.33	14.20	24.23	19.78	30.25	28.40	13.43	19.09	10.70	12.57	14.40	12.03	32.50
PH	107.85	97.47	130.40	126.90	144.67	114.87	124.07	96.67	117.87	148.73	121.27	76.67	120.77	152.67	77.77	74.20	166.50	73.23
PW	22.11	18.62	18.50	26.93	25.81	20.93	22.80	28.40	22.35	29.00	26.93	19.93	28.71	21.67	20.73	28.67	36.60	14.17
BYPP	39.29	31.35	36.83	46.20	42.78	39.67	45.20	54.80	34.33	46.17	53.60	35.13	52.49	33.80	36.93	53.40	58.73	24.63
SF	85.04	82.06	87.30	88.47	80.56	86.03	83.92	80.08	80.03	77.38	80.99	85.28	73.69	82.50	80.95	92.75	73.76	85.08
HI	53.48	51.60	56.82	45.71	49.97	56.41	55.37	53.79	46.70	39.45	54.78	55.97	48.20	29.20	54.83	50.06	36.17	64.32
PI	124.45	133.50	115.17	109.28	128.08	120.89	112.43	111.15	111.15	105.35	110.46	130.57	107.53	85.33	125.96	108.54	96.50	154.28
GYPP	21.05	16.26	21.07	21.00	21.28	22.60	25.13	29.33	15.90	18.00	29.47	19.87	25.38	10.37	20.53	26.87	21.33	16.13

DTF- Days to 50% flowering, FLL- Flag leaf length, FLW- Flag leaf width, SL- Stem length, PL- Panicle length, ST- Stem thickness, NSPP- No. of spikelet per plant, SF- Stem fertility, PW- Plant weight, PTPP- No. of productive tillers per plant, PWPP- Panicle weight per plant, PH- Plant height, SSPP- Sterile spikelets per plant, NTPP- No. of tillers per plant, FSPP- Fertile spikelet per panicle, PI- Panicle index, SD- Spikelet Density, TGW- Thousand grain weight, HI- Harvest Index, BYPP- Biological yield per plant, GYPP- Grain yield per plant, GL- Grain length, GB- Grain Breadth, DGL- Decorticated grain length, DGB- Decorticated grain breadth, LBR- L:B ratio of decorticated grain, H (%)- Hulling percentage, M (%)- Milling percentage, HRR (%)- Head rice recovery

milling percentage, head rice recovery percentage, length breadth ratio of decorticated grain, spikelet density and grains per plant had no contribution towards genetic divergence. This finding was not in agreement with the findings of Ranjith et al. (2018).

Variability

Principal component analysis was performed using yield and yield attributing components on the rice genotypes. Out of the twenty nine traits studied, only seven principal components (PCs) exhibited more than 1.5 eigen value and showed 73.18% of total cumulative variability among the traits studied (Fig. 2). The PC1 showed 22.43% variability, while the PC2, PC3, PC4, PC5, PC6 and PC7 exhibited 11.96%, 10.43%, 8.36%, 6.83%, 6.63% and 6.51% variability respectively. PC1 exhibited highest eigen value (6.506) which then declined gradually as 3.468, 3.025, 2.426, 1.982, 1.924, 1.888 and so on with successive PCs. Shoba et al. (2019) reported that, out of nine PCs, four displayed more than 1.0 eigen values and showed a total variability of 70% among nine characters of 67 rice germplasm.

Rotated component matrix

The rotated component matrix is depicted in Table 4 and shows that the PC1 which reported the highest variability (22.43%) was mostly associated with physiological and some yield related traits like days to

Table 4. Interpretation of rotated component matrix for the traits having highest value in each PCs.

	PC1	PC2	PC3	PC4	PC5	PC6	PC7
Traits	DTF	TGW	PTPP	M (%)	NTPP	HI	SSPP
	FLL	PW	PWPP	HRR (%)	FSPP		GL
	SL	FLW	PH	SD	DGL		
	PL		GB		LBR		
	ST		DGB		GYPP		
	NSPP		H (%)				
	BYPP		PI				
	SF						

DTF- Days to 50% flowering, FLL- Flag leaf length, FLW- Flag leaf width, SL- Stem length, PL- Panicle length, ST- Stem thickness, NSPP- No. of spikelet per plant, SF- Stem fertility, PW- Plant weight, PTPP- No. of productive tillers per plant, PWPP- Panicle weight per plant, PH- Plant height, SSPP- Sterile spikelets per plant, NTPP- No. of tillers per plant, FSPP- Fertile spikelet per panicle, PI- Panicle index, SD- Spikelet Density, TGW- Thousand grain weight, HI- Harvest Index, BYPP- Biological yield per plant, GYPP- Grain yield per plant, GL- Grain length, GB- Grain Breadth, DGL- Decorticated grain length, DGB- Decorticated grain breadth, LBR- L:B ratio of decorticated grain, H (%)- Hulling percentage, M (%)- Milling percentage, HRR (%)- Head rice recovery

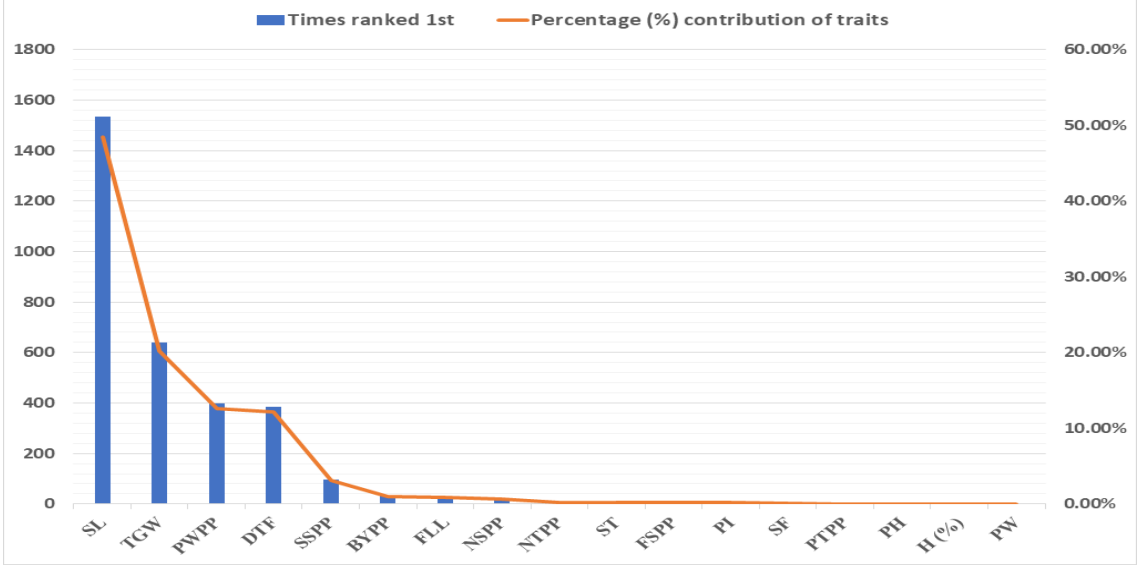

Fig. 1. Graphical Representation of Contribution of Different Characters towards Divergence.
SL- Stem length, TGW- Thousand grain weight, PWPP- Panicle weight per plant, DTF- Days to 50% flowering, SSPP- Sterile spikelets per plant, BYPP- Biological yield per plant, FLL- Flag leaf length, NSPP- No. of spikelet per plant, NTPP- No. of tillers per plant, ST- Stem length, FSPP- Fertile spikelet per panicle, PI- Panicle index, SF- Stem fertility, PTPP- No. of productive tillers per plant, PH- Plant height, H (%)- Hulling percentage, PW- Plant weight

Table 5. Genotypes selected on the basis of high PC score (>1.5) in each component contributing positive values

PC 1	PC2	PC3	PC4	PC5	PC6	PC7
Bagri	Amagaur	Basmati Purani	Basmati Purani	Dharudhan	BrajbhogJagdish	Bhaisan
BirajphoolShivram	BedharPapra	BedharPapra	Chhindphool	DubrajLallu	Chhindphool	Culture
Dihula Kala Mujra	Culture	BiranjphoolShivram	Doodh Newari Chopal	Kadanbhog	Dooth	JhargaRaghuraj
Faram	Dhaur Gaya Prasad	Chapti	Janki-2	Kailari Ram Madan	Janki-2	Lal LaichiRosar
JhargaRaghuraj	Hanskanak Newari	Dhaur Gaya Prasad	KardhanaBaldev	Mahuan	Kailari Ram Madan	Nawari-1
KailariPapra	JeeraPhool	Dihula Kala Mujra	KarondaBhudh	Methichoor	Lal LaichiRosar	Nawari-2
Karan Phool	KailariPapra	Dooth Newari Chopal	LohandiLalitpur	Soniya	LohandiRambhajan	Ponga
Kardhana	KariyaParvat	Janki-2	Nawari-2	Tendhaniyadhan	Rameshwar	SinduriMado
KarondaBhudh	Lal Dhan	JhargaRaghuraj	Safri	Vishnubhog	Ratua	
LohandiRambhajan	Luchai-1	Kailari Ram Madan	Sikiya		Shrijot	
Lohandi-1	Mahuan	LohandiLalitpur	Tharribharijag		Tendhaniyadhan	
Mula Pradhan	Mohanbhog	Mohanbhog				
Pisso	Ponga	SellowDhan				
Shera-2	Safri	Soniya				
SikiaKallu	Shrijot	Tharribharijag				
SikiaVishwanath	Soorag	Tinpakhia				
Sikiya						
ThoothdhanKaushal						

50% flowering, flag leaf length, stem length, panicle length, stem thickness, number of spikelets per panicle, biological yield per plant and spikelet fertility. The PC2 was dominated by yield related traits like thousand grain weight, plant weight. The PC3 was dominated by grain quality traits such as number of productive tillers per plant, panicle weight per plant, plant height, grain breadth, decorticated grain breadth and hulling percentage. The PC4 also contributed to grain quality characters like milling percentage, head rice recovery and spikelet density. Similarly PC5 was dominated by quality traits like number of tillers per plant, fertile spikelets per panicle, decorticated grain length, length and breadth ratio of decorticated grain and grain yield per plant. PC6 was dominated by quality traits like harvest index. PC7 is dominated by physiological traits and single trait sterile spikelets per panicle. These findings were in partial agreement with the findings of Nachimuthu et al. (2014), Pachauri et al. (2017), Tiruneh et al. (2019), Rahangdale et al. (2021), Surjaye et al. (2021) and Barrie et al. (2022). However, these findings were not in full agreement with the finding of Pathak et al. (2018) who reported that biological yield per plant was mostly dominated in PC2.

PC scores of rice genotypes selected on the basis of >1.5 in each PCs

In this study, the genotypes that contributed their presence in more than one principal component and also had >1.5 PC scores, were selected for further consideration. From this study, it was reported that PC1 and PC2 were constituted by most of the yield attributing traits and genotypes belonging to these PCs like Kardhana, SikiaVishwanath, Kailari Papra and Dooth Newari Chopal should be used for the development of high yielding promising genotypes. Similarly, PC3, PC4,PC5, PC6 and PC7 reported for the quality attributing traits, hence genotypes like Jharga, Raghuraj, Dihula, Kala Mujra, Lohandi, Lalitpur, Kashtriya, Lal Laichi, Rosar, Nawari-2, Biranjphool Shivram and Bedhar Papra should be selected from these PCs can be utilized for quality improvement programme. The genotypes recorded as having a common share of presence in more than one PC are Basmati Purani, Chhind Phool, Dhaur Gaya Prasad, Dihula Kala Mujra, Janki-2, Jharga Raghuraj, Kailari

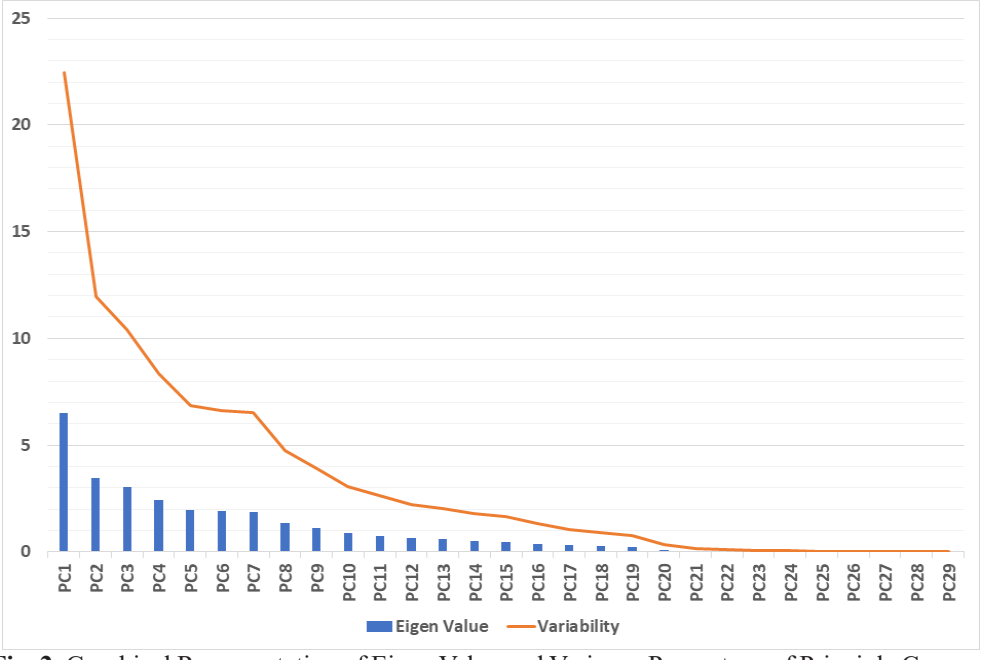

Fig. 2. Graphical Representation of Eigen Value and Variance Percentage of Principle Component

Papra, Karonda Bhudh, Lohandi Lalitpur, Lohandi Rambhajan, Nawari-2, Safri, Sikiya and Tharribharijag (Table 5). The characters with the most variability are highlighted by PC analysis as can be concluded. So, intensive selection procedures can be designed to bring about rapid improvements in yield and quality attributing traits.

CONCLUSION

The genotypes of cluster XVII (Bharaphool Karwahi) and cluster XVIII (Kardhana) showed the highest inter cluster distance. In order to increase the genetic diversity of rice, genotypes from these clusters may be crossed. The highest intra cluster distance has been recorded in cluster XIII hence, hybridization among the genotypes of cluster I may also result in superior recombinants for different yield and quality attributing traits. On the basis of PCA ranking and contributing their presence in more than one PC dominated with yield and quality traits, genotypes Biranjphool Shivram, Jeera Phool, Basmati Purani, Kardhana Baldev, Kailari Ram Madan, Sellow Dhan and Nawari-2 will be selected as donor for improving the yield and quality traits of rice. More study on the molecular characterization of the mentioned rice genotypes is needed to offer more accurate measurements of genetic diversity as well as validation of essential yield and quality contributing factors with associated molecular markers for precise selection.

DECLARATION

The authors declare no conflict of interest.

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Physiological and biochemical traits regulating preharvest sprouting resistance in rice

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ABSTRACT

In cereals, pre-harvest sprouting (PHS) or vivipary is a key physiological and agronomic trait that causes huge economic loss. PHS triggered by typhoons, cyclones, and high relative humidity at the late seed maturation stage is becoming a major threat to rice production in India. To explore the mechanism of PHS in rice, we evaluated 96 rice genotypes for PHS resistance and discovered 12 PHS resistant genotypes. These genotypes were classified into two groups susceptible and resistant, based on their phenotype. From the 96 genotypes, 16 contrasting genotypes were chosen, to unravel the underlying mechanism associated with PHS resistance. The results revealed that resistant genotypes had 0% germination at all the flowering stages (20 to 40 DAF), while susceptible genotypes had 4 to 87.5% germination from 20 to 40 DAF. In terms of pericarp color, 7 out of 8 resistant genotypes had red/pigmented pericarp color while the susceptible genotypes had white/non-pigmented pericarp color. The carotenoid content of leaves and seeds from 20 to 40 DAF was also measured and found to be significantly higher in resistant genotypes than susceptible genotypes. Carotenoids have been demonstrated to increase resistance by assisting in the synthesis of ABA and thereby seed dormancy. The 12 resistant genotypes were examined for germination to decide the duration of dormancy. The duration of dormancy varied in these 12 resistant genotypes varying from 10 days up to 40 days after harvest. These findings suggest that these novel PHS resistant genotypes (PB-68, HT-81, PB-50(1), HT-86, HT-20, Mahulata, PB-285, PB-47, NHN-279, PB-65, PB-259 and Budidhan) may be exploited as donors in the crop improvement programmes to generate PHS resistant genotypes.

Key words: *Pre harvest sprouting/vivipary, carotenoid, pericarp, abscisic acid, gibberellins, seed dormancy and germination*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the world's most important cereal crop because it feeds more than half of the global population and fulfill their need of balance caloric and nutritional intake (Hung et al., 20l6; Lin et al., 20l1). Rice production and quality are related to genotype and growing conditions prior to harvest (Falade et al., 20l4). However, as people's level of living has risen, consumers are becoming more concerned about rice quality (Kong et al., 2015). Germination that occurs in seeds while they are still attached to the panicle is known as pre-harvest sprouting (PHS). PHS is a major threat that has a main impact on rice production in India. PHS not only reduces rice production, but it also drastically lowers grain quality, which results in huge economic losses for farmers. PHS is found in the top five cereals farmed globally in 2017 [according to http://www.fao.org/faostat/en/#compare (3/9/20l9)]: maize, wheat, rice, barley, and sorghum, as well as rye. Japan, India, China, USA, Canada, Australia, North Africa, and most of Europe have all threatening cases of PHS (Wan et al., 2006; Biddulph et al., 2007; Benech-Arnold and Rodriguez, 2018; Nakamura, 2018).

According to earlier researchers, various mutants such as viviparous (vp) of maize, ABA-deficient (aba) of Arabidopsis, and ABA-insensitive (abi) lacks in production of abscisic acid and often resulting in seeds which germinate prematurely (McCarty, 1995). Singh et al., (2003), reported ten viviparous mutants of maize namely vp2, vp5, w3, y3, vp7, vp9, and y9 which were lacking in de novo ABA synthesis carotenoid precursors. The vp9 mutant of maize and the non-dormant-1 (nd-1) mutant of sunflower have mutations in the zeta-carotene desaturase gene (ZDS; Conti et al., 2004). In mature maize embryo, the Vp7/Ps1 gene encoding a lycopene-b-cyclase, necessary for the accumulation of both ABA and carotenoid zeaxanthin but the mutant lacking this gene produced pink kernels owing to the accumulation of lycopene (Singh et al., 2003). These mutants having defects in carotenoid precursor synthesis showed pleiotropic effects, like seedlings which were albino or light yellow, nonviable and vivipary which was result of deficiencies in carotenoids and ABA.

Carotenoid plays the role in precursors of the hormone ABA in addition to their role as accessory pigments in photosynthesis and photoprotectors that reduce photo-oxidative damage. After analyzing the mutant and transgenic plants Bewley, (1997) found that ABA synthesis and their responses to this phytohormone were strongly linked to the induction and dormancy improvement as well as the control of PHS. Mutant seeds of tomato (sitiens), Arabidopsis (aba), and maize (fluridone which is carotenoid biosynthesis inhibitor) which were ABA-deficient, germinate rapidly in water and often revealed vivipary (Groot and Karssen, 1992; Fong et al., 1983; Leon-Kloosterziel et al., 1996a).

Pleiotropic gene, *Os07g11020* that affects abscisic acid, flavonoid synthesis, and map-based cloning regulated the association between seed dormancy and pericarp color in weedy red rice (Gu et al., 2011). *Os07g11020* was characterized as Rc because of its qualitatively distinctive red color pericarp (Sweeney et al., 2006; Furukawa et al., 2007) and the quantitative attribute such as polygenes governing seed dormancy (SD7-1). Red pericarp color is connected with seed dormancy, which is aided by carotenoid concentration in the seed coat and acts as a precursor for β-carotenoid synthesis and, indirectly, ABA production, resulting in robust seed dormancy (Rodriguez-Concepcion et al., 2001). From tropical to temperate climates it was to be found that *Oryza* spp. known as a weedy red rice that competes with cultivated rice (*Oryza sativa* L. and *O. glaberrima* Steud) (Delouche et al., 2007). Red rice, which had a red pericarp color, is the most persistent species of weedy rice. Red rice has a high level of seed dormancy (Noldin et al., 2006). In red rice, genetic studies revealed associated between pericarp color to seed dormancy (Gu et al., 2005a). In wheat, this association was originally identified, where dormancy of red grain genotypes were more than white grain genotypes. This morphology helped to select pre-harvest sprouting resistance cultivars (Flintham, 2000).

Seed dormancy is an essential agronomic trait in cereal crops permitting many species seeds to stay dormant until the favorable conditions are there for germination. This entails a complex collection of physiological and biochemical processes that are regulated by innate seed dormancy as well as a number of external environmental stimuli (Finkelstein et al., 2008). The plant hormones abscisic acid (ABA) and gibberellic acid (GA) are the primary endogenous regulators that control seed dormancy and germination in a variety of plant species (Chen et al., 2020). Genetic factors affecting germination influence the degree of seed dormancy (Gu et al., 2003). Considering the significance of PHS resistance and characterization, the current study aimed to elucidate the physiological processes governing PHS resistance in rice genotypes.

MATERIAL AND METHODS

Plant material

A diverse group of 96 rice accessions from the Gene Bank of the ICAR-National Rice Research Institute, Cuttack, were used. Of these 96 genotypes, 16 contrasting genotypes were selected to study the influence of pericarp colour on PHS resistance. A field experiment was conducted during *kharif* 2021 and *kharif* 2022, in the ICAR-NRRI research farm (W4 block)and the average values of the two years' data are presented. Rice genotypes were laid out in an alpha lattice design with three replications. (Details of the genotype are provided in the Table 1).

Evaluation of rice genotypes for vivipary/PHS

A standardized *invitro* methodology (Hanjagi et al.,

Table 1. Rice genotype accession number along with their Vernacular name and code.

Rice accession number along with their Vernacular name and code	
Accession	Accession Code and vernacular name
IC-256580	PB-68
AC-35090	HT-81
BUDIDHAN	BUDIDHAN
MAHULATA	MAHULATA
IC-256559	PB-47
IC-256577	PB-65
IC-256771	PB-259
IC-256562	PB-50(1)
ANNADA	ANNADA
BALAM	BALAMA
CR DHAN-312	CR-312
GEETANJALI	GEETANJALI
IC-256575	PB-63
IC-256563	PB-51
BHAGAWATI	BHAGABATI
COTTONDORA SANNALU	NHN-288

2022) was used to evaluate rice genotypes for PHS. As depicted in the Fig. 1, at 20, 25, 30, 35, and 40 days after flowering (DAF), five panicles from each genotype were collected and sandwiched between two wet blotting sheets placed in 25 × 25 cm aluminum trays, and incubated for six days with a 12/12 photoperiod at 28°C. The optimal moisture content was maintained throughout the duration of treatment. To determine the PHS, the viviparity (number of grains germinated per panicle) and total number of grains were recorded at 20, 25, 30, 35, and 40 DAF and represented as a percentage of germination.

$$\text{Germination percentage} = \frac{\text{Total grains germinated in the panicle}}{\text{Total grains presented in the panicle}} \times 100$$

Quantification of carotenoid content (mg g^{-1}fw)

The calorimetric estimation of carotenoid concentration in leaves and seeds were measured by the modified form of the methodology published by Hiscox and Stam (1979). 300 mg of sliced leaf material and grinded seed powder was collected at the 20, 30, and 40 DAF stages, homogenised, and then immersed in 10 ml of pure dimethyl sulfoxide (DMSO) in covered test tubes. The sample and DMSO in test tubes were heated in a dry air oven at 60 - 65°C for three hours, while to quantify carotenoid content in the seed samples, they were separately boiled (1 hr) after heating. The carotenoid was determined by measuring the optical densities of the supernatant liquid at 480 and 510 nm using a Spectrophotometer (Make: Thermo-Evolution 300).

Pericarp color

Pericarp colour was visually graded as either red or white for each accession. Genotypes with red and white pericarp were assigned the values 1, 0.5 has assigned to the genotypes white but resistant and 0 to indicate resistance and susceptibility to PHS, respectively. Under a light microscope (Zeiss stemi 508, Germany) transverse sections of the seeds were taken and photographed to observe the pigmentation.

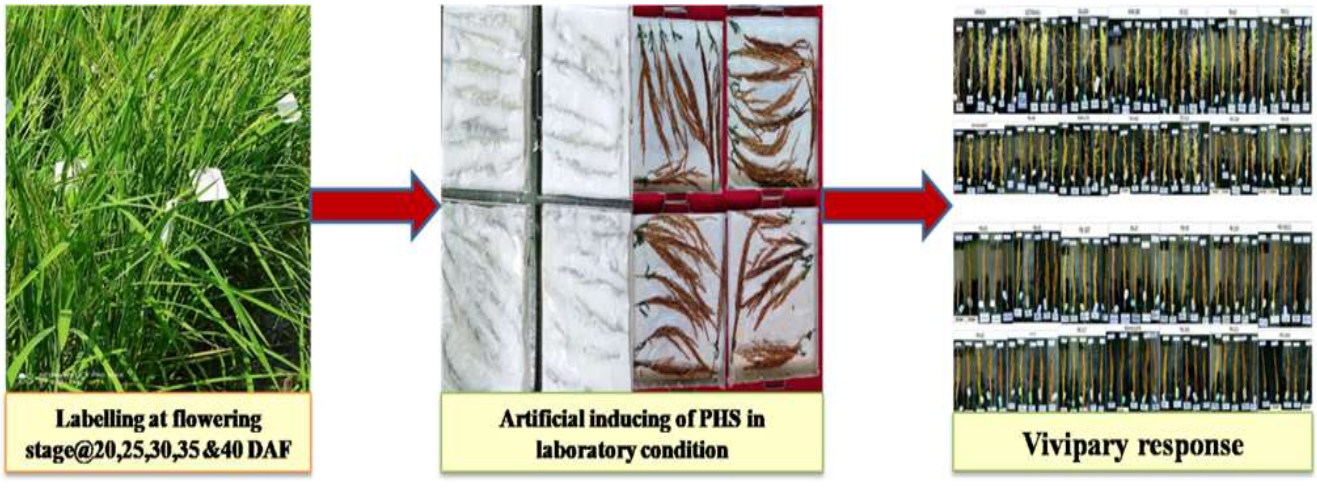

Fig. 1. depicts the laboratory methodo1ogy followed to screen rice genotypes for PHS resistance.

Evaluation of duration of dormancy for vivipary resistance genotypes

From harvest to the time when the germination attained the minimum Seed Certification Standard (80%) per ISTA, 1985, germination and day of germination were regularly assessed at 10-day intervals. The time from harvest to 80% germination in each genotype was used to determine the dormancy duration. Following the guidelines established by ISTA 1976, the 12 rice genotypes were divided into four groups according to the duration of dormancy: weak (0-10 DAH, days after harvest), moderate (10-20 DAH), strong (20-30 DAH), and very strong (30-40 DAH).

Statistical analysis

Advanced applications were used for the analysis of data (R Studio 4.2.2, Past 4.0 project and Blue sky statistics).

RESULTS AND DISCUSSIONS

PHS is a quantitative characteristic, and multiple factors, including seed dormancy, may contribute to PHS resistance. Temperature and moisture levels throughout the ripening stage may also influence the formation of PHS. Further more, Godinez-Palma et al. (2013) reported that environmental conditions during seed development have a substantial influence on seed dormancy and Gu et al. (2015) revealed that the trait's broad-sense heritability of seed germination on harvest day was low. As a result, repeated trials under diverse situations are crucial for improving PHS resistance estimate accuracy. Most accessions between 20 and 30 DAF had significantly decreased PHS resistance. This could imply that a lack of germination promoters in immature seeds, such as ABA regulators, was the reason for the low number of seeds that germinated at 20 DAF (Nakamura et al., 2011) rather than PHS resistance. Greenish seed coats were commonly detected in the 20 DAF group, which might indicate seed immaturity. In rice, Seed dormancy and pre-harvest sprouting resistance is an important physiological trait and also a complex phenomenon underlying mechanism of plants to delaying germination till optimal conditions are there for survival. Red rice has strong seed dormancy (Noldin et al., 2006). Genetic study has connected pericarp color to seed dormancy in red rice (Gu et al., 2005a). In our findings also observed that red pericarp color has showed strong dormancy there by PHS resistance. Here are the findings that were collected and discussed.

Phenotypic evaluation of pre harvest sprouting

PHS/Vivipary was evaluated at 20, 25, 30, 35 and 40 DAF. At 20 and 25 DAF less germination was observed in the susceptible genotypes. Annada, Balam and PB-63, CR Dhan-312 showed highest germination among all the susceptible genotypes across all the durations (20-40 DAF).While not a single grain was sprouted in the resistant genotypes between 20 and 40 DAF. Genotypes exhibiting 0% germination throughout all stages were classified as resistant genotypes and clustered together as shown in Fig. 2. Whereas PB-51, Geetanjali, NHN-288, and Bhagawati all exhibited germination percentages varied from 4 to 49% between 20 DAF and 40 DAF, annada showed the highest (22-86%) among susceptible genotypes within the same time intervals (20-40 DAF) followed by remaining all susceptible genotypes. Consistent with the results of Ju et al. (2000), we found that viviparity was more common between 30 and 40 DAF than at an early stage. As observed by Chen et al., 2020, 12 of the 96 genotypes evaluated for PHS did not germinate at any of the 5 phases over the course of two years from our study.

The chance of a getting significant variation in PHS between rice ecotypes is high because, other than the natural differences in dormancy that would be projected from wild progenitor populations which have tendency to adapting different environments, the effect of domestication on PHS in *indica* and *japonica* would have been different also because of the intensity of artificial selective pressure differ significantly between these two populations (Huang et al., 2012; Zhu et al., 2007). Interestingly, our findings corroborate with those of Lee et al., 2021. They had also screened few genotypes for PHS out of them four japonica genotypes were shown to have higher resistance to PHS, whereas the other genotypes exhibited viviparity.

Quantification of carotenoid content (mg g^{-1} FW)

Carotenoid content was estimated in the leaves and seeds at the stage of 20, 30 and 40 DAF and it was observed that during initial flowering stage carotenoid content was found increased in seeds and leaves [0.81

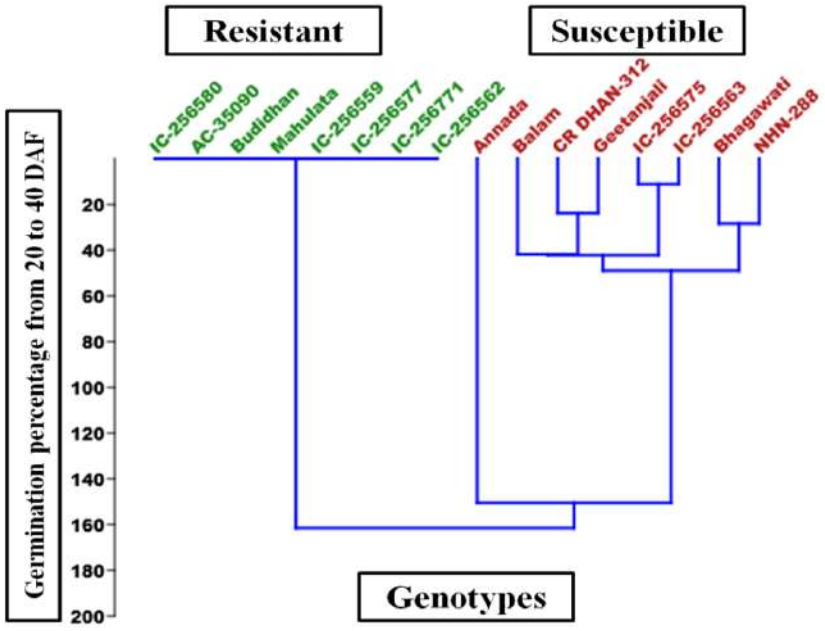

Fig. 2. Manhattan clustering analysis (UPGMA) representing the genotypes resistant and susceptible based on PHS percentage from 20 to 40 DAF.

to 1.29 in leaves; 0.77 to 0.91 in seeds (Fig. 3)]. Among the 8 resistant genotypes, the carotenoid content was in the descending order of PB-68> PB-47>HT-81> PB-65>Mahulata>Budidhan> PB-50(1)> PB-259. Whereas susceptible genotypes exhibited much lower amount of carotenoid content than the resistant genotypes. At 30 and 40 DAF, PB-68, HT-81 and PB-47, Mahulata recorded highest carotenoid content than remaining

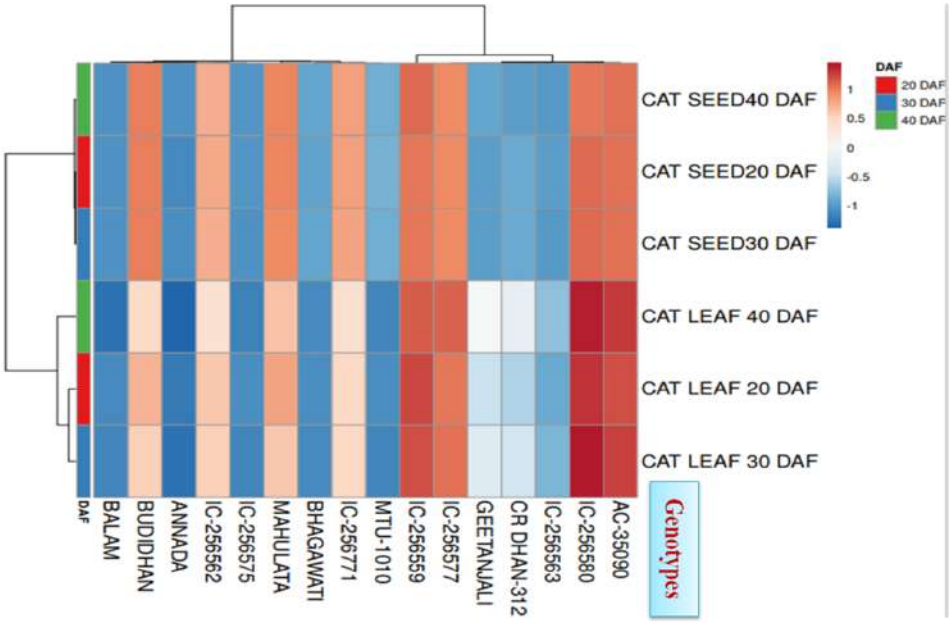

Fig. 3. Carotenoid content in leaf and seed from 20 to 40 DAF (red color representing the resistant genotypes; blue color-susceptible genotypes while dark red color indicates more carotenoid and dark blue color indicates the less carotenoid content).

resistant genotypes. While Annada, Balam, PB-63 showed less carotenoid content in both leaves and seeds at 30 and 40 DAF. The range of carotenoid content in leaves varied from 0.77 to 1.18 while in seed, it was varied from 0.67 to 0.81 mg of carotenoid in both the stages.

Agrawal et al. (2001) had observed that photosynthetic pigments like chlorophyll and carotenoid content were found in higher concentrations in PHS resistant genotypes than the PHS susceptible genotypes. Carotenoid was detected at all stages in both the leaves and the seeds, with higher values in resistant genotypes and much lower values in susceptible ones; nevertheless, early in the flowering stage, when the percent germination was lowest, carotenoids were highest in susceptible genotypes. Similar findings were observed by Fang et al. (2008), where he indicated that carotenoids not only act as a photosynthesis pigments and photoprotectors preventing photo-oxidative damage, but also precursors of the hormone ABA which regulates PHS through dormancy.

Qualitative analysis of pericarp/ seed coat color

The pericarp color and pigmentation of seeds was examined in 16 contrasting genotypes. Based on microscopic examination score of 1 was assigned to genotypes having red colour while score of 0 was assigned to genotypes with white pericarp colour (Fig. 4). To differentiate PHS resistant genotype Budidhan with white pericarp colour, a score of 0.5 was given. Red grain colour in cereal crops has been linked to seed dormancy for over a century. Our findings also showed that out of a total of 8 resistant genotypes, 7 had a red pericarp seed coat which is supports earlier finding (Fig. 5). One resistant genotype IC-256797 had moderately pigmented pericarp but had thicker pericarp, which could have acted as a physical barrier for the seed imbibition thereby imparting PHS resistance in this genotype. In contrast to resistant genotypes, the pericarp of susceptible genotypes was white, and observed no physical barrier between the seed and its water imbibitions. As the red pericarp aided in the production of carotenoid content, this in turn might have aided in the synthesis of ABA. As these results corroborated with the findings of Gu et al., 2011, it can be concluded that red grain colour and seed dormancy have been linked for a many years, which might be having qSD7-1/qPC7 cluster of QTLs which are known to affect seed dormancy/pericarp colour in red rice.

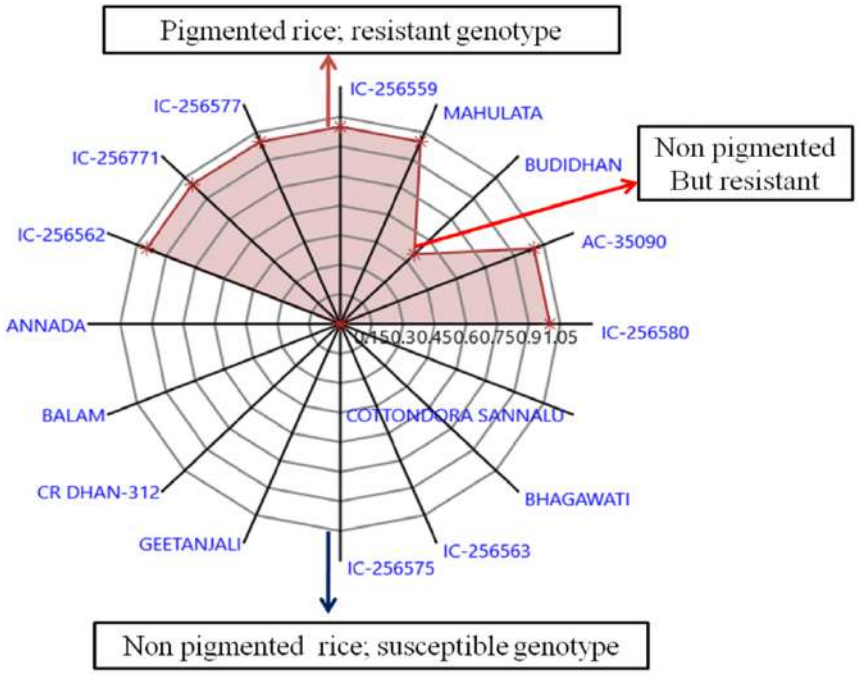

Fig. 4. Radar plot differencing the genotypes based on pigmentation white (susceptible) and red (resistant).

Evaluation of duration of dormancy for vivipary resistant genotypes

Resistant genotypes were evaluated for the duration of dormancy by recording the germination of seedlings at 10 days interval up to entries reached 80% germination. Genotypes had varied duration of dormancy and were grouped as weak, moderate, strong and very strong duration of dormancy based on the days taken to germinate (Table 2).

Recently, Scientists interests has been increased in the area of dormancy research because the production issues are connected with dormancy which is either short- or long-term and a significant portion has been devoted to studies on cereal grains. Hence, optimal dormancy is one of the important traits considered in rice breeding. If dormancy is too high, it may affect use of rice seeds for next season planting subsequently resulting in establishment of poor crop. On the other hand, seed quality is affected if dormancy is too low, such seeds will be prone to pre/post-harvest sprouting thereby (Gubler et al., 2005). Thomson et al., 2003 had detected QTLs and fine mapped genes could be used for crop improvement program in the PHS resistance. In the present work, 12 genotypes were evaluated for duration of dormancy and observed degree of dormancy ranging from 10 to 40 DAH. Based on the findings from the present study, 12 PHS resistant genotypes could be used as donors in breeding programmes for developing PHS resistance rice genotypes.

Table 2. classification of genotypes based on duration of dormancy.

	Duration of dormancy of PHS resistant genotypes			
S. no.	Weak (10 DAH)	Moderate (20 DAH)	Strong (30 DAH)	Very strong (40 DAH)
1	BUDIDHAN	Ambika (NHN-279)	MAHULATA	PB-68 (IC-256580)
2		PB-47 (IC-256559)	HT-20 (AC-34975)	(HT-81) AC-35090
3		PB-65 (IC-256577)	PB-285 (IC-256797)	PB-50 (1) IC-256562
4		PB-259 (IC-256771)		HT-86 (AC-35096)

Correlation analysis with PHS/vivipary, carotenoid content and pericarp colour

The association between germination percentage, carotenoid content and pericarp colour was studied using correlation analysis. The analysis as shown in Fig. 6 illustrates that vivipary germination is negatively correlated with carotenoid content and pericarp colour at all the durations of flowering which strongly states that carotenoid content and pericarp color are preventing viviparous germination in the resistant rice genotypes. Carotenoid content in leaf and seed at 20, 30 and 40 DAF were significantly correlated with carotenoid content in seed and pericarp color. In the correlation Fig. 6, positive correlation is represented by blue color line while negative correlation was represented by red colour line and thickness indicates the level of correlation.

Fig. 5. (A) lane is showing that HT-81, Mahulata, PB-50(1), PB-68, PB-259, PB-266, PB-285, Budidhan- Resistant genotypes, **(B)** Lane is showing that Annada, Balam, Bhagabati, CR-312, Geetanjali, NHN-288,PB-51, PB-63-susceptible genotypes (supplemetary table).

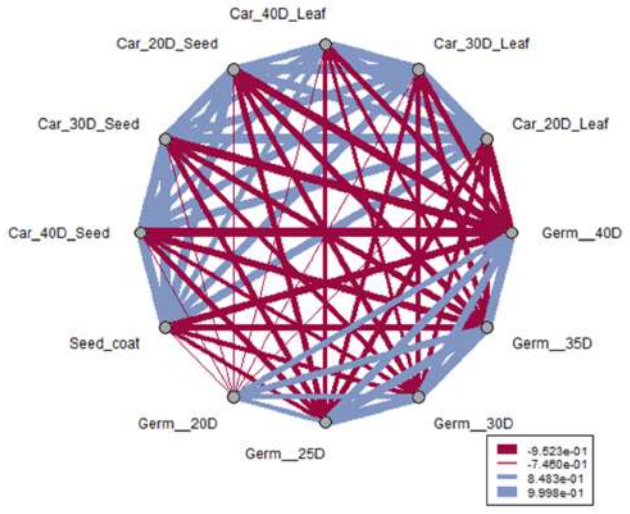

Fig. 6. Web plot is showing that correlation between the germination percentage, carotenoid content and seed coat/ pericarp colour from 20 to 40 DAF. (Legends: Germ; germination percentage 20 to 40, DAF; days after flowering, Car; carotenoid)

CONCLUSION

PHS/vivipary response in the 96 rice genotypes exhibited a wide range of variation. From the high through put screening methodology, 12 novel rice genotypes with very strong PHS resistance were found among a diverse set of genotypes with 0% germination from 20 to 40 DAF. In the 16 contrasting genotypes, 8 were resistant 8 were susceptible to PHS (Table 1). From the present study, it is very obvious that carotenoids not only plays role as a photosynthesis pigments and photoprotectors preventing photo-oxidative damage but also found to be the precursors of the hormone ABA there by regulating PHS by inducing dormancy. Genotypes with red pericarp had higher carotenoid content, which might have aided in the synthesis of ABA in turn imparting PHS resistance in rice genotypes. As these results corroborated with the findings of Gu et al., 2011, it can be concluded that red grain colour and seed dormancy have been linked for many years in regulating PHS resistance in cereals. We can conclude that red pericarp and high carotenoid content may have contributed in ABA production and thereby imparting PHS resistance via dormancy. To develop climate resilient rice varieties for changing climate, these novel genotypes (PB-68, HT-81, PB-50(1), HT-86, HT-20, Mahulata, PB-285, PB-47, NHN-279, PB-65, PB-259 and Budidhan) of rice which had very high levels of PHS resistance can be employed in the breeding programs for developing PHS resistant genotypes.

In terms of a conflict of interest: The authors stated that they do not have any conflicts of interest.

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Ion exclusion, osmoregulation and management of oxidative stress improve salt tolerance in rice at seedling stage

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ABSTRACT

Excess ion accumulation disturbs ionic homeostasis, creates an osmotic imbalance, and generates oxidative stress in plants under salinity stress. In the present experiment, the effect of salt stress at the seedling stage on the osmotic equilibrium and ROS scavenging potential was evaluated in ten differentially salt-sensitive rice genotypes. For this, the plants were grown hydroponically and salt stress equivalent to 12 dS m^{-1} was imposed at 3-4 leaf stages. The results showed that a few genotypes like FL478, AC41585, and AC39416A were able to maintain a lower Na^+/K^+ ratio in the leaf and thus proved more tolerant to salt stress than others. Additionally, these genotypes produced greater organic osmolytes (proline, glycine betaine, trehalose) and also had higher activities of key antioxidant enzymes (superoxide dismutase, catalase, peroxidase). On the contrary, Rashpanjor and CSR27 showed lesser ionic discrimination (higher leaf Na^+/K^+ ratio) but a moderate degree of salt tolerance, perhaps using Na^+ effectively as an inorganic osmoticum to overcome stress. The susceptible genotypes like IR29 and Sabita were found extremely poor in restricting the upward movement of Na^+, as well as the management of oxidative stress under saline conditions. From this study, we conclude that an efficient reactive oxygen species scavenging system along with greater osmotolerance helps to render salt tolerance at the seedling stage in rice.

Key words: *Antioxidant enzymes, ionic stress, osmolytes, reactive oxygen species, salinity*

INTRODUCTION

The rise in mean sea level and faulty irrigation practices are two major factors causing increased salinization of arable lands. Throughout the world, more than 1700 million hectares of land are salt-affected of which the highest area belongs to Asia and Australia (Negacz et al., 2022). Presently, 6% of the world's and 6.72 million hectares of land in India alone is salt-affected (Arora and Sharma, 2017). Out of that, nearly 70% of the salinity-affected area belongs to the coastal region due to the penetration of the sea into the land (Pathak et al., 2021). This invasion causes great crop loss and mostly rice is affected, as it is mono cropped popularly in the deltas and coastal plains during the wet season. Rice can thrive under salt stress up to 3-4 dS m^{-1}, beyond which its yield is severely affected (Gao et al., 2007). Mostly the early seedling (3-4 leaf stage) and reproductive (booting) stages are more sensitive to salt stress in rice (Chakraborty et al., 2019).

Excessive salt concentration severely affects the physiological and metabolic activities of the plant. Initially, changes in plant water status cause an osmotic imbalance which at later stages leads to ionic stress due to the high accumulation of toxic ions especially, Na^+ and Cl^- (Dash and Panda, 2001). Primarily to overcome the osmotic stress plant produces different compatible osmolytes like proline, trehalose, and glycine

betaine (GB) (Flowers and Colmer, 2008). Rapid accumulation of proline in the rice leaves and grains was noticed to maintain the osmotic adjustment in drought and salinity (Bohnert et al., 1995). Similarly, an increment in GB and trehalose was also seen to have a beneficial impact on balancing the osmotic potential (Chakraborty and Sairam, 2018). Subsequently, when the salt concentration in the rhizosphere exceeds the tolerable limit, plants adopt different osmoregulation and ion exclusion strategies to maintain a favourable Na^+ to K^+ ratio in actively growing parts. Plants may flush out the excess Na^+ from the xylem stream or may sequester some portion of Na^+ as an osmolyte in its vacuole to maintain ionic homeostasis based on their tissue tolerance potential (Fukuda et al., 2011; Chakraborty et al., 2016; 2020).

In response to salinity cascade of biochemical reactions takes place inside the cell. Production of harmful reactive oxygen species (ROS) in chloroplast and mitochondria due to the leakage in the electron transport chain under stress is one of the major concerns (Pradhan et al., 2019). Higher concentrations of ROS ($O_2^{\cdot-}$, H_2O_2, $^1O_2$, and $OH^{\cdot-}$) in the cell chiefly reduce the net photosynthesis by up to 50% and causes denaturation of proteins, destruction of DNA, and peroxidation of membrane lipids (Mittler et al., 2010). Hence detoxification of ROS is extremely important to overcome oxidative damage. Plants usually follow a well-coordinated antioxidant enzyme-mediated scavenging strategy to control the harmful ROS concentration inside the cell (Chakraborty et al., 2015). Enzymes like superoxide dismutase (SOD; EC1.15.1.1), Catalase (CAT; EC 1.11.1.6), Peroxidase (POX; EC 1.11.1.7), and some non-enzymes antioxidants like ascorbate and reduced glutathione play a major role in ROS scavenging cascade (Apse and Blumward, 2002). SOD is the major scavenger of $O_2^{\cdot-}$, whereas, both CAT and POX decompose the H_2O_2 into H_2O. However superior ROS scavenging system alone might not be sufficient for imparting salinity tolerance (Munns and Tester, 2008). Therefore, it is evident that genotypes having better osmoregulation and ROS scavenging strategy is likely to handle salinity stress more efficiently than others. In the present study, we took ten differentially salt-sensitive rice genotypes to study their osmoregulation and ROS scavenging potential under salt and finally to correlate these attributes with overall salinity tolerance.

MATERIAL AND METHOD

Plant materials and experimental condition

Ten differentially salt sensitive rice genotypes (FL478, AC41585, AC39416A, Sadri, Rashpanjor, CSR27, Binadhan8, Luna Suvarna, Sabita and IR29) were grown hydroponically in the net house of ICAR-NRRI, Cuttack in the year of 2021. For this experiment, the seeds were sown onto the suspended styrofoam panel of 10×10 and one seed in each hole was placed on the trays filled with Yosidha nutrient solution (pH of 5.0 ± 0.25) (Gregorio et al., 1997). The plants were maintained in this condition until they attained desired growth (*i.e*., 3-4 leaf) stage. After attaining a desirable vigour, one set of plants was allowed to grow normally in the Yosidha solution (control) and another set was grown in the above said condition but imposed with EC (electrical conductance) of 6 dS m^{-1} of (adding NaCl) salt stress initially. After two days, the same set of treated plants were imposed with 12 dS m^{-1} (equivalent to 110 mM of NaCl) of salt stress and maintained until at least half of the IR29 (universal susceptible check for salinity) (Gregario et al., 1997; Chakraborty et al., 2020) plants got an SES score of 9.

Standard evaluation score, plant vigour, biomass and Na^+/K^+ ratio

A standard protocol developed by IRRI was used for standard evaluation score (SES) scoring (Gregario et al., 1997). A SES score of 1 to 9 was given to all treated plant based on visual salt injury. Plants with no damage were scored 1 and considered highly tolerant whereas, a plant with severe damage scored 9 and considered highly susceptible and the plants in between were scored as 3, 5 and 7 based on the severity of the symptoms. The plant vigour (total plant length) and biomass were measured from each genotype × treatment at the end of salt treatment. The plants were taken out of the solution and the length of the plant was measured by a scale. The total dry biomass was estimated from the above said samples after keeping them in the hot air oven for 7-8 days at 80 °C until they attained a constant dry weight. Shoot portions of the same samples were used to measure Na^+ and K^+ concentrations individually using Flame Photometer (PFP7, Jenway, Cole Palmer, UK), and Na^+/K^+ ratio

was calculated (Mohanty et al., 2023).

Estimation of relative water content and organic solutes

The relative water content (RWC) of 2nd leaf from each genotype × treatment combination was estimated after 5 days of imposition of salt stress and data was presented as the mean of three independent biological replications Chakraborty et al., (2020) using the following formula.

$$RWC = [(Fresh\ wt. - Dry\ wt.)/ (Turgid\ wt.-Dry\ wt.)] \times 100$$

To estimate the proline concentration, 500 mg of fresh leaf (fully expanded 2nd leaf) samples of each genotype × treatment combinations, were taken after five days of the imposition of stress and crushed with 10 ml of 3% sulfosalicylic acid (Bates et al., 1973). The absorbance was taken at 520 nm of the upper toluene layer, and quantification was done by using the proline standard curve and expressed as mg g^{-1} FW of proline. Glycine betaine (GB) was estimated from the dried 2nd leaf tissues (0.5 g of leaf sample) from each genotype × treatment combinations and spectrophotometric observation was taken at 365 nm by following the protocol of Grieve and Grattan (1983). The amount of trehalose was estimated from 10 mg oven-dried leaf tissues and absorbance was taken at 620 nm following the method proposed by Ferreira (1997).

Estimation of hydrogen peroxide (H_2O_2) and lipid peroxidation

The H_2O_2 concentration was measured from the fresh leaf (200 mg of fresh leaves from 2nd leaf) tissues after five days of the imposition of salt stress by following the method described in Sarkar et al. (2013). The absorbance was taken at 410 nm and the H_2O_2 content was estimated from the standard curve. The MDA content was estimated from 500 mg fresh leaf (2nd leaf) samples from each genotype × treatment combination after 5 days of the imposition of stress by following the protocol of Heath and Packer (1968). The spectrophotometric observation was taken at 532 nm and 600 nm. The calculation was done by using the extinction coefficient of 155 mM^{-1} cm^{-1}.

Antioxidant enzyme assay and protein determination

The activities of three antioxidant enzymes (SOD, CAT, and POX) were estimated spectrophotometrically from the 2nd fully expanded leaf after 5 days of the imposition of salt stress following the protocols as described in Chakraborty et al. (2014). Extraction was done from fresh leaf samples (500 mg) by using 0.1 mM phosphate buffer (pH 7.5) containing 0.5 mM EDTA. Estimation of SOD (EC 1.15.1.1) was done by taking the absorbance at 560 nm. Catalase (EC 1.11.1.6) activity was measured by taking absorbance at 240 nm. The assay of Peroxidase (POX, EC 1.11.1.7) was done by measuring the increase in absorbance at 470 nm caused by guaiacol oxidation.

Statistical analysis

The experiment was conducted following a completely randomized design and the data were subjected to one-way factorial ANOVA. A post hoc analysis for pair-wise comparison of treatment × genotype combinations was performed by Tukey's multiple comparison tests using GraphPad Prism 9.5 software.

RESULT AND DISCUSSION

Effect of salt stress on plant biomass and vigour

Salinity brings severe damage to plants' health and restrains growth and development. It causes a high degree of mortality due to poor crop establishment at the early growth stage and severe yield loss in the later stages. Hence genotype with a greater ability to retain vigour and biomass under stress showed better salt-tolerance ability (Hu et al., 2012). In this present study each of the genotypes was scored based on visual salt injury expressed interms of SES score and the results revealed that after seven days of imposition of salt stress, IR29 was the first genotype to get SES the score of 9 and thus, was the most susceptible one among tested genotypes in response to salt stress followed by Sabita (7) and Sadri (7). With a lesser degree of damage, genotypes like Rashpanjor, CSR27, and Binadhan 8 were given a score of 5; while, FL478, AC41585 and AC39416A were scored 3, indicating their higher salt-tolerance potential against 12 dS m^{-1} of salt stress (Table I). In accordance with the SES score, a similar kind of response was observed in plant vigour and biomass accumulation. Least reduction in

shoot length (<10%) was recorded in FL478 followed by AC39416A (12.6%) as compared to the control. On the contrary, the shoot growth was highly compromised (~30% reduction as compared to control) in Sabita and IR29 under stress (Table I). A significant reduction was observed in the dry biomass accumulation in all the genotypes under salt stress, but the highest reduction was observed in IR29 (>50%), while it was ~30% Rashpanjor, CSR27, Binadhan 8 and Luna Suvarna and <20% in FL478 (Table I). Therefore, it is quite evident that greater initial vigour and higher dry matter retention ability under stress gave an added advantage to the tolerant and moderately tolerant genotypes to sustain the salt stress as reported previously by Ali et al. (2014).

Ionic homeostasis and tissue Na^+/K^+ ratio

Excessive uptake of Na^+ competes with the K^+ ion and creates ionic toxicity, which disturbs essential cellular functions (Adams and Shins, 2014). Glycophytes that can channel a lesser amount of Na^+ and more K^+ to the upper parts are considered to be tolerant by maintaining a lower ratio Na^+/K^+ ratio. Hence, the Na^+/K^+ ratio is considered to be one of the major factors in determining tolerance to salt stress (Munns, 2002). In the present study, the lowest Na^+/K^+ ratio was observed in the shoot of FL478 (0.38) followed by AC41585 (0.40) and AC39416A (0.45) (Table 1). While in moderately tolerant genotypes like Rashpanjor and CSR27 the Na^+/K^+ ratio was about 0.70 followed by Biandhan 8 (0.80) and Luna Suvarna (0.98). Contrastingly, in susceptible genotypes like IR29 and Sabita, much higher Na^+/K^+ ratio of 2.01 and 1.38 respectively, in the shoot was reported. This could probably be due to the higher uptake of Na^+ as compared to K^+, which hampered the ionic equilibrium in these genotypes (Nakhoda et al., 2012; Chakraborty et al., 2020). The moderately tolerant genotypes (Rashpanjor and CSR27) were able to sustain under the salt stress despite having a considerably higher ratio of Na^+/K^+ in their leaf tissues, indicating the ability of vacuolar sequestration of excess Na^+ in these genotypes (Chakraborty et al., 2020).

Osmoregulation and plant water status under stress

The plant faces osmotic stress immediately after exposure to salinity due to the presence of excess solutes in the rhizosphere and a high concentration of Na^+ makes it difficult for the plants to draw water at the root zone. Hence maintaining optimum water content is considered one of the essential traits for salt tolerance (Uddin et al., 2016). In the present study, we found that tolerant genotypes like FL478 and AC41585 were able to maintain 86 and 81% RWC and were able to cope with the salinity much more efficiently than the susceptible genotype IR29, where the RWC dropped to 45% after 7 days of salt stress (12 dS m^{-1}) (Table 1). Besides maintaining a better plant water status, the synthesis of organic osmolytes like proline, trehalose and glycine betaine was found to maintain favourable osmotic potential between the cell and the surrounding (Shivakumar et al., 2001). In the present study, the highest production of proline was observed in the leaves

Table 1. Effect of salt stress (12 dS m^{-1}) on plant morphology and tissue ion content.

Genotype	Score	Plant DW (g)		Plant vigour (cm)		RWC		Na+/K+ ratio (Leaf)	
	Stress	Control	Stress	Control	Stress	Control	Stress	Control	Stress
FL478	3	0.297^{ef}	0.245^{gh}	21.4^{fgh}	19.6^{ghi}	93.33^{ab}	86.47^{bc}	0.186^{a}	0.383^{ab}
IR29	9	0.209^{i}	0.095^{l}	21.8^{fgh}	15.6^{i}	91.63^{ab}	45.88^{g}	0.193^{a}	2.010^{f}
AC41585	3	0.341^{bc}	0.265^{g}	29.6^{ab}	25.6^{cdef}	90.10^{ab}	81.22^{cd}	0.194^{a}	0.406^{ab}
Sadri	7	0.309^{de}	0.180^{jk}	26.3^{bcde}	22.3^{efgh}	89.08^{ab}	70.33^{f}	0.175^{a}	1.014^{d}
AC39416A	3	0.376^{a}	0.276^{fg}	28.8^{abc}	25.2^{cdef}	92.63^{ab}	78.34^{de}	0.186^{a}	0.453^{b}
Rashpanjor	5	0.284^{fg}	0.195^{ij}	31.4^{a}	26.2^{bcde}	93.29^{ab}	77.69^{def}	0.177^{a}	0.726^{c}
CSR27	5	0.345^{b}	0.235^{h}	22.8^{efg}	20.5^{gh}	91.62^{ab}	77.91^{de}	0.171^{a}	0.791^{cd}
Binadhan 8	5	0.247^{gh}	0.168^{k}	24.9^{cdef}	18.3^{ih}	91.62^{ab}	80.84^{cd}	0.182^{a}	0.807^{cd}
Luna Suvarna	5	0.323^{cd}	0.208^{i}	28.6^{abc}	22.1^{efgh}	94.11^{a}	75.64^{def}	0.177^{a}	0.987^{d}
Sabita	7	0.352^{b}	0.207^{i}	27.3^{abcd}	19.1^{ih}	93.09^{ab}	71.21^{ef}	0.185^{a}	1.386^{c}

*Same lowercase letter(s) within a column indicates non-significant difference at $p<0.05$ level by Tukey's multiple comparison test.

of FL478 (24.48 mg g^{-1} FW). Surprisingly, in genotypes like Sabita and IR29, there was no significant increment in proline content was observed under salt stress. Similarly, about 60% increment in glycine betaine (GB) concentration was observed in tolerant genotypes (FL478, AC39416A and AC41585), whereas about 40% increase was recorded in moderately tolerant genotypes like Rashpanjor, Luna Suvarna and Binadhan 8 and the increase was lowest in IR29 (27%) (Fig. 1). Previous studies reported the higher amount of glycine betaine in the leaf might protect the photosynthetic apparatus (fluidity of thylakoid membrane) under drought and salt stress (Wang et al., 2010). Similarly, the trehalose content was found comparatively higher in genotypes like FL478, AC41585 and AC39416A under stress. However, most of the moderately tolerant genotypes like Rashpanjor, CSR27 and Binadhan 8 were able to maintain the optimum water status with minimal accumulation of organic osmoprotectants. The comparatively lower accumulation of organic osmolytes with a higher Na^+/K^+ ratio in the leaf indicates the ability of a few genotypes to use some portion of the excess Na^+ as cheap compatible osmolytes under stress (Solis et al., 2021).

Oxidative stress and ROS scavenging enzymes

Salinity causes oxidative damage to plants by producing reactive oxygen species (ROS) like H_2O_2, OH^- and O_2^- (Chakraborty et al., 2014). In rice hyper production of NaCl induces H_2O_2 and disturbs the membrane stability and hampers growth and development (Vaidyanathan et al., 2003). In the present study, H_2O_2 content and lipid peroxidation level was significantly increased upon imposition of salt stress as compared to control plants (Fig. 2A). The H_2O_2 content was highest in IR29 (18.17 µmol g^{-1} FW), followed by Luna Suvarna (14.94 µmol g^{-1} FW) and Sabita (14.01 µmol g^{-1} FW) showing the vulnerability of these genotypes to oxidative stress. We also estimated malondialdehyde (MDA) content, another good indicator of oxidative damage, produced during the process of membrane lipid peroxidation. Jain et al. (2001) reported that sensitive genotypes were observed to have more membrane disintegration and MDA concentration as compared to the tolerant genotypes. In our case increment in MDA content in the leaves of FL478 was not significant under salt stress (Fig. 2B). However significant induction was evident in AC41585, AC39416A, Rashpanjor, and CSR27 under 12 dS m^{-1} of salt stress. But the highest increment was recorded in the leaves of IR29 (~85%) followed by Sadri (~79%) and Sabita (72%).

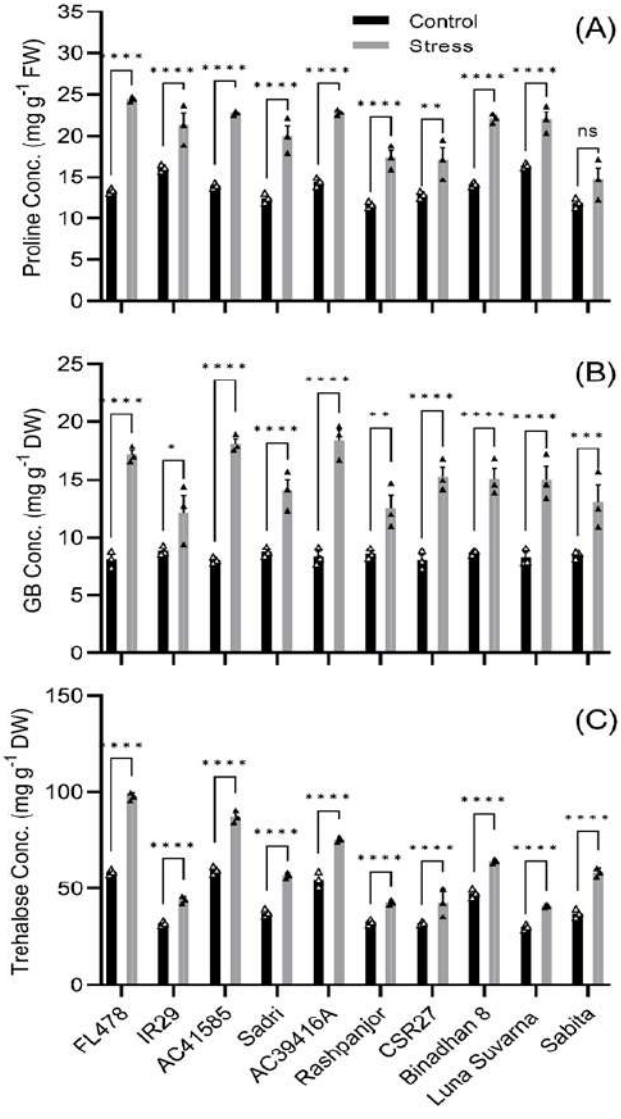

Fig. 1. Changes in Proline concentration (A), Glycine betaine concentration (B), and Trehalose concentration (C) of ten rice genotypes subjected to seven days period of salt stress (12dS m-1) at the seedling stage. Bars represented the mean (± SE) for three biological samples. Bars with asterisk '*, **, *** and ****' and lines are significantly different at $p < 0.05$, < 0.01, < 0.001, and < 0.0001 levels as compared to their respective control. 'ns' represents not significant.

Changes in activities of ROS scavenging enzymes under salt stress

Stress-induced hyperactivities of key antioxidant enzymes like SOD, CAT, and POX were reported to scavenge the ROS effectively in rice under salt stress (Wi et al., 2006). SOD acts as a first line of defense against the elevated level of ROS (especially superoxide radicals) and removes the superoxide radicals by reducing them into H_2O_2 and O_2 (Stepien and Klobus, 2005). In the present study, the increase in SOD activity under stress was highly significant in AC41585 at $p<0.001$ which was ~2.5 times as compared to the control. Besides, significant induction ($p<0.05$) was observed in both FL478 and AC39416A after five days of the imposition of salt stress, while there were no significant changes in SOD activity were noticed in the rest of the genotypes (Fig. 3A). According to Sairam et al. (2002), tolerant genotypes with higher activities of SOD can effectively protect them from oxidative stress. But on the other hand, CAT activity showed a significant level of increase in all the genotypes except for Binadhan 8 and Sabita (Fig. 3B). In FL478, AC41585, AC39416A, and Rashpanjor CAT activity was highly upregulated ($p<0.0001$) implying the effective detoxification of ROS (especially H_2O_2) under salt stress in these genotypes. On the contrary, lower CAT activities in other genotypes perhaps resulted in greater H_2O_2 accumulation that led to severe lipid peroxidation and membrane injury under salt stress (Turkana and Demiral, 2009). Similarly, the POX activity was significantly induced ($p<0.0001$) in a few tolerant genotypes (FL478, AC41585, and AC39416A), while moderate upregulation ($p<0.05$) was noticed in genotypes like Rashpanjor, CSR27 and Binadhan 8 (Fig. 3C). But, the POX activity under salt stress, was not significantly induced in the susceptible genotypes (IR29 and Sabita). From the present study, it is very clear that higher induction of POX and other antioxidant enzymes improved ROS scavenging potential and played a key role in stress tolerance in these genotypes.

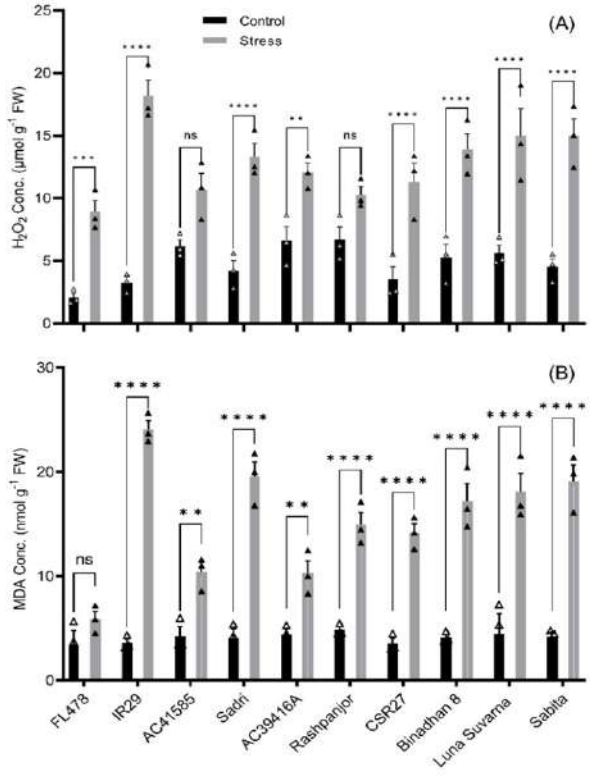

Fig. 2. Changes in H_2O_2 concentration (A) and MDA concentration (B) of ten rice genotypes subjected to seven days period of salt stress (12dS m^{-1}) at the seedling stage. Bars represented the mean (± SE) for three biological samples. Bars with asterisk '*, **, *** and ****' and lines are significantly different at $p<0.05$, <0.01, <0.001, and <0.0001 levels as compared to their respective control. 'ns' represents not significant.

Correlation studies

From the correlation analysis (Table 2), we found a

Table 2. Correlation between some physiological and biochemical parameters of 10 genotypes under salt stress.

Parameters	DW	Plant vigour	Na+/K+ (Leaf)	CAT	SOD	POX	H_2O_2
Plant vigour	0.734**						
Na^+/K^+ (shoot)	-0.816**	-0.699**					
CAT	-0.272	-0.217	0.125				
SOD	-0.709**	-0.632**	0.778**	0.297			
POX	-0.037	0.157	-0.197	0.584**	-0.125		
H_2O_2	-0.751**	-0.548**	0.895**	-0.428*	-0.738**	-0.138	
MDA	-0.803**	-0.606**	0.925**	-0.275	-0.650**	-0.124	0.929**

*Significant at $p<0.05$. ** Significant at $p<0.01$

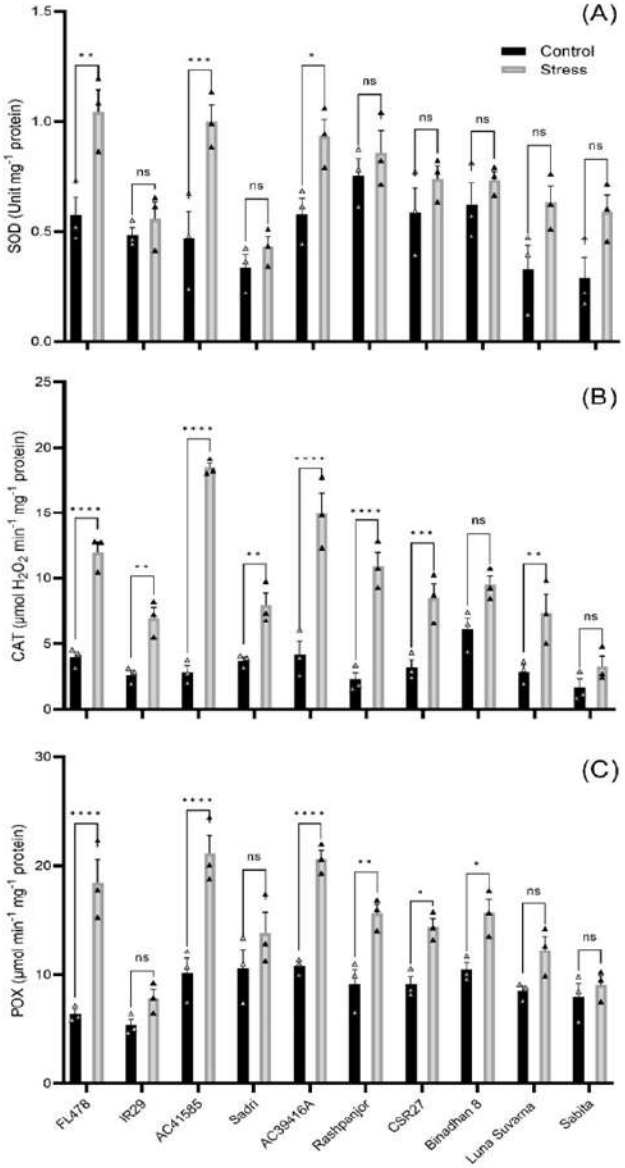

Fig. 3. Changes in Superoxide dismutase (A), Catalase (B), and Peroxidase activity (C) of ten rice genotypes subjected to seven days period of salt stress (12 dS m^{-1}) at the seedling stage. Bars represented the mean (± SE) for three biological samples. Bars with asterisk '*, **, *** and ****' and lines are significantly different at p <0.05, <0.01, <0.001, and <0.0001 levels as compared to their respective control. 'ns' represents not significant.

strong negative correlation between shoot Na^+/K^+ ratio with plant dry weight and vigour, indicating a high Na^+/K^+ ratio in the shoot is detrimental to plant health and vigour. A significant positive correlation between Na^+/K^+, MDA, and H2O2 production indicates excessive Na+ absorption induced the H_2O_2 level and caused membrane lipid peroxidation as reported previously by Sarkar et al. (2013). A negative correlation between MDA level with all the anti-oxidant enzymes (CAT, SOD, and POX) suggested the association of an efficient enzyme-driven ROS scavenging mechanism with salt tolerance in rice.

CONCLUSION

From the above study, it can be concluded that there was enough variability in salt tolerance potential in the studied genotypes. A few genotypes *viz*., FL478, AC41585, and AC39416A were found more tolerant to seedling stage salt stress (12 dS m-1) owing to their greater ionic and osmoregulation potential. Other genotypes like Rashpanjor and CSR27 with comparatively lesser ionic discrimination ability, were found to use Na^+ as osmoticum and hence can maintain a fair osmotic equilibrium with lesser organic osmolyte production. On the contrary, susceptible genotypes like IR29 and Sabita were found poor in both aspects and thus succumb to stress. Besides, we observed ROS scavenging ability is also a critical factor in oxidative homeostasis and salt tolerance in rice. The genotypes with a more efficient ROS scavenging system were found more tolerant to salt stress.

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A novel insecticide seed treatment formulation (Chlorantraniliprole 625 g/L FS) for yellow stem borer and leaf folder management in rice

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ABSTRACT

The potential yield loss of 15-25% rice production is noticed due to different pest infestation in South Asian countries. This is due to the favorable climatic conditions for pests. It compels farmers to use a major chunk of pesticides to prevent/recover from pest attack. Hence, the field experiments were carried out to test the insecticide, Chlorantraniliprole 625 g/L FS as seed treatment formulation. It is evident from the studies undertaken during rabi 2018-19 and kharif 2018, that Chlorantraniliprole provided excellent control over yellow stem borer and leaf folder in conventional variety of rice (CR Dhan 304) and hybrid rice (28P67). Among the different doses, Chlorantraniliprole @ 75.0 g a.i/ha recorded significantly better control registering least dead heart of 5.41, 4.77, 3.43, 2.98% and 2.47% at 14, 21, 28, 35 and 42 days after transplanting, respectively. Similarly for Hybrid (28P67) trial the treatment recorded the least count of leaf folder with 0.57, 0.68, 0.74, 0.81 and 0.89 larvae/hill during the intervals of 14, 21, 28, 35 and 42 days after transplanting, respectively and the highest yield (5.46 t/ha) was registered in treatment which was significantly superior over the other entire dose rates of Chlorantraniliprole and the market standard. Thus, from this study, Chlorantraniliprole @ 75 g a.i /ha can be recommended for controlling stem borer and leaf folder in paddy (both for conventional variety and hybrid).

Key words: *Seed treatment, yellow stem borer, leaf folder, paddy, Chlorantraniliprole 625 g/L FS*

INTRODUCTION

Rice pests are major bottlenecks in escalating the productivity of rice under all ecosystems in the tropics. Pest population changes both spatially and temporally over a period of time in rice. There are more than 100 insect species feeding on rice but few of these are considered to be the major pests. The number of major pests is increasing day by day. About one third of major problems in rice cultivations are related to rice pests (Herdt, 1991). At present number of major insect pests are 20 and major diseases are 10 based on the information generated from All India Coordinated Project on Rice between 1965 to 2017 (Jena et al., 2018). The major concern of the farmers is frequent occurrence of pests with increased severity in recent years. The mean yield reduction in rice had been estimated to vary between 21-51 per cent and out of which 10-15% yield reduction occurred due to the infestation of insect pests. Among the different insect pests, stem borer, plant hoppers, gall midge, leaf folder accounted for 30, 20, 15, 10% of the crop losses, respectively at national level (Krishnaiah and Varma, 2014).

Among the major insect pests, yellow stem borer (YSB), *Scirpophaga incertulas* (Walker) a monophagous pest is known to be most damaging pest in different rice ecosystems since it attack at seedling stage causing dead hearts & white ear-head symptoms during reproductive stage. Similarly rice leaf folder, *Cnaphalocrocis medinalis* (Guenee) has recently emerged as an important major pest status in Asian countries. As there is no holistic approach to get rid of these two pests either through a resistant variety or through certain biological agents. Hence, the use of

insecticides becomes unavoidable to control these pests. In India, Central Insecticide Board and Registration Committee has recommended more than 90 pesticides or combination product to tackle wide range of pest problems, but not even a single insecticide seed treatment formulation is recommended against rice insect pests. Always there exists a thrust to search for a viable and cost-effective alternative strategy to manage the insect pests, paving way for reduced use of insecticides without compromising the natural enemies' suppression. One such management tactic is the seed treatment which is very much successful in managing rice water weevil, *Lissorhoptrus oryzophilus* at Louisiana State of USA (Lanka et al., 2013 and Hummel et al., 2014). Even though we selected good quality seeds for sowing, it is always advised to go for seed treatment for better germination and further to prevent crop from seed and soil borne diseases and insect pests. Seed treatment enhances the seed viability and vigor which are the two most important factors in plant health management.

In western countries to achieve early season defense of corn from feeding damage by wireworms, cutworms, armyworm (*Mythimna unipuncta*) and seed corn maggot. Chlorantraniliprole (Lumivia), as seed treatment is used in approximately 80% of the rice-producing area in Southwest Louisiana (Wilson et al., 2019). Chlorantraniliprole persists in the plant long enough to affect late season pests. In fact, a greenhouse study conducted by Sidhu et al. (2014) reported that 70-80% mortality on sugarcane borer larvae in chlorantraniliprole treated rice plants at the mid-tillering stage of development. The tested product Chlorantraniliprole 625 g/L FS for seed treatment is proved to have positive effect on rice yellow stem borer and rice leaf folder under multi-location trials. In this context, the present work was conducted to explore the possible utilization of the product for the management of yellow stem borer (*S. incertulas*) and leaf folder (*C. medinalis*) in paddy.

MATERIAL AND METHODS

The field experiments were carried out during *kharif* 2018 and *rabi* 2018-19 seasons in a RBD design with 4 replications at the Crop Protection Division fields (K & L Block), of ICAR-National Rice Research Institute (NRRI), Cuttack (Odisha). Cuttack is located in East and Southern Coastal plain of Odisha state at 20° 46'25'N, latitude and 85°88¹30¹¹E, longitude at an altitude of 36 m above mean sea level. This place is considered to have hot and humid climate with mean annual rain fall of 1577 mm, average maximum summer temperature of 39°C and average minimum winter temperature of 11.5°C. The soil is diverse ranging from saline, lateritic, alluvial, red, mixed red and black.

Seed treatment with Chlorantraniliprole 625 g/L FS

Seed treatment was done to test the bio-efficacy of test insecticide on Variety (CR Dhan 304) and Hybrid (28P67) as per the standard protocol. Details of the treatments imposed for the bio-efficacy trials on variety (CR Dhan 304) and hybrid (28P67) experiments during *rabi* 2018-19 are mentioned hereunder in Table 1. The required quantity of Chlorantraniliprole 625 g/L FS was calculated for different treatments and mixed with water to make 25 ml slurry which was sufficient to treat 1 kg seed. Then slurry was applied onto seeds and thoroughly mixed to ensure uniform coating on the seeds. The treated seeds were spread under shade (12 hours) for proper drying and seeds were used for sowing in nursery. After germination crop management was done as per standard practice including the control of non-target insect and diseases though foliar sprays or other standard practices.

Observations recorded

For YSB, after transplanting, randomly selected 20 hills from each plot, number of dead-hearts and healthy tillers count was made at 14, 21, 28, 35 and 42 days after transplanting. The percent dead-hearts for each

Table 1. Treatment details.

Treatment number	Treatment detail	Dose (g a.i. / ha)	Dose of formula-tion* (ml/ kg seed)
T1	Chlorantraniliprole 625 g/L FS	52.50	2.80
T2	Chlorantraniliprole 625 g/L FS	60.00	3.20
T3	Chlorantraniliprole 625 g/L FS	67.50	3.60
T4	Chlorantraniliprole 625 g/L FS	75.00	4.00
T5	Cartap hydrochloride 4% GR	750.00	18750g/ ha.
T6	Untreated Check	NA	NA

*Seed rate of 30 kg/ha for conventional variety (CR Dhan 304) and 15 kg/ha for hybrid (28P67).

plot was calculated. Similarly, for leaf folder, we randomly selected 20 hills from each plot and observed for number of leaf folder larvae per hill and estimated the approximate damage done by leaf folder on randomly selected hills at 14, 21, 28, 35 and 42 days after transplanting. Finally, plot wise grain yield was recorded at the time of harvest and represented as yield in terms of tonnes/ha after proper drying and threshing.

Data analysis

The data was showed as mean ± standard error (SE). For analysis of data Microsoft excel and SAS, software was used. The significance of observed difference was assessed by analysis of variance (ANOVA).

RESULTS AND DISCUSSION

YSB and leaf folder infestation

During *kharif* 2018 trial, for CR Dhan 304 variety, at different time intervals of 14, 21, 28, 35 and 42 days after transplanting, all the treatments recorded superior results in terms of dead-heart control in comparison to the untreated check and even the lowest dose of Chlorantraniliprole 625 g/L FS was found superior over the standard check (T5). Among the different dose rates of Chlorantraniliprole treatments, Chlorantraniliprole @ 75.0 g a.i/ha was recorded significantly best control with a least dead-heart per cent of 5.41, 4.77, 3.43, 2.98 and 2.47 at 14, 21, 28, 35 and 42 days after transplanting, respectively. Similarly, for hybrid (28P67) trial, among the different dose rates of treatments Chlorantraniliprole @ 75.0 g a.i/ha recorded significantly best control with a least dead-heart per cent of 4.77, 3.43, 2.81, 2.37 and 2.02 at 14, 21, 28, 35 and 42 days after transplanting, respectively. The similar trend was observed during *rabi* 2018-19 trials (Table 2 and Table 3).

Similarly, during *kharif* 2018 trial, for CR Dhan 304 variety, at different time intervals of 14, 21, 28, 35 and 42 days after transplanting, untreated check recorded the highest incidence of leaf folder with 4.45, 4.55, 4.77, 4.92 and 5.02 larvae/hill, respectively. Whereas, treatment Chlorantraniliprole @ 75.0 g a.i/ha registered the least count of leaf folder recording 0.87, 0.68, 0.78, 0.53 and 0.61 larvae/hill, respectively. Similarly, for Hybrid (28P67) trial the treatment Chlorantraniliprole @ 75.0 g a.i/ha recorded the least count of leaf folder with 0.57, 0.68, 0.74, 0.81 and 0.89 larvae/hill during the intervals of 14, 21, 28, 35 and 42 days after transplanting, respectively. The similar trend was observed during *rabi* 2018-19 trials (Table 4 and Table 5).

The seed treatment of paddy with

Table 2. Yellow stem borer infestation during *kharif* 2018 field experiment trial.

Sl. no.	Treatments	Variety trial					Hybrid trial				
		Dead heart (%)					Dead heart (%)				
		14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
1	Chlorantraniliprole 625 g/L FS @ 52.5 g a.i./ ha	4.06 (11.62)*	3.02 (10.01)	2.50 (9.10)	2.24 (8.61)	2.08 (8.29)	3.02 (10.01)*	2.53 (9.15)	2.26 (8.65)	2.03 (8.19)	1.87 (7.86)
2	Chlorantraniliprole 625 g/L FS @ 60.0 g a.i./ ha	3.10 (10.14)	2.21 (8.55)	1.42 (6.84)	1.17 (6.21)	1.26 (6.44)	2.21 (8.55)	1.71 (7.52)	1.24 (6.38)	1.13 (6.11)	1.26 (6.44)
3	Chlorantraniliprole 625 g/L FS @ 67.5 g a.i./ ha	2.77 (9.58)	1.24 (6.38)	1.15 (6.15)	0.90 (5.44)	1.15 (6.15)	1.24 (6.38)	1.15 (6.15)	1.03 (5.83)	0.96 (5.62)	1.14 (6.13)
4	Chlorantraniliprole 625 g/L FS@ 75.0 g a.i./ ha	0.89 (5.41)	0.69 (4.77)	0.36 (3.43)	0.27 (2.98)	0.19 (2.47)	0.69 (4.77)	0.36 (3.43)	0.24 (2.81)	0.17 (2.37)	0.12 (2.02)
5	Cartap hydrochloride 4% GR @ 750 g a.i./ ha (Standard check)	5.26 (13.26)	6.53 (14.81)	6.83 (15.15)	8.82 (17.28)	9.51 (17.97)	5.60 (13.69)	6.75 (15.06)	7.07 (15.42)	10.12 (18.55)	11.07 (19.44)
6	Untreated Check	9.86 (18.30)	10.25 (18.68)	13.97 (21.95)	15.05 (22.83)	17.33 (24.60)	9.86 (18.30)	11.03 (19.40)	13.50 (21.56)	14.90 (22.71)	16.50 (23.97)
	SEm±	0.38	0.37	0.29	0.22	0.25	0.29	0.34	0.30	0.25	0.24
	CD (5%)	1.15	1.11	0.88	0.67	0.67	0.88	1.02	0.90	0.75	0.72
	CV	7.44	7.31	5.77	4.23	4.58	5.14	6.44	5.71	4.72	4.36

DAT- Days after transplanting. * Figures in the parenthesis are Arcsine transformed values.

Table 3. Yellow stem borer infestation during *rabi* 2018-19 field experiment trial.

Sl no	Treatments	Variety trial					Hybrid trial				
		Dead heart (%)					Dead heart (%)				
		14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
1	Chlorantraniliprole 625 g/L FS @ 52.5 g a.i./ ha	3.42 (10.66)*	4.25 (11.90)	4.06 (11.62)	3.02 (10.01)	2.50 (9.10)	7.71 (16.12)*	7.20 (15.57)	5.70 (13.81)	4.08 (11.65)	3.32 (10.49)
2	Chlorantraniliprole 625 g/L FS @ 60.0 g a.i./ ha	2.81 (9.64)	3.33 (10.51)	3.17 (10.26)	2.21 (8.55)	1.26 (6.44)	6.14 (14.34)	6.26 (14.49)	4.81 (12.67)	2.81 (9.65)	2.09 (8.31)
3	Chlorantraniliprole 625 g/L FS @ 67.5 g a.i./ ha	2.49 (9.07)	2.89 (9.79)	2.53 (9.15)	1.24 (6.38)	1.15 (6.15)	4.22 (11.86)	5.37 (13.40)	3.45 (10.71)	1.72 (7.54)	1.51 (7.05)
4	Chlorantraniliprole 625 g/L FS@ 75.0 g a.i./ ha	1.01 (5.77)	1.85 (7.83)	1.36 (6.71)	0.69 (4.77)	0.36 (3.43)	2.51 (9.12)	2.63 (9.34)	1.71 (7.52)	0.84 (5.25)	0.34 (3.35)
5	Cartap hydrochloride 4% GR @ 750 g a.i./ ha (Standard check)	10.80 (19.18)	19.60 (26.28)	16.84 (24.23)	11.75 (20.04)	9.97 (18.41)	16.27 (23.79)	17.71 (24.89)	16.96 (24.32)	15.24 (22.98)	14.30 (22.22)
6	Untreated Check	13.07 (21.19)	27.98 (31.93)	28.70 (32.39)	16.29 (23.80)	16.50 (23.97)	22.77 (28.50)	30.27 (33.38)	27.71 (31.76)	17.81 (24.96)	15.87 (23.48)
	SEm±	0.44	0.45	0.66	0.40	0.34	0.89	0.81	0.58	0.48	0.49
	CD (5%)	1.32	1.30	1.98	1.20	1.01	2.67	2.44	1.73	1.45	1.49
	CV	6.95	5.36	8.39	6.50	5.98	10.28	8.79	6.86	7.05	7.93

DAT- Days after transplanting. * Figures in the parenthesis are Arcsine transformed values.

Chlorantraniliprole is widely recommended in locations where high incidence of leaf folder and stem borer incidence is anticipated (Wilson et al., 2019). The study revealed that Chlorantraniliprole 625 g/L FS seed treatment can effectively manage the key pests of rice.

Present study is in line with studies by Lanka et al. (2013) and Hummel et al. (2014) who reported the reduction in rice root weevil densities in chlorantraniliprole treated plots. Similarly, Thrash et al. (2013) reported reduced survivorship of fall armyworm

Table 4. Leaf folder infestation during *kharif* 2018 field experiment trial.

Sl. no.	Treatments	Variety trial					Hybrid trial				
		Leaf folder larvae per hill					Leaf folder larvae per hill				
		14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
1	Chlorantraniliprole 625 g/L FS @ 52.5 g a.i./ ha	1.64 (1.78)*	1.75 (1.82)	1.83 (1.85)	1.89 (1.87)	1.96 (1.90)	1.92 (1.89)	1.96 (1.90)	2.04 (1.93)	2.17 (1.97)	2.26 (2.00)
2	Chlorantraniliprole 625 g/L FS @ 60.0 g a.i./ ha	1.49 (1.72)	1.58 (1.75)	1.62 (1.77)	1.74 (1.82)	1.88 (1.87)	1.57 (1.75)	1.68 (1.79)	1.94 (1.89)	2.06 (1.94)	2.13 (1.96)
3	Chlorantraniliprole 625 g/L FS @ 67.5 g a.i./ ha	1.23 (1.61)	1.42 (1.69)	1.48 (1.71)	1.67 (1.79)	1.13 (1.56)	1.41 (1.69)	1.53 (1.74)	1.67 (1.79)	1.73 (1.82)	1.84 (1.86)
4	Chlorantraniliprole 625 g/L FS@ 75.0 g a.i./ ha	0.87 (1.43)	0.68 (1.32)	0.78 (1.38)	0.53 (1.23)	0.61 (1.28)	0.57 (1.25)	0.68 (1.32)	0.74 (1.36)	0.81 (1.40)	0.89 (1.44)
5	Cartap hydrochloride 4% GR @ 750 g a.i./ ha (Standard check)	3.14 (2.27)	3.24 (2.30)	3.57 (2.39)	3.84 (2.46)	3.94 (2.49)	2.94 (2.21)	3.01 (2.23)	3.19 (2.29)	3.27 (2.31)	3.35 (2.33)
6	Untreated Check	4.45 (2.61)	4.55 (2.63)	4.77 (2.68)	4.92 (2.72)	5.02 (2.74)	5.18 (2.77)	5.25 (2.79)	5.34 (2.81)	5.50 (2.85)	5.62 (2.87)
	SEm±	0.03	0.03	0.02	0.02	0.03	0.02	0.03	0.02	0.02	0.02
	CD (5%)	0.10	0.08	0.07	0.07	0.09	0.06	0.09	0.07	0.07	0.07
	CV	3.37	2.94	2.44	2.39	3.08	1.97	2.89	2.36	2.42	2.33

DAT- Days after transplanting. * Figures in the parenthesis are square root transformed values.

Table 5. Leaf folder infestation during *rabi* 2018-19 field experiment trial.

Sl. no.	Treatments	Variety trial					Hybrid trial				
		Leaf folder larvae per hill					Leaf folder larvae per hill				
		14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
1	Chlorantraniliprole 625 g/L FS @ 52.5 g a.i./ ha	1.20 (1.60)*	1.13 (1.56)	1.16 (1.58)	1.15 (1.57)	1.14 (1.57)	1.05 (1.52)	1.11 (1.55)	1.13 (1.56)	1.15 (1.57)	1.19 (1.59)
2	Chlorantraniliprole 625 g/L FS @ 60.0 g a.i./ ha	1.14 (1.57)	1.16 (1.58)	1.12 (1.56)	1.12 (1.56)	1.13 (1.56)	1.03 (1.52)	1.13 (1.56)	1.14 (1.57)	1.17 (1.58)	1.11 (1.55)
3	Chlorantraniliprole 625 g/L FS @ 67.5 g a.i./ ha	1.11 (1.55)	1.11 (1.55)	1.14 (1.57)	1.00 (1.50)	0.80 (1.39)	1.15 (1.57)	1.14 (1.57)	1.11 (1.55)	1.14 (1.57)	1.15 (1.57)
4	Chlorantraniliprole 625 g/L FS@ 75.0 g a.i./ ha	0.17 (0.91)	0.44 (1.16)	0.35 (1.09)	0.28 (1.03)	0.38 (1.11)	0.24 (0.99)	0.42 (1.14)	1.06 (1.53)	0.68 (1.32)	0.55 (1.24)
5	Cartap hydrochloride 4% GR @ 750 g a.i./ ha (Standard check)	3.20 (2.29)	2.70 (2.14)	2.90 (2.20)	3.10 (2.26)	3.25 (2.30)	2.50 (2.08)	2.75 (2.16)	2.85 (2.19)	3.00 (2.23)	2.85 (2.19)
6	Untreated Check	5.40 (2.82)	4.90 (2.71)	5.20 (2.78)	5.30 (2.80)	4.40 (2.60)	4.25 (2.56)	4.50 (2.62)	4.00 (2.50)	3.75 (2.44)	4.15 (2.54)
	SEm±	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.02	0.03	0.03
	CD (5%)	0.08	0.08	0.09	0.08	0.08	0.08	0.06	0.07	0.09	0.08
	CV	3.00	2.84	3.15	3.20	3.20	2.99	2.16	2.61	3.43	2.93

DAT- Days after transplanting. * Figures in the parenthesis are square root transformed values.

larvae at both vegetative as well as reproductive stages of soybean plants due to treatment of seeds with chlorantraniliprole. The above results corroborate with work of Singh et al. (2004) who reported that, imidacloprid 600 FS @ 10 ml/ kg seed is the most effective in pearl millet against termites. Sundria and Acharya (2012) also reported imidacloprid 70 WS @ 10 gm/ kg seed as highly effective on wheat. Panigrahi (2010) found that imidacloprid 10 ml/ kg seed was superior. Choudhary and Dashad (2002) proved that seed treatment with chlorpyriphos @ 7 ml/ kg seed has significantly proved to be effective in chickpea resulting in minimum termite damage and maximum grain yield of chickpea.

Yield determined under Chlorantraniliprole 625 g/L FS treated plots

During *kharif* 2018 trial, for CR Dhan 304 variety the highest yield (5.46 t/ha) was recorded in treatment Chlorantraniliprole @ 75.0 g a.i/ha (T4) which was significantly superior over the other doses of Chlorantraniliprole and the check treatment (Table 6). Similarly in hybrid (28P67) trial also, all the dose rates of Chlorantraniliprole and the check treatment recorded superior yield in comparison to the untreated check. The highest yield (6.85 t/ha) was recorded in treatment Chlorantraniliprole @ 75.0 g a.i/ha (T4) which was significantly superior over the other doses of Chlorantraniliprole and the check treatment. The similar trend was observed for yield parameters during *rabi* 2018-19 trials.

Due to the high efficacy of chlorantraniliprole seed treatment in suppression of leaf folder and stem borers population under field conditions significantly increase in yield under chlorantraniliprole treated plots were recorded in both seasons (*Kharif* and *Rabi*) and in both variety and hybrid trials. Mazzanti et al. (2012) evaluated the efficacy of thiamethoxam seed treatment on paddy water weevil control in conventional and hybrid rice and showed that the hybrid paddy had significantly more tiller number and dry weight when compared with the conventional variety hence, shown significantly higher larval density of paddy water weevil. Similarly, Parsai et al. (2014) achieved maximum grain yield of chickpea with seed treatment of thiamethoxam 30% FS @ 2.5 ml/ kg seed. Mishra et al. (2007) observed maximum plant stand with maximum grain yield in wheat due to seed treatment against termite. Hence, seed treatment with Chlorantraniliprole 625 g/ L FS (seed treatment formulation) can be recommended for managing stem borer and leaf folder pests in paddy.

CONCLUSION

It is apparent from the studies undertaken during *kharif* 2018 and *rabi* 2018-19, that Chlorantraniliprole 625 g/ L FS (seed treatment formulation) provided an excellent

Table 6. Yield data observed during Chlorantraniliprole 625 g/L FS field experiment trial.

Sl no	Treatments	*Kharif* 2018		*Rabi* 2018-19	
		Yield (t/ha)		Yield (t/ha)	
		Variety (CR Dhan 304)	Hybrid (28P67)	Variety (CR Dhan 304)	Hybrid (28P67)
1	Chlorantraniliprole 625 g/L FS @ 52.5 g a.i./ ha	4.64	5.86	4.55	5.78
2	Chlorantraniliprole 625 g/L FS @ 60.0 g a.i./ ha	4.72	5.98	4.61	5.85
3	Chlorantraniliprole 625 g/L FS @ 67.5 g a.i./ ha	4.87	6.03	4.73	5.91
4	Chlorantraniliprole 625 g/L FS@ 75.0 g a.i./ ha	5.46	6.85	5.34	6.49
5	Cartap hydrochloride 4% GR @ 750 g a.i./ ha (Standard check)	4.05	5.15	4.18	5.13
6	Untreated Check	3.70	4.43	3.39	4.41
	SEm±	0.14	0.17	0.10	0.15
	CD (5%)	0.41	0.51	0.30	0.44
	CV	5.91	5.84	4.37	5.23

control of stem borer and leaf folder in conventional variety of rice (CR Dhan 304) and hybrid rice (28P67). Similarly, treatment of Chlorantraniliprole @ 75 g ai/ha recorded highest yield (both in variety and hybrid rice). Hence, seed treatment with Chlorantraniliprole @ 75 g ai/ha can be recommended for managing stem borer and leaf folder pests in paddy.

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CONFLICTS OF INTEREST

Individual authors declare no conflict of interest with regards to this publication.

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Bacterial synthesized silver nanoparticle inhibits *Rhizoctonia solani* Kuhn, the causal organism for sheath blight disease of rice

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ABSTRACT

Rhizoctonia solani Kuhn (AG 1-IA) is one of the most devastating pathogens of rice causing sheath blight disease and being a prime reason for the unsatisfactory productivity of rice in India. The goal of the present study was to investigate the efficacy of silver nanoparticles (AgNPs) synthesized using an agriculturally important bacterium, Pseudomonas fluorescens OKC, in managing the sheath blight disease of rice. Successful biosynthesis of AgNPs was monitored by UV-visible spectroscopy, showing a peak at 432 nm. The AgNPs were further characterized using a Transmission Electron Microscope (TEM), Dynamic Light Scattering (DLS) and Fourier Transform Infrared spectroscopy (FT-IR). The TEM result confirmed that the size of the synthesized nanoparticles was less than 100nm. DLS results revealed that the average particle size of the AgNPs was 74 nm and the zeta potential was -23.6 mV, indicating that the synthesized nanoparticles were of good stability at room temperature. The antifungal potential of AgNPs was tested against the test fungus in vitro and maximum growth inhibition was recorded in AgNPs treatment (69.09%) as compared to the control. Moreover, this result was further authenticated under net house conditions, where AgNPs successfully reduced the incidence of R.solani Kuhn. The findings showed that the biosynthesized AgNPs inhibited the growth of R. solani Kuhn and could be useful in the management of sheath blight disease in rice.

Key words: *Silver nanoparticles, biosynthesis, Pseudomonas fluorescens OKC, antifungal potential, Rhizoctonia solani Kuhn*

INTRODUCTION

The rise in human population demands higher production of rice (*Oryza sativa* L.), as it is one of the most essential staple foods and feeds more than half of the world's population (Singh et al., 2021). India is among the largest producers of rice, with an average production of 120.544 million metric tons of milled rice over the last five years (USDA, 2022). However, its productivity remains unsatisfactory, being less than many of the neighbouring countries (DRR, 2017). Among the major bottlenecks for achieving unsatisfactory productivity and food security, the susceptibility of the crop to diseases resulting in yield loss occupies a prime position. Sheath blight disease caused by *Rhizoctonia solani* Kuhn {Teleomorph: *Thanatephorus cucumeris* (Frank) Donk)} is considered as a significant biotic hindrance to rice crops, which causes severe economic loss to the farmers. This pathogen is a universal soil saprotrophic and facultative plant parasite. The majority of the promising cultivars fail to survive the disease, and resistant cultivars against this disease have not yet been reported. The substantial use of chemical fungicides such as validamycin, azoxystrobin, trifloxystrobin, propiconazole, carbendazim, hexaconazole, etc. appears to be a viable strategy for closing the production gap caused by *Rhizoctonia solani*, since it considerably boosts rice crop protection and productivity (García and Argüelles, 2021). Unfortunately, our environment is threatened by the

increased use of such harmful chemicals in agriculture by causing a variety of pollutions in addition to their high economic expenses, which are still a major barrier for farmers in less developed countries (Shuqin and Fang, 2018; Almaraz et al., 2018; Benson and Mogues, 2018). Thus, exploration of innovative ways for controlling these diseases is needed to solve the problems of environmental and ecological sustainability as well as the expenses associated with pesticides.

The agriculture sector is currently utilizing the cutting-edge technique of nanotechnology-based solutions to address such challenges (Mishra et al., 2017). Nanoparticles, especially metallic nanoparticles like silver, gold and copper are known to have antimicrobial potential and are finding applications in plant and animal disease management (Mahanty et al., 2013). There are a number of strategies for nanoparticle synthesis, such as physical method, chemical method and biological method. The chemical method of nanoparticle synthesis uses chemicals that could have harmful environmental effects, whereas biological method produces nontoxic, clean, and biocompatible nanoparticles that are quick, easy, and economical. Thus, biologically synthesized nanoparticles are becoming popular and a variety of biological entities, such as plants and microorganisms, are being used for the synthesis of nanoparticles. Biosynthesized nanoparticles have been used successfully and effectively in agriculture, particularly for managing plant diseases and promoting plant development (Masum et al., 2019).

In the present study, the efficacy of silver nanoparticles biosynthesized using a beneficial bacterium, *Pseudomonas fluorescens* OKC, was tested against *Rhizoctonia solani*, the devastating pathogen of rice.

MATERIALS AND METHODS

Extracellular biosynthesis of silver nanoparticles from *P. fluorescens* OKC

A pure culture of *P. fluorescens* OKC (Accession number JN128891) was obtained from the Hoffman laboratory of the Department of Mycology and Plant Pathology, Institute of Agricultural Sciences, BHU, Varanasi. The bacterial culture was maintained in King's B medium containing proteose peptone, dipotassium hydrogen phosphate (K_2HPO_4), Magnesium sulphate ($MgSO_4.7H_2O$) and Glycerol. The bacterium was inoculated in 120 ml of King's B broth in 250 ml conical flasks and agitated in a mechanical shaker for 48 hours to achieve the early stationary phase for the extracellular synthesis of silver nanoparticles. By centrifuging the bacterial culture at 12,000 rpm for 12 minutes, the supernatant was obtained and collected in sterilized conical flasks. 1 mM silver nitrate was prepared and mixed with culture supernatant in 3:1 ratio in a conical flask. The flask was kept on a magnetic stirrer. Dark condition was maintained and synthesis of silver nanoparticles was monitored by the visual observation of a change in colour from yellow to brown.

Characterization of biosynthesized silver nanoparticles (AgNPs)

Biosynthesized AgNPs were characterized using a range of analytical techniques. UV-vis spectrophotometer (Evolution 300, Thermo Scientific, USA) was used to analyze the optical property of the fabricated AgNPs synthesized from *P. fluorescens* OKC and absorbance was measured in the range of 300-600 nm. For size and morphological characterization, Transmission Electron Microscope (TEM; JEM-1011, Jeol, Japan) was used and the sample image was captured at 5.0 kV. Dynamic Light Scattering (DLS) was used for particle size distribution (Nano ZS; Make: Malvern Instruments, UK). The zeta potential and z-average particle size of the generated nanoparticle solutions were quantified with a particle size analyzer (Nano ZS; Make: Malvern Instruments, UK). To evaluate the chemical groups associated with the biosynthesis of AgNPs, Fourier Transmission Infrared (FTIR) spectroscopy (Prestige 21, Shimadzu Japan) was used. The IR spectra of the powdered samples were recorded with FTIR spectrometer at a wavenumber of 400-4000 cm^{-1} with a diffuse reflectance mode attachment at 4 cm^{-1} resolution.

In vitro antifungal efficacy of biosynthesized AgNPs

The antifungal effect of bacterium-synthesized AgNPs was examined *in vitro* against the mycelial growth of *R. solani* (NCBI Accession Number: MK478903.1) using the poisoned food technique as per the protocol given by Nene and Thapliyal (1993). PDA medium was used for this experiment. After sterilization of the PDA

medium and before solidification, biosynthesized AgNP was added to the PDA medium to obtain the desired concentration of 30 PPM. After solidification of the medium, a uniform disc (about 5 mm in diameter) containing a four-day-old *R. solani* culture was put in the centre of the Petri dish containing PDA mixed with AgNP. For comparison purposes, one set of Petri dishes containing PDA mixed with Validamycin 3% L at its recommended dose of 2 ml/lit was used. Another set of Petri dishes containing only PDA was used as the control. Then the Petri plates were incubated at 28±1ºC for four days. After that, using the formula given by Vincent et al. (1927) per cent reduction of mycelial growth of the test fungus was calculated.

Per cent reduction of mycelial growth of test fungus= (C - T) /C × 100

Where, C= Mycelial growth of the test fungus in control

T= Mycelial growth of the test fungus in treatment

Microscopic visualization of interactions between biosynthesized AgNPs and *R. solani*

Interactions between biosynthesized AgNPs and *R. solani in vitro* Interactions between biosynthesized AgNPs and *R. solani* were studied by observing the hyphal deformations under optical microscope. Sterilized glass slides were taken and a drop of glycerol was put on each slide. Mycelia from four-day-old untreated *R. solani* cultures were taken using sterilized needle and were mounted on glycerol drop and covered using a cover slip. Similarly, sets of slides were prepared for 30 PPM AgNPs and Validamycin 3% L treated groups. The slides were observed under RADICAL RXLr-4 optical microscope (10X magnification).

Net house experiment

The antimicrobial property of biosynthesized AgNPs was evaluated against the sheath blight disease of rice caused by *R. solani* under net house conditions. The experiment was conducted in the crop protection division net house at ICAR- National Rice Research Institute, Cuttack. Highly susceptible rice var. Pusa Basmati 1 was used for this experiment. Seeds were sown in nursery beds, and after about 21 days, seedlings were transplanted into earthen pots containing sterilized soil and kept inside the net house. Plants were inoculated with the pathogen *R. solani* in the tillering stage. For this, a small portion of fungal mycelial mat of about five days old was taken and inoculated beneath the leaf sheath using forceps, covered with moistened cotton and tied with a thread. Inoculated plants were then covered with poly bags to maintain desirable humidity for the growth of the pathogen for 48 hours. After the appearance of first symptoms, the plants were treated with 30 ppm of biosynthesized AgNPs with two sprayings. The first spraying was immediately after the appearance of the first symptom and the second one was 48 hours after the first spraying. One set of plants were treated with validamycin 3% L @ 2ml/liter of water for comparison purpose. Untreated pathogen inoculated and untreated pathogen un-inoculated plants were used as positive and negative controls, respectively. Each treatment was replicated five times. Per cent disease index (PDI) was calculated 28 days post inoculation using the 0-9 disease scoring scale as per the Standard Evaluation System of the International Rice Testing Programme (IRRI, 2014).

Per cent Disease Index (PDI) = (Sum of all individual disease scoring)/(Total no. of plants observed x maximum scoring) x 100

RESULTS AND DISCUSSIONS

Synthesis and characterization of silver nanoparticle

In the present study, *P. fluorescens* OKC, a bacterium

Table 1. Effect of biosynthesized AgNPs on growth parameters of rice plants after challenged with *R. solani*.

Treatment	Shoot length (cm)	Root length (cm)	Shoot dry weight (g)	Root dry weight (g)
Uninoculated plant (Control)	94.76 ± 3.00^{a}	21.3 ± 1.25^{a}	7.85 ± 0.86^{a}	1.88 ± 0.46^{a}
Pathogen challenged plants	82.25 ± 1.90^{c}	15.4 ± 1.03^{c}	6.25 ± 0.63^{b}	1.17 ± 0.29^{b}
AgNPs+ Pathogen challenged plants	96.65 ± 2.87^{a}	20.1 ± 1.48^{ab}	8.1 ± 0.79^{a}	1.84 ± 0.46^{a}
Validamycin 3% L+ Pathogen challenged plants	91.18 ± 2.14^{b}	18.5 ± 1.14^{b}	7.7 ± 0.72^{a}	1.76 ± 0.37^{a}
CD (0.05%)	3.378	1.672	1.005	0.532

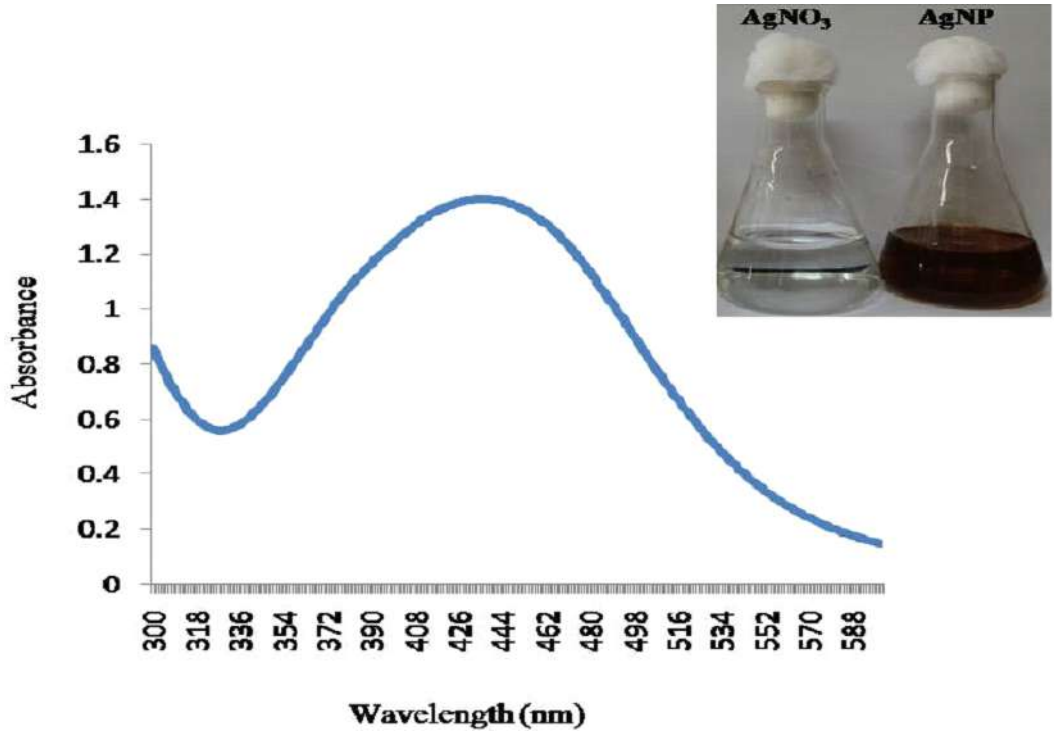

Fig. 1. UV-visible absorption spectrum of biosynthesized silver nanoparticles.

having antimicrobial properties, was used with the aim of utilizing it for the extracellular synthesis of silver nanoparticles. The colour of the culture supernatant of *P. fluorescens* when mixed with 1 mM $AgNO_3$ solution changed from yellow to dark brown (Fig. 1). This result confirmed the successful biosynthesis of silver nanoparticles. Because of the synthesis of the biomolecules necessary for the fabrication of nanoparticles and the reduction of Ag^+ to Ag^0, variations in the colour of AgNPs have been recorded (Ibrahim et al., 2020b). The nitrate ions (NO_3^-) are converted to nitrite (NO_2^-) during the reduction of $AgNO_3$ by initially taking two protons and then emitting two electrons as well as water. Finally, the silver element Ag^0 is formed when the electrons released during the reduction event are transferred to Ag^+ (Roy et al., 2019; Ibrahim et al., 2020a).

The synthesis of AgNPs was further validated by examining them in UV-visible spectrophotometer, which exhibited a spectrum of surface plasmon resonance (SPR) peaks at 432 nm (Fig. 1), which is within the range reported earlier (Kasithevar et al., 2017; Hossain et al., 2019; Masum et al., 2019). The biosynthesized AgNPs were morphologically, structurally and functionally characterized using different analytical techniques like TEM, DLS and FTIR. The TEM image confirmed that the silver nanoparticles synthesized from *P. fluorescens* OKC were of size <100 nm (Fig. 2a). By using DLS and zeta potential analyzer, the particle size and zeta potential of AgNPs in colloidal suspension were each measured. The average size of biosynthesized AgNPs is 74 nm and their mean zeta potential is -23.6 mV as shown in Fig. 2b and 2c. The negative zeta potential confirmed that the biosynthesized AgNPs are stable at room temperature (Ashraf et al., 2020).

The FTIR spectra of biosynthesized AgNPs produced from *P. fluorescens* culture supernatant following treatment with $AgNO_3$ are displayed in Fig. 2d. The spectrum analysis identifies the number of biological functional groups that serve as capping or stabilizing agents for nanoparticle stabilization. FTIR results revealed different absorption peaks at 2978, 1571, 1429, 1392, 900, 862, 772, 685 and 554 cm^{-1}. In case of synthesized AgNPs, a strong absorption peak was observed at 2978 cm^{-1} indicating silver ion (Ag^+) binding with hydrocarbon (C-H) groups present in the *P. fluorescens* OKC culture supernatant, while the

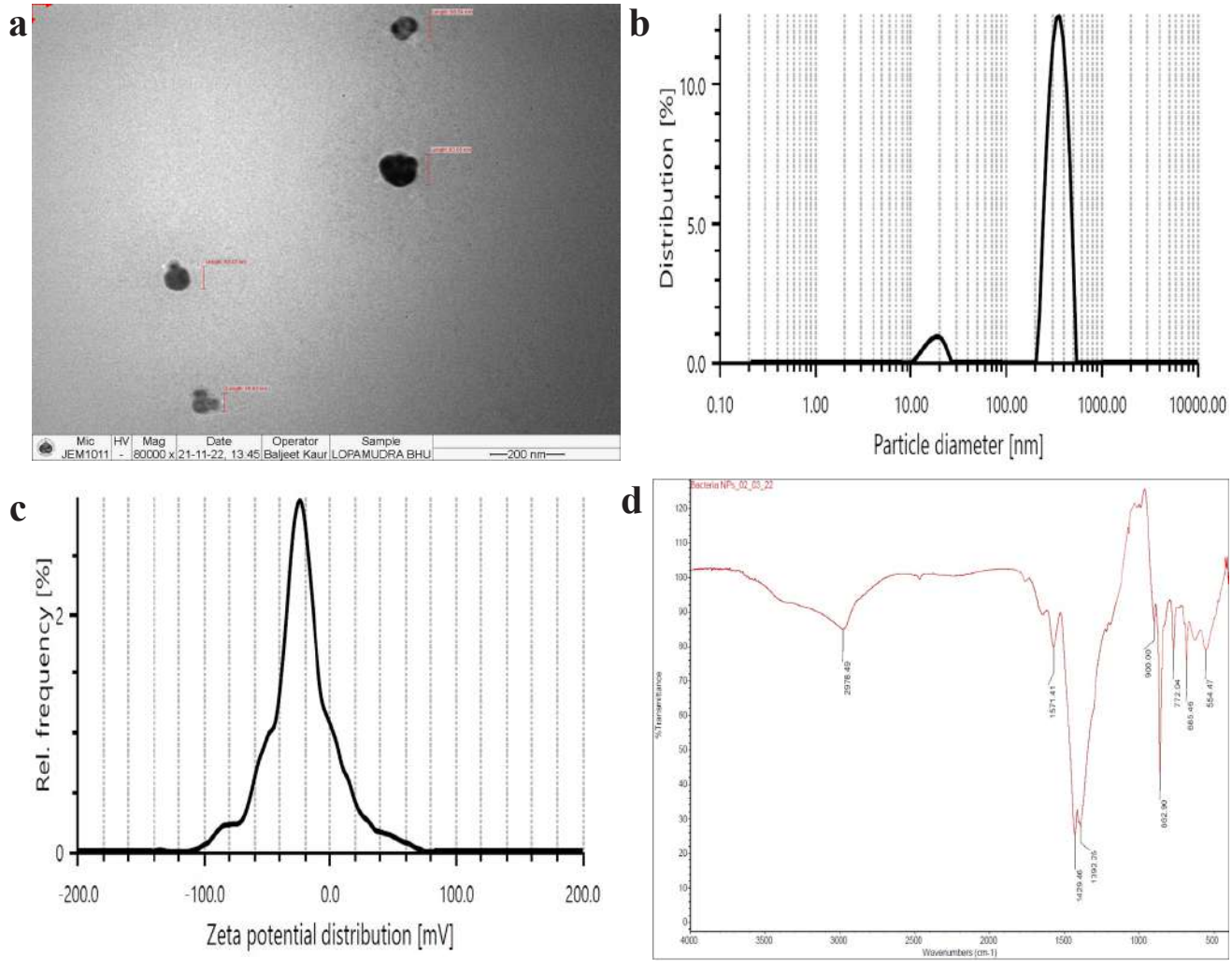

Fig. 2. Analytical characterization of biosynthesized silver nanoparticles. a. Morphological characterization of AgNPs using TEM, b. Particle size distribution, c. Zeta potential d. FTIR spectrum of the nanoparticle.

peak at 1571 cm^{-1} represents the involvement of amide-I arising due to carbonyl groups (-C=O) in proteins and enzymes. Small peaks between 1000 cm^{-1} to 1300 cm^{-1} correspond to aliphatic C-N stretching vibrations of the amines. Overall, FTIR spectroscopic research reveals that the release of extracellular proteins in the culture supernatant of *P. fluorescens* OKC may accomplish simultaneous tasks of AgNPs production and stabilization in the aqueous reaction media (Mishra et al., 2017; Adak et al., 2020).

Antifungal activity against *Rhizoctonia solani*

In the present study, using the poisoned food technique, the antifungal potential of biosynthesized silver nanoparticles was tested against *R. solani* and compared with the control (only PDA) and validamycin 3% L. The results revealed that both AgNPs (30 PPM) and validamycin 3% L treatments significantly inhibited the growth of *R. solani* under *in vitro* conditions (Fig. 3a). Maximum growth inhibition was recorded in AgNPs treated plates (69.09 %) followed by validamycin treated petri plates (59.02 %) (Fig. 3c). The majority of researchers believe that AgNPs' greater surface area, which improves their interaction with pathogens, is what gives them their antimicrobial characteristics (Panacek et al., 2006). Additionally, its effectiveness is influenced by particle size, shape, and preparation technique. AgNPs with a spherical form and a smaller size demonstrated greater antimicrobial effectiveness (Simon-Deckers et al., 2009). AgNPs may

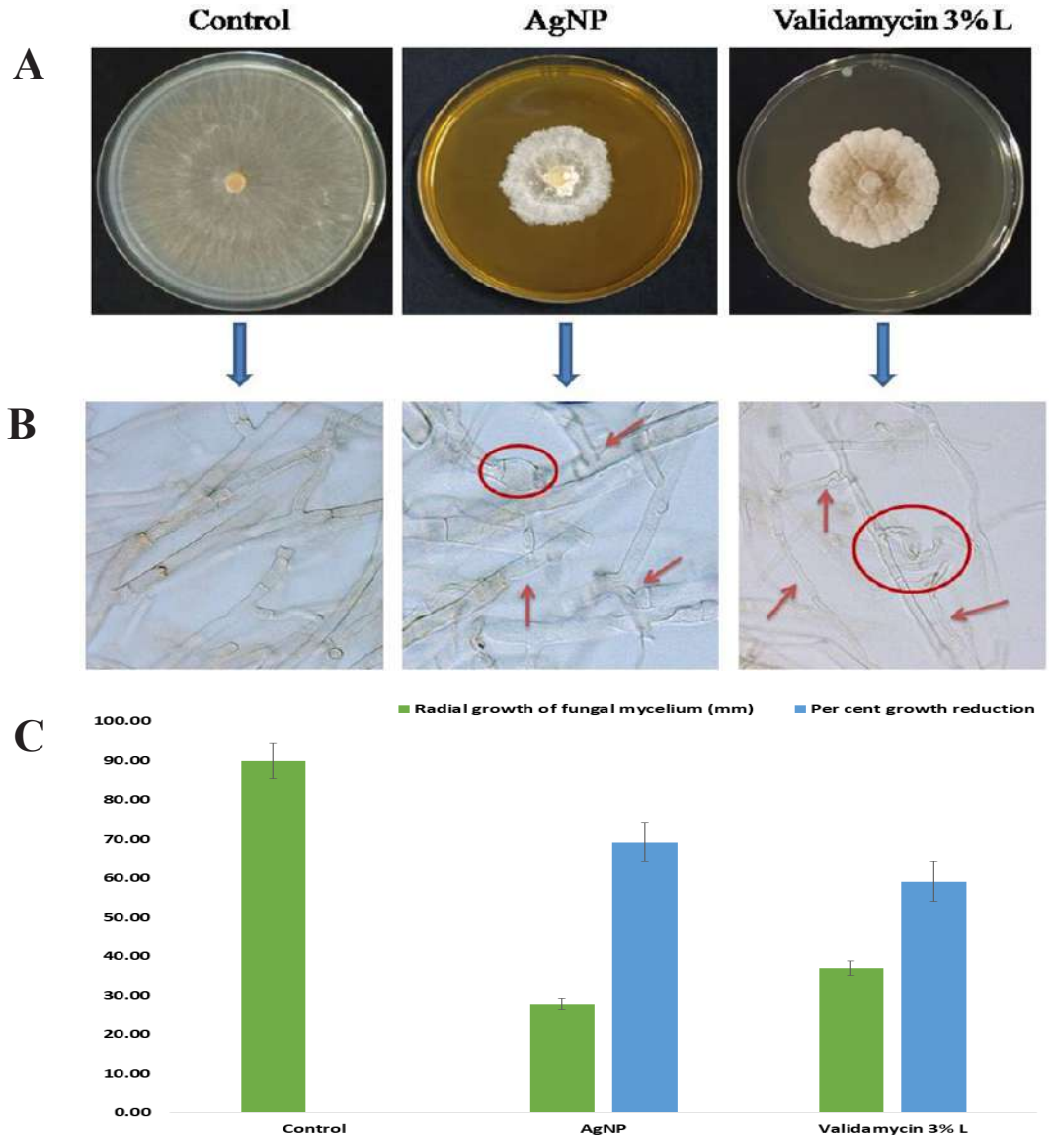

Fig. 3. Antifungal potential of biosynthesized AgNPs *in vitro*. **A**. Inhibitory effect of AgNPs on growth of *R. solani* on PDA, **B**. Microscopic observation of effect of AgNPs on mycelial deformities of *R. solani*, **C.** Graphical representation of impact of AgNPs on per cent growth.

interact with cell components that include sulphur or phosphorus within the cell, or they may produce reactive oxygen species (ROS), which may ultimately have an antimicrobial impact (Adak et al., 2020).

When the interaction between fungus and AgNPs was studied under light microscope, deformations like swelling, twisting and thinning of hyphae were observed (Fig. 3b); whereas, in the control there were no such deformations. These results confirmed the antifungal efficacy of biosynthesized AgNPs. The primary components of the complexly dynamic fungal cell wall are chitin, glycoproteins, mannans, and glucans. AgNPs might affect the biosynthesis of chitin and when chitin production is disrupted, fungal cells become distorted and osmotically unstable as well as their walls disintegrate (Ashraf et al., 2020).This is corroborated by the findings of Elgorban et al. (2016), who have demonstrated that

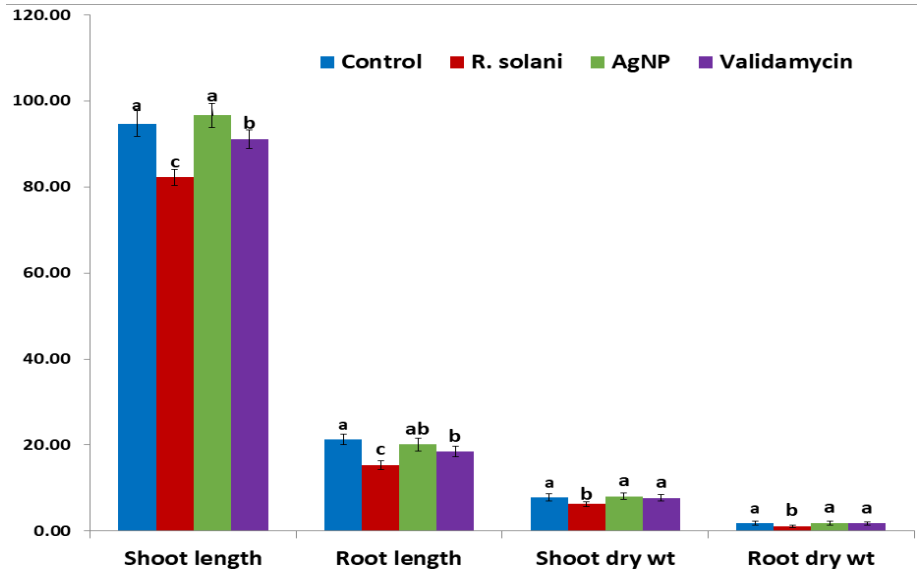

Fig. 4. Effect of different treatments on plant growth parameters (Shoot length and Root length in cm; Shoot dry wt and Root dry wt in g).

the antifungal activity of AgNP could be attributed to its ability to impair the pathogen's membrane integrity.

Under net house conditions, the findings were once again validated, where the treatment with 30 PPM AgNPs successfully managed the incidence of *R. solani*. Due to *R. solani* infection, there was 13.20, 27.7, 20.38 and 37.77% significant decrease in shoot length, root length, shoot dry weight and root dry weight, respectively when compared with uninoculated plants. It is noteworthy to mention that when rice plants were treated with AgNPs, the decline in plant growth caused by a pathogen challenge was reversed and 17.51, 30.52, 29.6 and 57.26% increases in shoot length, root length, shoot dry weight and root dry weight were noticed when compared with pathogen challenged plants (Table 1 and Fig. 4). In the last few years, many researchers have successfully synthesized silver nanoparticles utilizing plant extracts and cell free extracts of microorganisms. Phull et al. (2016) investigated the antifungal effectiveness of *Bergenia ciliata*-derived AgNPs against a variety of fungi, and their findings showed that the nanoparticles were more effective than the *B. ciliata* extract alone. *Bipolarissorokiniana*, a pathogen that causes spot blotches on wheat, was completely inhibited by the bio-fabricated AgNPs at various doses (Mishra et al., 2014). Balashanmugam et al. (2016) also conducted such type of experiment, which revealed that nanoparticles synthesized from *Cassia roxburghii* aqueous leaf extracts possessed antifungal properties and were potent enough to manage several phytopathogenic fungi.

CONCLUSION

In the present study, *P. fluorescens* OKC culture supernatant was successfully utilized for synthesizing silver nanoparticles. The study showed that at a concentration of 30 PPM, the inhibitory potential of biosynthesized silver nanoparticles was better than validamycin (at its recommended dose), indicating its potential application for the management of sheath blight disease in rice. Overall, the findings of this study indicate that bacterium-synthesized AgNPs may be able to shield rice plants against fungal infection and can be used as a substitute for different harmful chemicals used in managing sheath blight disease of rice.

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Effect of silicon on morphological, physiological and biochemical characteristics in salinity tolerance in *indica* rice

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ABSTRACT

Silicon application can increase plant tolerance to abiotic stress and enhance productivity. Seeds of two Rice varieties (ADT53: salinity susceptible and Co 53: salinity tolerance) were exposed to different concentrations of NaCl (25, 50, 75 and 100 mM), Silicon (Sodium metasilicate; 1, 1.5 and 2 mM) and a combination of both NaCl and Silicon to investigate the effect of silicon on seed germination, morphological, physiological and biochemical characteristics under salinity stress imposed for 15 days and also subjected to fresh water alone (control). Application of silicon enhances the germination rate of ADT 53 and Co 53 (90%) in 50mM NaCl + 2mM Si. Maximum shoot length was observed in ADT 53 (6.54 cm) and Co 53 (10 cm) in 25 mM NaCl + 2mM Si. Maximum root length was observed in ADT 53 (12.9 cm) and Co 53 (10 cm) in 50mM NaCl + 1mM Si. ADT 53 (33.3%) showed the highest percentage of chlorophyll-a in 25 mM NaCl + 1 mM Si and Co 53 (30%) in 75mM NaCl + 2 mM Si 100 mM NaCl + 2 mM Si. The highest percentage of Chlorophyll b was observed in ADT 53 (104%) in 25 mM NaCl + 2mM Si and Co 53 (95%) in 100 mM NaCl + 2 mM Si. Total chlorophyll content was maximum in ADT 53 (133%) in 25mM NaCl + 2mM Si and Co 53 (130%) in 75 mM NaCl + 2mM Si. Maximum carotenoid was observed in ADT 53 (240%) in 100 mM NaCl + 1.5 mM Si and Co 53 (273%)in 25 mM NaCl + 2 mM Si. Chlorophyll stability index was increased in ADT 53 (185.7%) in 100 mM NaCl + 2 mM Si and Co 53 (205.38%) in100 mM NaCl + 1.5 mM Si. Proline content was decreased in ADT 53(13%) in 25 mM NaCl + 1.5 mM Si and Co 53 (11%) in 100 mM NaCl + 1.5mM Si. MDA was found low in ADT 53 (11%) in 100 mM NaCl + 1.5 mM Si for and Co 53 (14%) in 50 mM NaCl + 2 mM Si. CAT activity was found low in ADT 53 (14%) in 25mM NaCl + 1.5 mM Si and Co 53 (16%) in 25 mM NaCl + 2 mM Si , 100 mM NaCl + 1.5mM Si. ADT 53 showed the on-par performance with salinity tolerant variety Co 53.

Key words: *Silicon, NaCl, salinity, morphology, physiology, biochemical, ADT 53 and Co53*

INTRODUCTION

Rice is considered a staple food crop across major countries worldwide. Rice covers a global area of 160 million hectares of land producing about 650 million tons of crop. India is the second-largest rice producer worldwide (USDA) (Pathak et al., 2020). In India alone, 6.74 million hectares of land are salt-affected (Razzaque et al., 2017). As an economically and industrially important crop of India, rice provides about 23% of total world rice production and 45% of the total Indian food grain production. (Bhambure et al., 2016). In Tamil Nadu, rice is grown in an area of 2.04 million hectares with a total production of 9.98 million tonnes (Umamageshwari, 2020). Saline soil is predominantly found in coastal districts of Tamil Nadu.

Salinity is the major abiotic stress which affects plant growth from seed germination to vegetative and reproductive development (Razzaque et al., 2017). It causes osmotic stress, oxidative stress, ion toxicity, nutrient deficiency and poor water and nutrient uptake from the soil. As a result of salinity-induced osmotic stress, water uptake by plant is affected and suffers from physiological drought. Under osmotic stress, regulation of water transport becomes a vital adaptive strategy of rice plant because a sufficient amount of water is indispensable for the cells to maintain their growth and cellular functions such as photosynthesis

and metabolisms. It also induces stomatal closure which results in the reduction of evaporation and water transport. A set of hormonal regulations also associated with these processes is upregulated many-fold under salt stress (Pathak et al., 2020). If high levels of sodium chloride (NaCl) are present on the external sides of the cell, sodium ion inactively moves towards the plant roots via nonspecific channels due to the poor affinities of sodium transporters. (Subramanyam, 2019 and Swain, 2018). Na and Cl concentrations are usually higher under saline conditions than other mineral elements resulting in reduced uptake of essential nutrients (Ca, Mg, Mn, and K). High levels of Na cause direct damage to plant cell membranes, disrupt cell metabolism, and impair plant growth, fertility, and free radical generation (Farouk, 2018)

The effect of salinity in plants interferes with the yield and production of the crops. Both Cl^- and Na^+ salts harm the rice root. The Cl^- activated damage is recognizable by the broad leaf cutting edge indicating burning while the accumulation of Na^+ causes leaf mottling and rolling. If heavy amounts of NaCl move inside via transpiration, it causes damage to the leaves, bringing about the additional decline of the developmental rate (Greenway and Munns, 1980).

Salinity stress induces stomatal closure resulting in increased temperature of the leaves and reduced elongation. It acts as a pro-oxidant and induces oxidative burst through hyper-accumulation of reactive oxygen species (ROS) that cause injuries to cellular ultrastructure, organic compounds and impaired a variety of metabolic reactions (Gupta et al., 2017; Ahmad et al., 2018; Kaya et al., 2018). The activities of antioxidant enzymes (CAT, APX, SOD and GPX) have been increased which provides a safeguard to the plants from damages caused by salt-induced reactive oxygen species (Li, 2011).

Silicon (Si) becomes a vital factor in plant protection against many biotic and abiotic stresses such as diseases, pests, drought, salinity and heavy metals toxicity (Alsaeedi et al., 2017a and Raven et al., 1983). It is considered a beneficial quasi-essential nutrient for plant growth and development when plants are grown under adverse environmental conditions (Adrees et al., 2015 and Bakhat et al., 2017). Si application under high saline stress induces resistance in plants against oxidative injury by suppressing ROS generation, regulating antioxidant enzyme activities (Kamran, 2020).

In the present study, two rice cultivars ADT 53 (salinity susceptible) and Co 53 (salinity tolerant) were exposed to different concentrations of NaCl, Silicon (Sodium metasilicate) and a combination of both NaCl and Silicon to investigate the effect of silicon on seed germination, morphological, physiological and biochemical characteristics under salinity stress. ADT 53 is a short-duration rice variety (110 - 115 days) and evolved from the parentage of ADT 43 / JGL 384. Co 53 is a short-duration variety (115 - 120 days) and evolved from the parentage of PMK (R)3 / Norungan.

MATERIALS AND METHODS

Sample collection

The rice seeds ADT 53 and Co 53 were collected from the Rice Research Station in Thiruvallur in Tamil Nadu. The seeds were grown in plant growth champer at 25°C under 16hr photoperiod. Morphological, physiological and biochemical characteristics were studied in Department of Biotechnology, Stella Maris College (Autonomous), Chennai.

Seed germination

Rice seeds ADT 53 and Co 53 were surface sterilized with 70% of ethanol for 2 minutes and 1% mercuric chloride for 10 minutes and then rinsed several times with distilled water. In Experiment 1, sterilized seeds were placed in petri plates with filter paper irrigated with different concentrations of NaCl and Si. In Experiment 2, sterilized seeds were inoculated in MS media prepared along with different concentrations of NaCl and Si (Kalaiyarasi et al., 2019). Rice seeds were kept for incubation at 25°C under dark for 3 days and then placed under 16h photoperiod. Salinity stress imposed for 15 days and also subjected to fresh water alone (control). The rate of germination was assessed in experiment 1 on the 3rd, 6th and 9th days after sowing and expressed in percentage (Xu et al., 2020).

Different concentrations of sodium chloride are as follows,

N1 - 25mM NaCl (EC: 25.6mS/cm)

N2 - 50mM NaCl (EC: 42.6mS/cm)

N3 - 75mM NaCl (EC: 59.1mS/cm)

N4 - 100mM NaCl (EC: 76.6mS/cm)

Different concentrations of Sodium metasilicate (Si) are as follows,

S1 - 1mM Si

S2 - 1.5mM Si

S3 - 2mM Si

Different concentration of NaCl + Si are as follows,

NS1 - 25mM NaCl + 1mM Si,

NS2 - 25mM NaCl + 1.5mM Si

NS3 - 25mM NaCl + 2mM Si

NS4 - 50mM NaCl + 1mM Si

NS5 - 50mM NaCl + 1.5mM Si

NS6 - 50mM NaCl + 2mM Si

NS7 - 75mM NaCl + 1mM Si

NS8 - 75mM NaCl + 1.5mM Si

NS9 - 75mM NaCl + 2mM Si

NS10 - 100mM NaCl + 1mM Si

NS11 - 100mM NaCl + 1.5mM Si

NS12 - 100mM NaCl + 2mM Si

MORPHOLOGICAL CHARACTERISTICS

Morphological characteristics such as shoot and root length were recorded.

Shoot and root length

The shoot and root length of both the rice cultivars were measured on the 15 days after the seed germination (Subramanyam et al., 2019).

PHYSIOLOGICAL CHARACTERISTICS

Physiological characteristics such as photosynthetic pigments and CSI (Cholophyll Stability Index) were observed in Experiment 1.

Photosynthetic pigments

100mg of fresh leaf sample was crushed in 20 ml of 80% acetone and extract centrifuged for 10 mins at 1000 rpm. The absorbance was recorded at 645, 663 and 470 nm in the spectrophotometer. The chlorophyll a, chlorophyll b and carotenoid were estimated (Arnon et al., 1949).

$$\text{Chlorophyll a } \left(mg/g^{-1}\right) = 12.7\left(A_{663}\right) - 2.69\left(A_{645}\right) \times \frac{V \times W}{1000}$$

$$\text{Chlorophyll B } \left(mg/g^{-1}\right) = 22.9\left(A_{645}\right) - 4.86\left(A_{663}\right) \times \frac{V \times W}{1000}$$

$$\text{Carotenoid} = 46.95\left(A_{470} - 0.268 \times \text{chl a + b}\right)$$

$$\text{Total chlorophyll } (a+b) = \frac{20.2\left(A_{645}\right) - 8.02\left(A_{663}\right) \times VW}{1000}$$

A- absorbance, V- Final volume of 80% acetone (in ml), W- Weight of plant tissue (in grams).

Chlorophyll stability index (CSI)

200mg of fresh leaf samples with 10 ml of distilled water is taken in a test tube. One set was left as control under room temperature and another set was kept in a water bath at 80°C for 30 minutes. The absorbance is recorded at 652 nm (Sairam et al., 1997).

$$TCC = \frac{A_{652} \times 1000 \times V}{3.45 \times 1000 \times W}$$

$$CSI\ (\%) = \frac{\text{Total cholorophyll content (heated)}}{\text{Total cholorophyll content(control)}} \times 100$$

BIOCHEMICAL CHARACTERISTICS

Biochemical characteristics such as proline, malondialdehyde and catalase activity were observed. in Experiment 1.

Proline

0.5g of a fresh leaf sample is homogenized in 2 ml of 3% of an aqueous sulfosalicylic acid solution and centrifuged at 3000 rpm for 10 minutes. 300µl of supernatant mixed with 300µl of glacial acetic acid and 300µl of acid ninhydrin. The mixture was incubated for 1 hr. in a boiling water bath at 100°C and cooled in an ice bath. 600µl of toluene is added and vortexed for 2

mins. The absorbance was measured at 520 nm against toluene (Bates et al., 1973).

Proline content = [(µg proline/ml) x ml toluene)/ 115.5 µg/µmole] / [(g sample)/5].

Malondialdehyde

500mg of fresh leaf sample homogenized with 10ml of 5% trichloroacetic acid. The extract is centrifuged at 4000 rpm for 10 mins. 2ml of supernatant mixed with 2ml of 0.6% Thiobarbituric acid. The mixture is kept in a boiling water bath for 10 minutes at 95°C. Absorbance reads at 532, 600 and 450nm separately. MDA content was calculated using an extinction coefficient of 155 mM^{-1} cm^{-1} and the results were expressed as nmol g^{-1} of FW (Draper et al. 1993).

MDA (nmol/ml) = Abs (test) - Abs (blank) / 1.56 x 1000000

Catalase activity

The leaf samples of about 1g were homogenized in the extraction buffer. The extraction buffer is composed of 50mM sodium phosphate buffer (pH 7.0), 0.1 mM EDTA, 5 mM beta-mercaptoethanol, 2% PVP and 1mM PMSF. The homogenate was centrifuged at 12,000 rpm for 20 mins and the supernatant was used to assay the enzyme activity. The reaction mixture continued 0.5 ml of 0.1 M sodium phosphate buffer, 1 ml of 3% Hydrogen peroxide (H_2O_2) and 1 ml of enzyme extract. After incubation at 250 C for 1 min, absorbance was recorded at 240 nm with a spectrophotometer (UV-Vis-SYSTRONICS). The decomposition of H_2O_2 was determined after absorbance decreased at 240 nm. The reaction was stopped by adding 3ml of 10% Sulphuric acid (H_2SO_4). The residual H_2O_2 was titrated against 0.02 (N) $KMnO_4$. The catalase activity was calculated using the extinction coefficient of 39.4 M^{-1} cm^{-1} and the enzyme activity was expressed in terms of mg H_2O_2 decomposed mg^{-1} protein min^{-1} (Velikova et al., 2000).

RESULTS AND DISCUSSION

SEED GERMINATION

Experiment - 1

The NaCl treatment N1(25mM), N2(50mM) and N4(100mM), Si treatment S1(1mM) and combined NS treatments NS1(25mM+1mM), NS5(50mM+1.5mM), NS6(50mM+2mM) achieved a maximum of 100% germination on the 6th day compared to control - fresh water (90%) in ADT 53. Most of the treatments achieved 100% germination in ADT 53 on the 9th day (Table 1; Fig. 1).

Among all the treatments N1(25mM) showed maximum germination percentage in Co 53 on the 9th day. Overall seed germination rate was greatly increased in ADT 53 when compared to Co 53 (Table 1). The Co 53 showed a gradual reduction in seed germination at different concentrations of NaCl and silicon. The germination rate in NS6 (50mM + 2mM) increased 90% whereas N2 (50mM) reduced 60% in Co 53 (Table 1; Fig. 2).

On the 9th day, complete germination of seeds was determined in both the rice varieties. NaCl treatment N1 showed the best germination rate in ADT 53 and Co 53 (Table 1).

Experiment - 2

ADT 53 and CO 53 were surface sterilized and inoculated into MS media with different concentrations of NaCl, Si, and NaCl + Si. The growth of both the rice cultivars enhanced in MS medium when compared to

Table 1. Seed germination percentage for ADT 53 and Co 53 under different concentration and time intervals.

Different treatments	Seed germination percentage (%)					
	ADT 53			Co 53		
	Day 3	Day 6	Day 9	Day 3	Day 6	Day 9
Control	90	90	100	60	80	80
N1	90	100	100	70	90	100
N2	80	100	100	50	60	60
N3	80	90	90	60	70	70
N4	70	60	50	60	50	50
S1	90	100	100	60	70	70
S2	90	90	100	60	70	80
S3	90	90	100	50	60	70
NS1	70	100	100	70	80	80
NS2	30	60	90	30	40	60
NS3	70	90	90	70	70	70
NS4	80	80	80	60	60	80
NS5	60	100	100	40	50	60
NS6	90	100	100	90	90	90
NS7	70	80	90	50	60	60
NS8	30	60	80	50	60	80
NS9	80	90	100	20	50	70
NS10	60	70	90	50	50	60
NS11	40	70	90	50	60	80
NS12	40	70	90	40	60	70

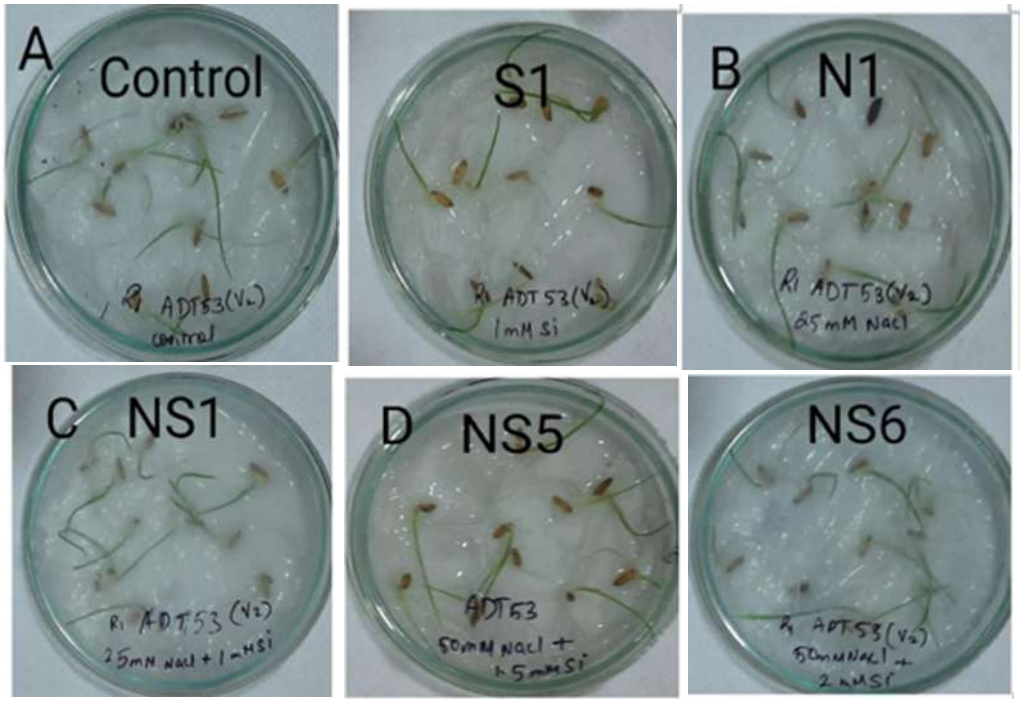

Fig. 1. Seed germination of ADT53 in different concentrations of NaCl, Si and NaCl + Si. Control and Si (A), NaCl (B) and NaCl + Si (C, D).

the plant irrigated with different treatments.

Similarly, Kalaiyarasi et al. 2019 reported that the germination percentage decreased with the increasing salt concentration. ADT 45 shows a maximum seed germination rate at 200mM NaCl and higher germination rate at 150mM NaCl. Seeds soaked with 20mM silicon showed the highest germination rate in rice (Xu et al., 2020).

MORPHOLOGICAL CHARACTERISTICS

Shoot and root length

Experiment - 1

The maximum shoot length was recorded in NS4-50 mM + 1 mM (6.54 cm) followed by NS12-100 mM + 2 mM (6.4 cm), NS6-50 mM + 2 mM (6.18 cm) when compared to control (6.14 cm) in ADT 53 (Fig. 3A).

In Co 53, increased shoot length was observed in NS3 - 25 mM + 2 mM (10 cm) followed by NS9-75 mM + 2 mM (9.3 cm), NS4-50 mM + 1 mM (8.62 cm) and NS2-25 mM + 1.5 mM (8.5 cm) compared to control (7.4 cm). The Si treatment resulted in gradual increase in S1 (1 mM), S2 (1.5 mM) and S3 (2 mM) by 7.86, 8.28 and 7.7 cm respectively (Fig. 4).

The shoot length of ADT 53 showed no significant difference in NaCl and combined treatments. Addition of silicon with NaCl increased shoot length in NS3-25 mM + 2mM (10 cm) and NS9 - 75mM + 2 mM (9.3 cm) compared to N1 - 25mM (5.9cm) and N3-75 mM (6.13 cm) in Co 53. The shoot length of Co 53 is greatly increased when compared to ADT 53. NS4 recorded maximum shoot length in ADT 53 (6.54 cm) and NS3 in Co 53 (10 cm) (Fig. 3 A, C and Fig. 4).

Sienkiewicz-Cholewa et al., 2018 reported, the length of the shoot is considerably reduced 50% in 100 mM NaCl and root length reduced 70% in 100 mM NaCl compared to control. The Si application increases the biomass of shoot and root length by 1.5 mM Si. The concentration of 1.5 mM Si efficiently alleviates the saline stress. The addition of 1mM silicon in arsenic treatment increases the shoot length and biomass by 10% and 20% (Preeti et al., 2012). The shoot length of ADT 45 increased at 150 mM and 200 mM NaCl (6 and 3 cm). The root length of ADT 45 and BPT 5204 increased (5 and 4 cm) at 200 mM NaCl (Kalaiyarasi

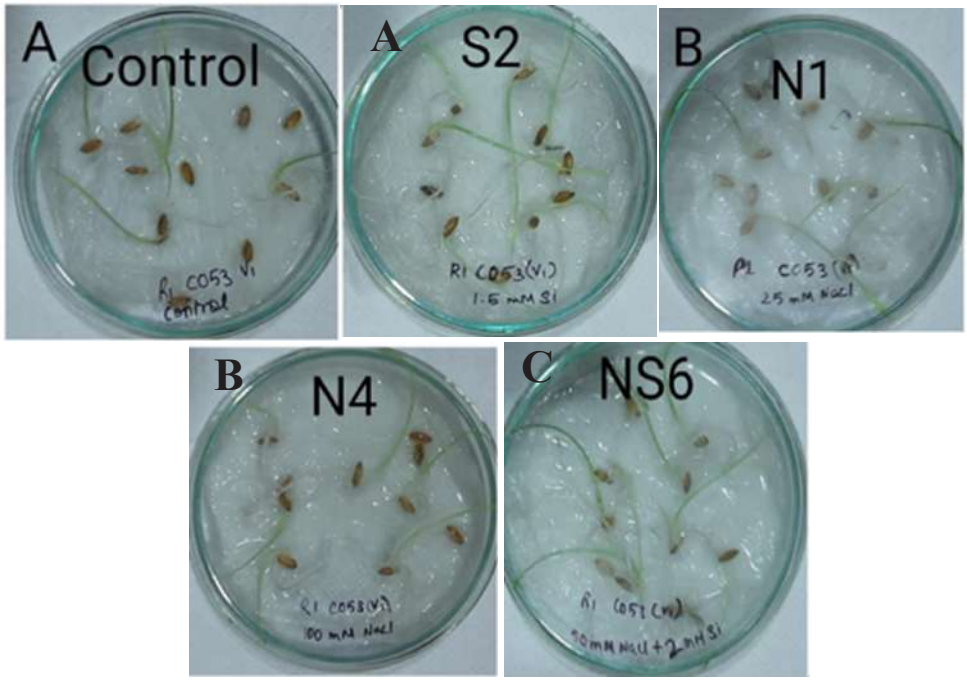

Fig. 2. Seed germination of CO 53 in different concentrations of NaCl, Si and NaCl + Si. Control and Si (A), NaCl (B) and NaCl + Si (C).

et al., 2019).

The increased root length was observed in NS6 (12.9 cm) followed by S1 (11.7 cm), S2 (10.46 cm), N1 (9.04 cm) and NS4 (9 cm) compared to control (8.58 cm) in ADT 53 (Fig. 4 B). In Co 53, root length was increased in NS5 (10 cm) followed by NS8 (9.48 cm), S1 (9.4 cm), NS3 (8.4 cm) and NS6 (8.4 cm) when compared to control (6.6 cm) (Fig. 5).

The silicon (S1 and S2) in both varieties result in a maximum root length. The combined treatment increased the root length of ADT 53 in NS4 (12.9 cm) and NS10 (7.7 cm) when compared to N2 (8 cm) and N4 (4.6 cm) (Fig. 6). In Co 53, root length was increased in NS3 (8.4 cm) when compared to N1 (5.64 cm). The higher root length is observed in NS4 for ADT 53 (12.9 cm) and NS5 for Co 53 (10 cm). The root length of ADT 53 was increased as compared to Co 53 (Fig. 3 A, D and Fig. 5).

Experiment - 2

The maximum increases of shoot length were observed in N2 for ADT 53 and NS8 for Co 53 (20.2 and 24.5 cm). The maximum increase of root length was observed in both ADT 53 and Co 53 in NS12 (16.4 and 14.2 cm) (Fig. 6). The shoot and root length of ADT53 and Co 53 was rapidly increased in experiment 2 when compared with experiment 1.

Kalaiyarasi et al., 2019 reported that the germination percentage was decreased with increasing salt concentration. At 150mM NaCl concentration ADT 45 showed maximum seed germination.

PHYSIOLOGICAL CHARACTERISTICS

Photosynthetic pigments

Physiological activities under the influence of salinity include changes in membrane permeability leading to destabilization of membrane proteins and reduction in the process of phothosynthesis.

The inhibitory effect of Si on NaCl uptake by plants leads to improved leaf chlorophyll content (Xu et al., 2020).

Chlorophyll a

The chlorophyll-a content was increased in NS1(33.3%) followed by S1(30%), NS3 (30%) as compared to

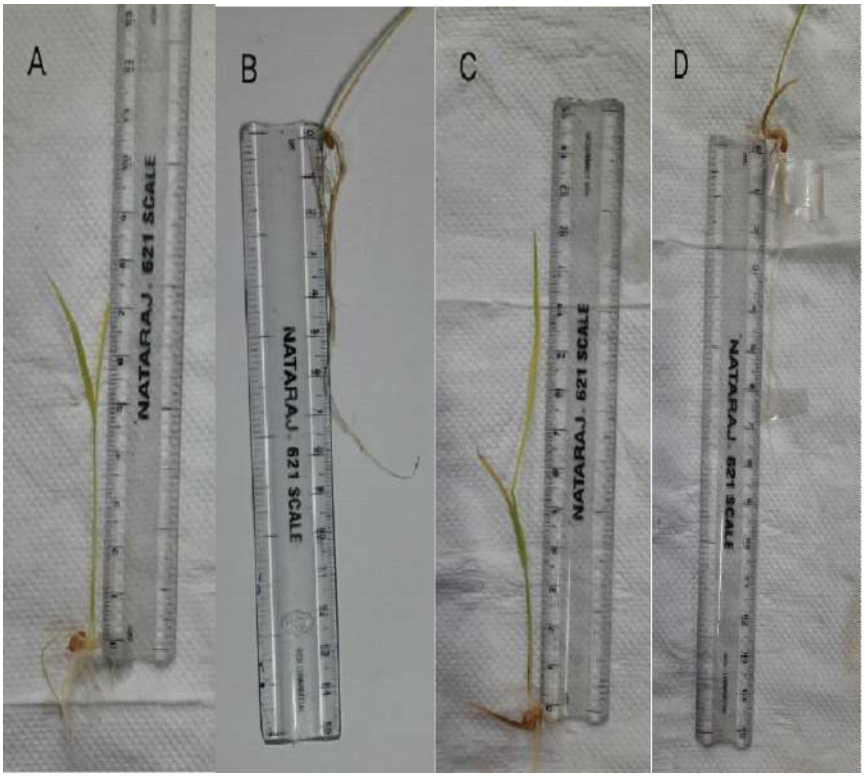

Fig. 3. Shoot length of ADT 53 in NS4 (7.8 cm) (A), Root length of ADT 53 in NS6 (12.9 cm) (B), Shoot length of CO 53 in NS3 (12.6 cm) (C) and Root length of CO 53 in NS5 (10 cm) (D).

control (32%) in ADT 53 (Fig 7 C and E). The application of silicon along with NaCl increased chlorophyll a in NS1 (33.3%) and NS3 (30%) compared with N1 (0.17%) (Fig. 7 A, E and F).

In Co 53, chlorophyll-a was increased in NS9 (30%) and NS12 (30%) as compared to control (23%). The combined treatment increased the chlorophyll a (30%) in NS9 when compared to N3 (12%). (Fig. 7 B, D, G and H).

Khan et al., 2018 reported Si with NaCl increases chlorophyll-a in maize genotype EV1089 when compared to the same variety treated with NaCl alone. The silicon concentration 15 mM Si increases pigment content after 48 hrs in saline condition (Xu et al., 2020)

Chlorophyll b

The chlorophyll b was increased in control (114%) followed by NS3 (104%), N3 (96%), NS6 (95%) and N4 (89%) in ADT 53. N3 (96% and 89%) showed the increased chlorophyll b when compared to combined treatment NS9 (75%), NS11 (30%) and NS12 (23%) (Fig. 7 A, E and F).

The chlorophyll b was increased in Co 53, S3 (110%), NS4 (103%), NS9 (105%) when compared to control (80%) (Fig. 7 D, G and H) (Fig 7 B and G). It is increased by 110% (S3) in Co53 when compared to other treatments (Fig. 7 D). NS3 (104%) for ADT 53 and NS9 (105%) for Co 53 recorded high percentage of chlorophyll b pigment (Fig. 7 E and H).

Khan et al. 2018 reported chlorophyll b pigment increased 6 folds in silicon treatment under the saline condition in Syngenta 8441 genotype. Xu et al., 2020 observed that 10 and 15 mM Si increased chlorophyll b at 24h after salinity stress.

Total chlorophyll content

Total chlorophyll content recorded maximum in S1 (230%) followed by control (147%), NS3 (133%), N3 (122%), NS6 (110%) in ADT 53 (Fig. 7 A, C, E and F). N1, N2, N3 and N4 resulted in a maximum increase of 104%, 99%, 122% and 109% in total chlorophyll content (Fig. 7 A). S1 results in a higher value (230%) of total chlorophyll content in ADT 53 (Fig. 7 C).

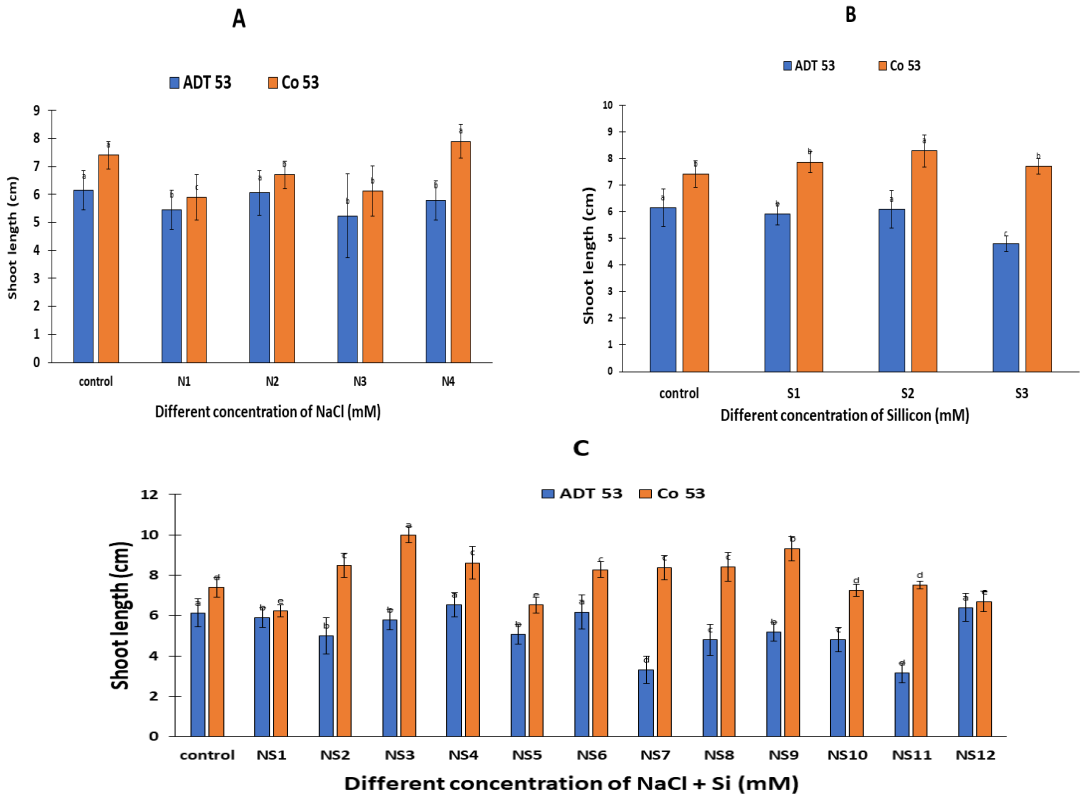

Fig. 4. Effect of different concentrations of NaCl, Si and NaCl + Si on shoot length. Shoot length of ADT 53 and CO 53 in (A) NaCl (B) Silicon (C) combination of NaCl + Si. Bars represent the mean values of three replication with standard error. Different letters indicate significantly different values between treatments ($P \leq 0.05$).

In Co 53, total chlorophyll content in S2 (130%) followed by NS9 (130%), NS12 (122%) and NS4 (116%) compared to control (103%) (Fig. 7 B, G and H). NS4 showed increase in total chlorophyll by 116% when compared to N2 (69%) in Co 53. The combined treatment increased the total chlorophyll content in ADT 53 by 133% in NS3 (Fig. 7 B, G and H).

A similar result was reported by Farooq et al., 2018. The combined stress NaCl and B toxicity reduced photosynthetic pigment by 65% and Si application did not provide a significant improvement in pigment under combined stress. 100mM NaCl results in a reduction of total chlorophyll content. The highest concentration of total chlorophyll pigment was observed in 100mM NaCl and Si. The addition of Si enhances 30% of the total chlorophyll content compared to a plant grown in saline condition without silicon (Sienkiewicz-Cholewa et al., 2018). Saleh et al. 2019 reported accumulation of Na^+ and Cl^- ions cause a decrease in chlorophyll content. Silicon application at 300 mg kg^{-1} increased leaf chlorophyll content.

Carotenoid

The carotenoid pigment recorded high in control (280%) as compared to other treatments NS11-240%, NS5-215%, NS8-213% in ADT 53. NS11-240% and NS5-215% showed high carotenoid content when compared to N2-113% and N4-113% (Fig. 7 A, E and F).

In Co 53, carotenoid was increased in NS3-273%, NS4-260%, NS9-260%, NS12-260%, NS10-230%, NS7-224%, N3-222%, NS11-220% when compared to control (127%) (Fig. 7 B, G and H). The combined treatment recorded an increase in NS4-260% and NS5-198% compared to N2-87%.

In both varieties combined treatment showed the gradual increase of carotenoid. A higher range of

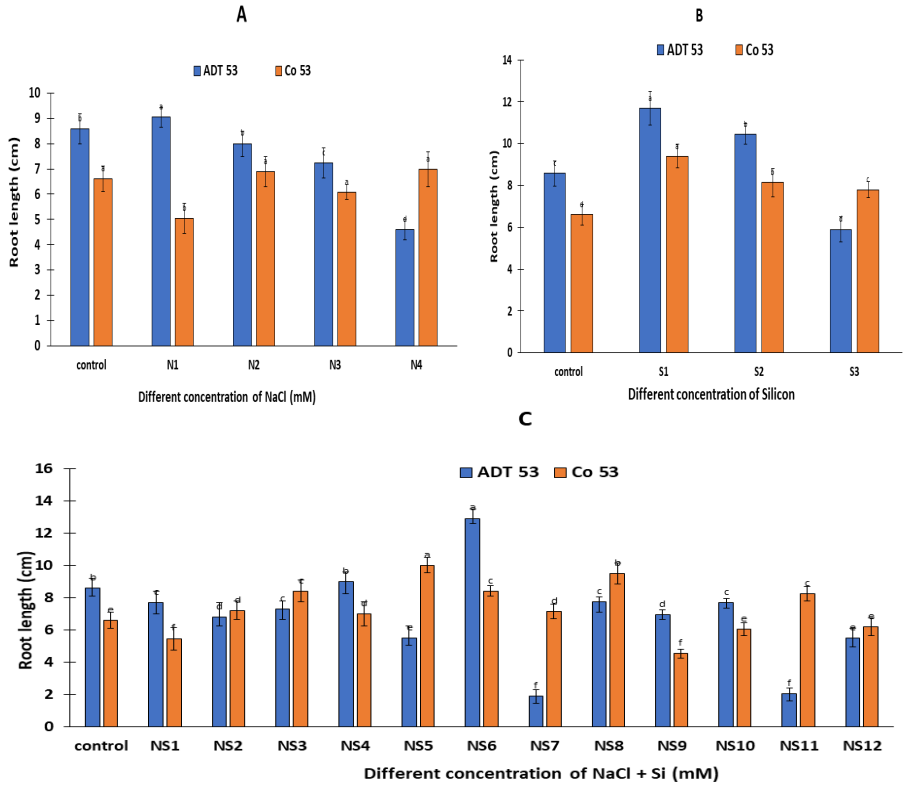

Fig. 5. Effect of different concentrations NaCl, Silicon and NaCl + Si on root length. Root length of ADT 53 and CO 53 in (A) NaCl (B) Si (C) combination of NaCl + Si. Bars represent the mean values of three replication with standard error. Different letters indicate significantly different values.

carotenoid (273%) was observed in NS3 in Co 53 (Fig. 7 E, F, G and H).

X u et al., 2020 reported that 10 or 15mM Si pretreatment significantly increased carotenoid content after 48hr exposure in salinity stress. Abdel-Haliem et al., 2017 reported that the silicon treatment under alkaline stress increases the carotenoid pigment by 40.74% at 1.5 mM Si concentration.

Overall photosynthetic pigments are recorded in a higher range when compared to chlorophyll a, chlorophyll b and total chlorophyll content. In ADT 53, S1 improved total chlorophyll content compared with carotenoid. The rice cultivar Co 53 shows an increased total chlorophyll and chlorophyll b content in S3 compared to carotenoid pigment. The chlorophyll-a shows a drastic reduction in both the varieties in all the treatments.

Chlorophyll stability index (CSI)

The chlorophyll stability index resulted in higher range in control (222.8%) followed by NS12 - 185.7% and NS9 - 173.14% in ADT 53 (Fig. 9 C). In Co 53, the CSI recorded the maximum in N4 - 388%, N1 - 300%, N3 - 266.53% as compared with control (238.8%). The silicon treatment has not shown a significant increase in CSI (Fig. 8 B). Among all the combined treatments in Co 53, NS11 results in an increase of 205.38% in CSI (Fig. 8 C).

BIOCHEMICAL CHARACTERISTICS

Proline

Proline content in leaves significantly increased in response to salinity stress when compared to control. In ADT 53, N3 showed the maximum proline content (105%) (Fig. 9 A). The combined treatment decreased

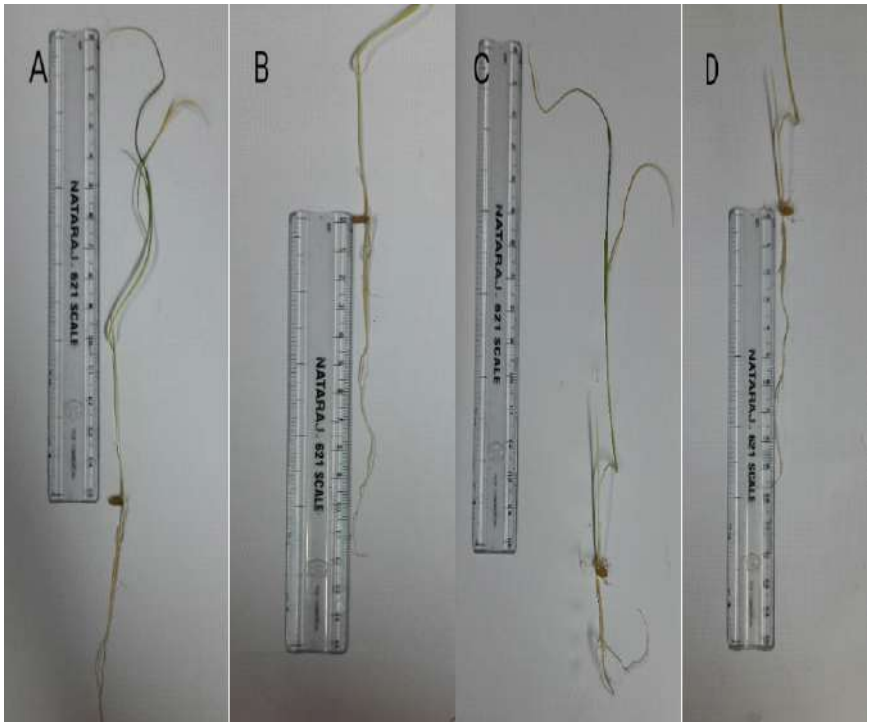

Fig. 6. The root and shoot length of rice in MS medium. Shoot length of ADT 53 in N2 (A), root length of ADT 53 in NS12 (B), Shoot length of Co 53 in NS8 (C) and root length of Co 53 in NS12 (D).

the proline by 34% in NS7 and 62% in NS9 (Fig. 9 C). Si reduces the proline accumulation (17%) (Fig. 9 B). Among the treatments, the accumulation of proline was reduced by the addition of silicon in combined treatments NS1(18%), NS2 (13%) and NS3(18%) (Fig. 9 C).

The highest range of proline content was observed 111% in N4 in Co 53 (Fig. 10A). NS12 and NS10 showed reduced the proline content by 11% and 32% respectively compared to N4 (111%) (Fig. 9C). The Si reduced the proline content by 16% and 12% in S1and S2 (Fig. 9B). Application of silicon under saline stress reduces proline production in NS1 (12%), NS8 (13%), NS9 (14%) and NS12 (11%) (Fig. 9C). Si reduced the proline production in both the rice varieties. Among the combined treatments, NS2 for ADT 53 and NS12 for Co 53 resulted in a significant decrease in proline (Fig. 9C).

The increased NaCl 100 mM causes a higher accumulation of proline in leaves. The addition of silicon to 75mM and 100mM NaCl saline stress considerably decreased the proline content (Sienkiewicz-Cholewa et al. 2018). Saleh et al. 2019 reported a decline in proline content due to the exogenous application of silicon in saline stress. Si pretreatment for 5 days with 15mM Si reduced the proline content after exposure to salinity stress (Xu et al. 2020). Nxele et al. 2017 reported the total proline content increased 63% in salinity stress, accumulation of proline concentration was higher in saline conditions. The Na2SeO4 treated plant under saline condition resulted in increased proline content compared to the plant grown under NaCl stress alone (Subramanyam et al., 2019).

Malondialdehyde (MDA)

The maximum MDA content was observed in NS8 (4.27), N3 (3.87), NS4 (3.66), NS12 (3.54), NS9 (3.41) and S2 (1.5) compared to control (2.32) in ADT 53 (Fig. 10 A, B and C). The application of Si reduced the MDA in NS7 (2.70) and NS11(1.08) compared to N3 (3.84) and N4 (2.98) (Fig. 10 A and C).

In Co 53 the maximum MDA content was observed in NS12 (5.74) followed by NS9 (4.53), NS5 (3.92), NS10 (3.7) and N3 (3.46) compared to control (2.54) (Fig. 10 A and C). The reduction of MDA was

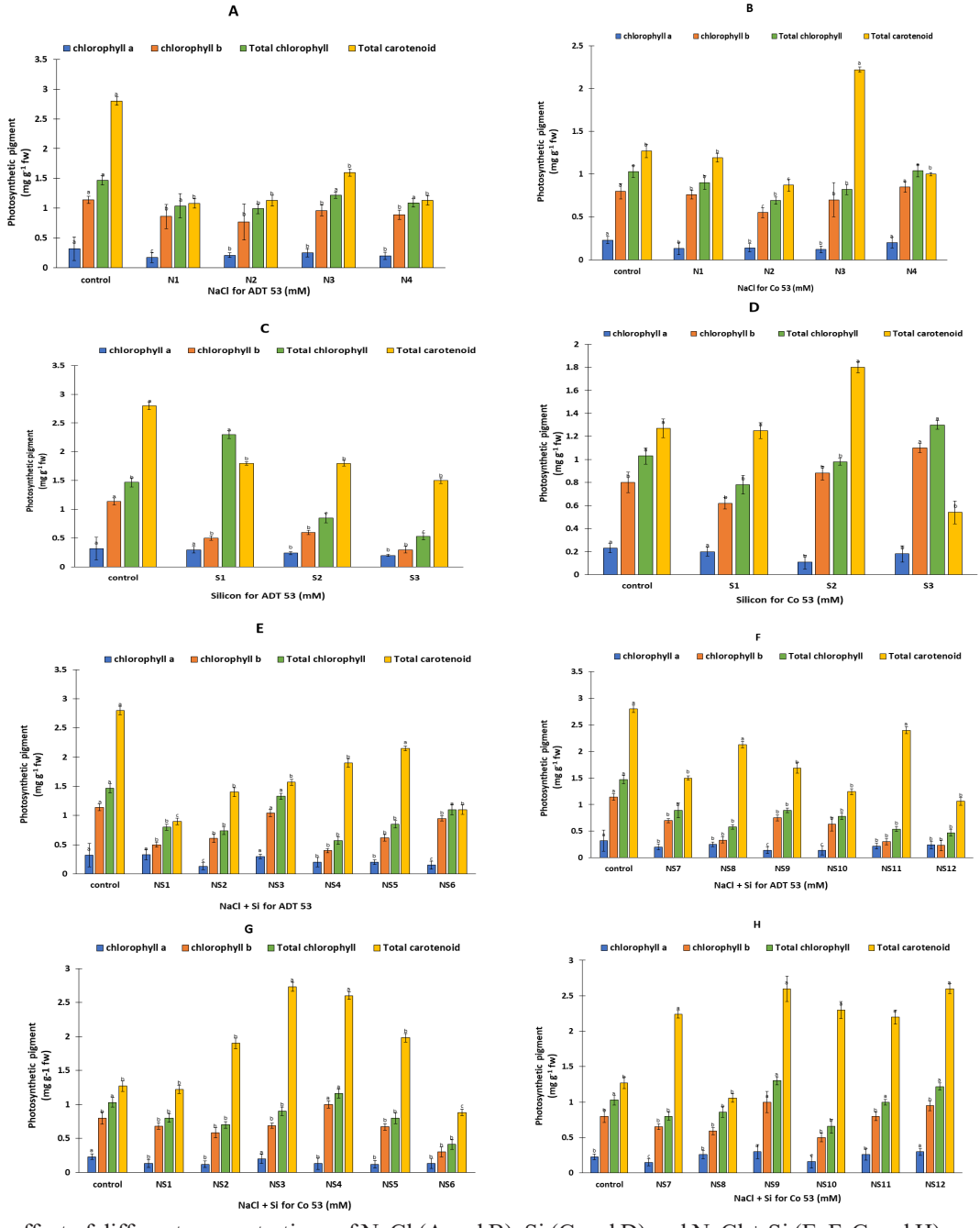

Fig. 7. The effect of different concentrations of NaCl (A and B), Si (C and D) and NaCl + Si (E, F, G and H) on chlorophyll a, chlorophyll b, total chlorophyll and carotenoid content of ADT 53 and CO 53. Bars represent the mean values of three replication with standard error. Different letters indicate significantly different values between treatments ($P \leq 0.05$).

observed in NS7 (1.52) and NS11(2.56) when compared to N3 (3.46%) and N4 (3.03%) (Fig. 10 C).

In both rice varieties some of the treatments NS2, NS3 and NS6 showed a similar reduction in MDA (Fig. 10 C).

Sienkiewicz-Cholewa et al. 2018 observed the MDA concentration increased 43% at 100 mM NaCl stress. Si -1.5 mM causes a greater decrease in MDA content (Sienkiewicz-Cholewa et al., 2018). Preeti et al., 2012 reported 1 mM Si reduces MDA content in

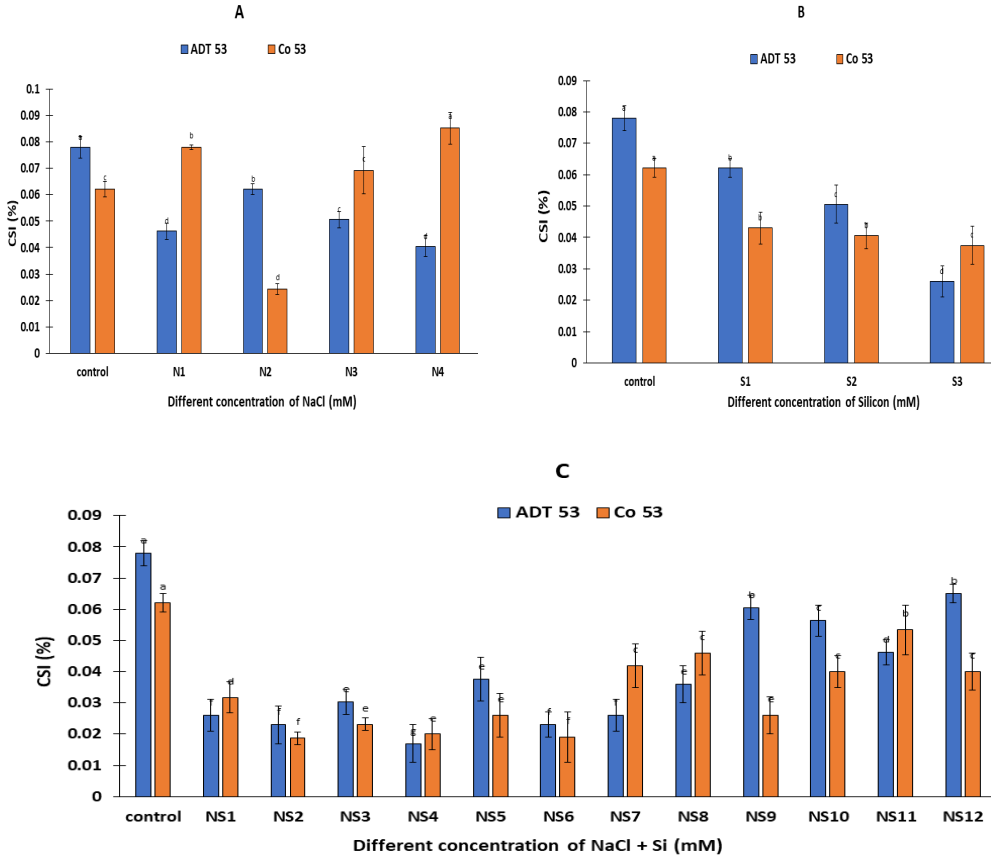

Fig. 8. The effect of different concentrations of NaCl (A), Si (B)and NaCl + Si (C) on CSI (%) content of ADT 53 and CO 53. Bars represent the mean values of three replication with standard error. Different letters indicate significantly different values between treatments ($P \leq 0.05$).

plants grown in arsenic stress by 18% in Triguna and 13% in the IET-4786 rice. The Si pretreatment with 10 and 15mM reduced MDA content (43% and 55%) after exposure to saline condition for 5 days (Xu et al., 2020). MDA content in leaves increased 106% in salinity stress (Nxele et al., 2017).

CAT activity

CAT activity showed maximum in NS11 (5.17) followed by NS7 (4.87), NS6 (4.47), NS12 (4.4), N3 (4.24) compared to control (2.15) in ADT 53 (Fig. 12 A and C). The application of Si reduced the CAT activity in NS2 (1.14) followed by NS5 (2.43) and NS3 (2.17) in ADT 53 compared to N3 (4.43), N2 (4.24) and N4 (4.21) (Fig. 11 C).

In Co 53, the maximum CAT activity was observed in NS8 (5.77) followed by S3 (5.12), NS10 (4.93), N3 (4.33), N2 (4.21), S2 (4.15) compared to control (2.28) (Fig. 11 A, B and C). The application of Si reduced the CAT activity in NS3 (1.56) followed by NS11 (1.86), NS2 (2.17), NS1(2.19) and NS6 (2.42) in Co 53 compared to N3 (4.33) and N2 (4.21) (Fig 11 A and C). In both the variety combined treatment NS3 showed reduction in CAT activity (Fig. 11 C).

Na_2SeO_4 treated plants under NaCl stress reported increased CAT activity in the mode III of combination of seed priming and foliar (82.9%), mode I foliar spray (43.3), mode II seed priming (56.3%) when compared to control (Subramanyam et al. 2019). The improved activity of CAT resulted in P1574 (15%) and Hycorn 11 (13.3%) maize cutivars under saline condition with application of silicon (Ali et al., 2020). Saline condition greatly increases the CAT activity in Skha 1 rice cultivar with or without application of nano silica

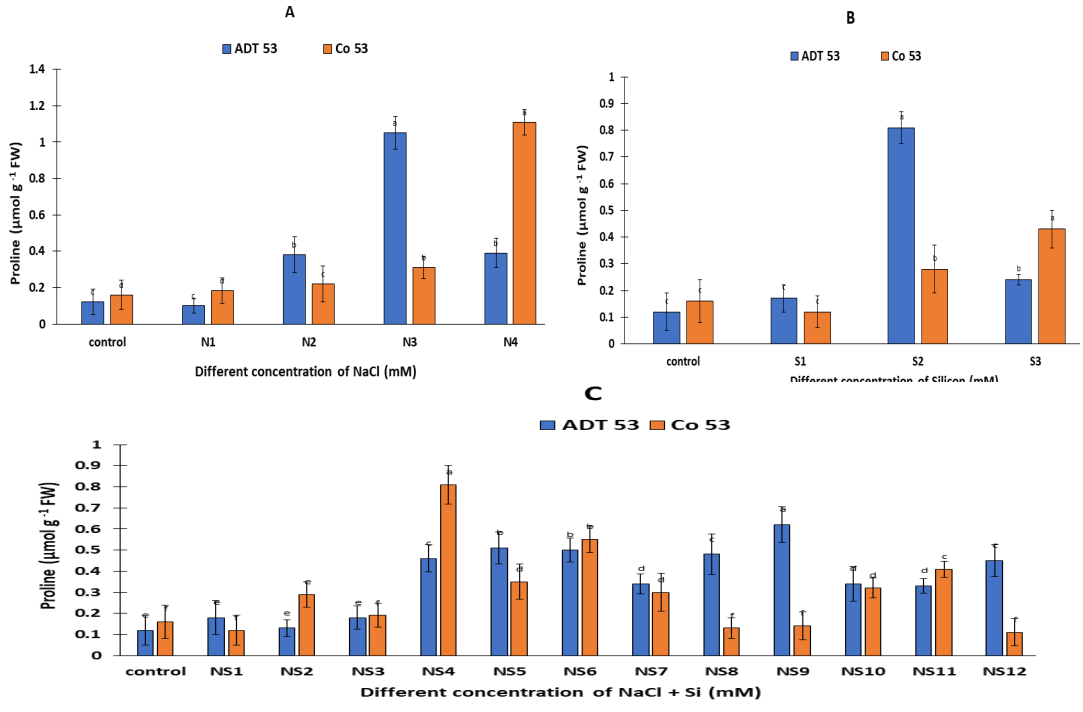

Fig. 9. The effect of different concentrations of NaCl (A), Si (B) and NaCl + Si (C) on proline content (µmol g^{-1} FW) of ADT 53 and CO 53. Bars represent the mean values of three replication with standard error. Different letters indicate significantly different values between treatments ($P \leq 0.05$).

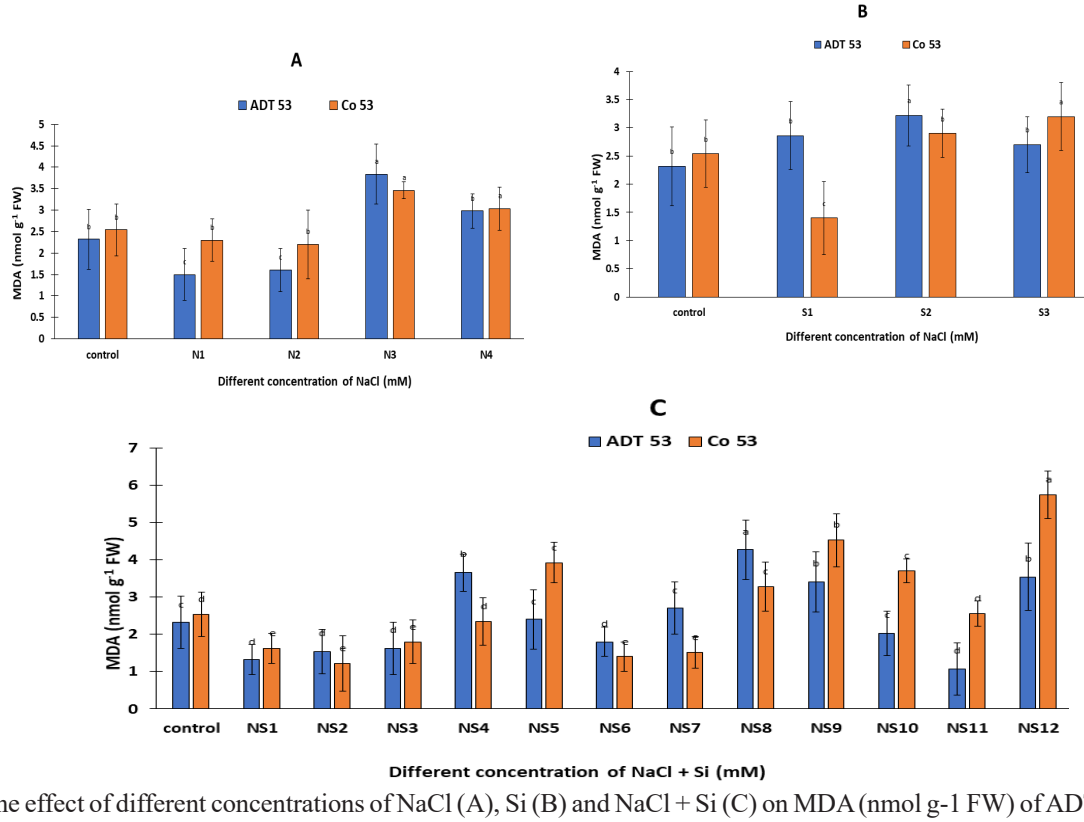

Fig. 10. The effect of different concentrations of NaCl (A), Si (B) and NaCl + Si (C) on MDA (nmol g-1 FW) of ADT 53 and CO 53. Bars represent the mean values of three replication with standard error. Different letters indicate significantly different values between treatments ($P \leq 0.05$).

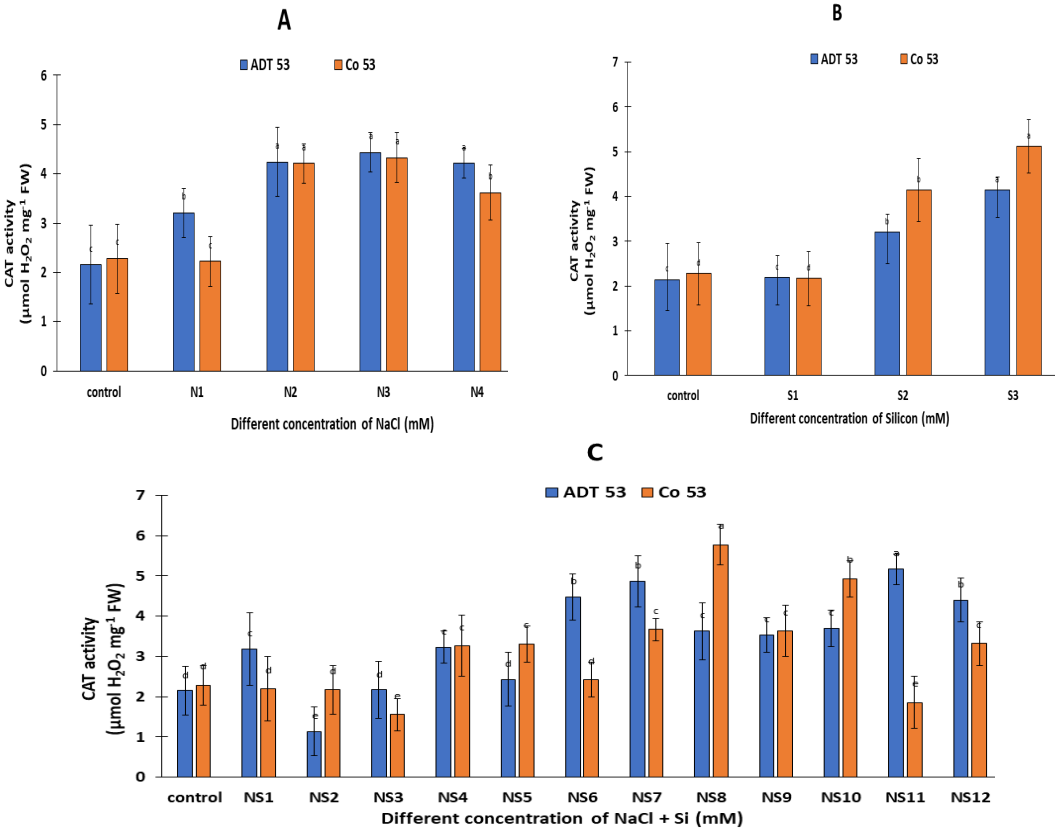

Fig. 11. The effect of different concentrations of NaCl (A), Si (B) and NaCl + Si (C) on CAT activity (µmol H_2O_2 mg^{-1} FW) of ADT 53 and CO 53. Bars represent the mean values of three replication with standard error. Different letters indicate significantly different values between treatments ($P \leq 0.05$).

(Abdel-Haliem et al., 2017). CAT activity gradually decreased in application of silicon under saline condition and boron toxicity in IR29 rice cultivar (Farooq et al. 2015). MTU 1010 rice cultivar resulted reduced CAT activity (43%) at 100µM arsenic concentration (Choudhury et al., 2011).

CONCLUSION

The findings of the present study suggested that salinity stress affected the seed germination, morphological, physiological, and biochemical characteristics in rice plants. In addition, silicon pre-treatment significantly improved several important morpho-physiological (shoot and root length and photosynthetic pigments) and biochemical (proline and MDA and CAT) characteristics in salinity susceptible variety ADT 53. Thus, varying levels of Si application might be beneficial in recovery of the salinity - impacted rice plants and thus safeguarding salinity - induced yield loss of rice in the saline-prone areas.

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Effect of nitrogen level on growth, yield attributes and yield of hybrid varieties of rice (*Oryza sativa* L.)

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ABSTRACT

A field experiment was conducted during two consecutive seasons of kharif 2020 and 2021 at Agronomy Research Farm, Acharya Narendra Deva University of Agriculture and Technology, Kumarganj Ayodhya (U.P.) to evaluate the effect of nitrogen level on growth, yield attributes and yield of different hybrid varieties of rice (Oryza sativa L.). The experiment was conducted in factorial randomized block design which comprised of 4 levels of nitrogen viz., 0 % RDN, 50 % RDN, 75 % RDN and 100 % RDN and 4 varieties viz., Arize-6444 Gold, Ankur-7576, 27P31 and Shahi-Dawat. Experiment was replicated three times. Results revealed significantly higher growth parameters, yield attributes and yield at 100% of RDN (150 kg N ha^{-1}) which is at par with 75 % RDN (112 kg N ha^{-1}) and significantly superior over 0 % RDN and 50 % RDN (75kg N ha^{-1}) were increased at all stages except days taken to 50% flowering. The growth parameters, yield attributes and yield were increased significantly with Ankur-7576 variety except for length of the panicle (cm) and test weight (g). Ankur-7576 gave a good response in low level of nitrogenous fertilizer and showed good efficiency in utilization of available and applied nitrogen to the crop and is best suitable for obtaining higher yield of hybrid rice.

Key words: *Growth parameters, hybrid rice, nitrogen levels, yield attributes*

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important cereal crops in the world and it is the staple food in South-East-Asia at present more than half of the world's population depends on this crop (Tahir et al., 2007). It has been suggested that by 2025, global rice production must increase by more than 50% for mid-1990 levels to meet that demand (Peng and Yang, 2003; Walker et al., 2008). Rice cultivation in India is predominantly practiced under the transplanting method that involves raising, uprooting and transplanting seedlings. This technique requires continuous ponding of water. The slogan "Rice is life" is most appropriate for India as this crop plays a vital role in our national food security and is a means of livelihood for millions of rural households. This has to be done against the backdrop of declining natural resource bases such as land, water, labour and other inputs and without adversely affecting the quality of environment. There is an urgent need to adopt some innovative technologies to break the yield ceiling in rice.

Among the available technological options to enhance rice production and productivity, hybrid rice is the most practically feasible and readily adoptable technology. To realize the maximum possible benefits of heterosis and to obtain a higher yield, it is essential to adopt recommended package of practices for the successful cultivation of rice hybrids.

Among the essential nutrients, nitrogen is one of the most important major essential nutrients which is required in large quantities for rice crop. A major reason for nitrogen limitation in these systems is low recovery efficiency of applied N (Fageria et al., 2011). Nitrogen is one of the most important plant nutrients and plays a vital role in plant photosynthesis and biomass production. Increasing panicle numbers in per unit area is the main factor of yield increment as a result of

nitrogen application (Bindra et al., 2000; Laroo and Shivay, 2011). Nitrogen is mostly lost by leaching, gaseous loss through volatilization and surface run off. Now a day's consumption of N fertilizer is in increasing trend, but its use efficiency is low in most of the production systems. Nitrogen is the most important and yield-limiting nutrient in rice production worldwide (Lin et al., 2006). Hence, experiment was conducted to assess the suitable dose of nitrogen and varietal performance of different hybrid varieties of rice for higher growth, yield attributes and yield.

MATERIALS AND METHODS

A two-year field experiment was conducted during *kharif* season of 2020 and 2021 at Agronomy research farm of Acharya Narendra Deva University of Agriculture and Technology, Kumarganj, Ayodhya, Uttar Pradesh, India (26^0.47' N, 82^0.12' E, 113 m MSL). The climate of the site is semi-arid type with hot summer and cold winter with average rainfall received during the cropping period (June-September) was 853.8 mm. The soil of the experimental field is silty loam in texture having slightly alkaline in reaction (pH 8.11), low in organic carbon (0.35%) and available nitrogen (145.81 kg ha^{-1}), medium in available phosphorus (13.71 kg ha^{-1}) and available potassium (247.21 kg ha^{-1}).

The experiment was conducted in a factorial randomized block design (FRBD) with 16 treatment combinations and three replications. Four levels of nitrogen as N_1: 0% of RDN; N_2: 50% of RDN (75kg ha^{-1}); N_3: 75% of RDN (112 kg ha^{-1}) and N_4: 100% of RDN (150 kg ha^{-1}), while Four varieties V_1: Arize -6444 gold; V_2: Ankur-7576; V_3: 27P31 and V_4: Shahi Dawat were taken. After 25 days, old seedlings were removed carefully from nursery bed for transplanting in the experimental plots. Two seedlings were transplanted in each hill with plant geometry of 25 cm and 10 cm. Recommended dose of N, P and K @ 150: 75: 75 kg ha^{-1} (full doses of P_2O_5 and K_2O were applied at the time of transplanting along with 50% of N and rest 25% of N at active tillering stage and 25% of at panicle initiation stage as top dressing) were applied during the experiment.

The hybrids namely Arize -6444 gold, Ankur-7576, 27P31 and Shahi Dawat were tested for various levels of Nitrogen. The recommended agronomical practices were adopted to raise healthy crop for conducting the experiment. The Phosphorous, Potash were applied in full dose and Zn were applied @ 25 kg ha^{-1}. The nutrients N, P and K were supplied through the chemical fertilizer urea, single super phosphate and muriate of potash, respectively.

RESULTS AND DISCUSSIONS

Growth indices

Different nitrogen levels were found to have a significant (p=0.05) effect on plant height in different hybrid rice varieties. (Table 1). Plant height increased successively with an increase in nitrogen levels in different varieties. Application of 100% RDN (150 kg N ha^{-1}) recorded more height (111.3 cm) compared to 75% RDN (112 kg N ha^{-1}), while recorded less height (94.8 cm) at 0 % of RDN. The highest plant height (108.8 cm) was recorded by Ankur-7576 variety followed by Arize 6444 gold (104.9 cm) and the lowest plant height (100.4 cm) was recorded by Shahi Dawat variety. Similar effects of hybrid rice varieties and nitrogen levels in accelerating the height of rice plant have also been reported by Mishra et al. (2014) and Kant et al. (2018). Total number of tillers $hill^{-1}$ recorded at maturity was significantly (p=0.05) affected by different nitrogen levels in hybrid varieties of rice (Table 1). The higher number of tillers hill-1 (9.8) was recorded

Table 1. Growth and yield attributes of transplanted Rice as affected by Nitrogen levels and hybrid varieties. (Average two-year data).

Treatments	Plant ht. (cm) at harvest	No. of total tillers $hill^{-1}$	Days taken to 50% flowering	No. of effective tillers $hill^{-1}$	No. of effective tillers m^{-2}
Nitrogen level					
0% RDN	94.8	8.6	97.3	6.3	250.7
50% RDN	100.9	9.0	97.5	7.2	287.4
75% RDN	108.6	9.7	97.6	8.0	310.6
100% RDN	111.3	10.3	97.7	8.6	330.7
SEm ±	1.11	0.12	1.30	0.43	9.25
CD (p=0.05)	3.22	0.35	NS	1.25	26.82
Hybrid Varieties					
Arize 6444 Gold	104.9	9.6	96.4	7.8	312.4
Ankur 7576	108.8	9.8	103.5	8.9	315.7
27 P 31	103.6	8.8	94.4	6.7	264.0
Shahi Dawat	100.4	9.2	95.7	7.3	287.4
SEm ±	1.11	0.12	1.30	0.43	9.25
CD (p=0.05)	3.22	0.35	3.77	1.25	26.82

by Ankur 7576 variety followed by Arize 6444 gold (9.6) at 100% of RDN. The lowest number of tillers hill-1 (8.8) was recorded by 27P31 variety at 0 % of RDN. This result was also agreed with the findings of Kant et al. (2018).

Yield attributes and yield

No. of effective tillers hill^{-1} was recorded significantly (p=0.05) affected by different levels of nitrogen in hybrid rice varieties (Table 1). Application of 100% RDN have significantly more effective tiller hill^{-1} (8.6) compared to 75 % RDN, while less effective tiller hill^{-1} recorded (6.3) under 0 % of RDN. The higher effective tillers hill^{-1} (8.9) was recorded by Ankur 7576 variety followed by Arize 6444 gold (7.8) at 100% of RDN. The lowest effective tillers hill^{-1} (6.7) was recorded by 27P31 at 0 % of RDN. It indicates that, the Ankur-7576 has capacity to maintain its unique feature of modified new plant type.

No. of effective tillers m^{-2} was recorded significantly (p=0.05) affected by different nitrogen levels in hybrid varieties of rice (Table 1). Application of 100% RDN have significantly higher effective tiller m-2 (330.7) as compared to 75 % RDN. The lowest effective tillers m^{-2} (264.0) was recorded by 27P31 at 0 % of RDN. Similar results of hybrid rice varieties and nitrogen levels on number of effective tillers m^{-2} have also been reported by Banerjee and Pal (2011) and Reddy et al. (2018).

Days to 50% flowering as affected by different levels of nitrogen in different hybrid varieties of rice have been presented in Table 1. Application of 100% RDN (150 kg N ha^{-1}) have non-significantly (P=0.05) more no. of days to 50% flowering (97.7) compared to 75 % RDN (112 kg N ha^{-1}), while less no. of days to 50% flowering (97.3) recorded under 0 % of RDN. Ankur-7576 was earlier (103.5 DAT) under 75% RDN compared to 100% RDN, which is evident from days to 50% flowering. Similar pattern was observed in other hybrids also. These results are in close conformity with the findings of Mishra et al. (2014) and Reddy et al. (2018).

Panicle length was affected by different nitrogen level and hybrid varieties have been presented in Table 2. The maximum panicle length was recorded in 100% RDN (30.8 cm) followed by 75% RDN (29.7 cm) and lowest panicle length was observed in 0% RDN (27.8 cm). The panicle length was non-significantly (P=0.05) recorded with hybrid varieties of rice. The results of present investigation in respect of these yield attributes are in close conformity with the findings of Tripathi and Jaiswal. (2006), Kant et al. (2018) and Reddy et al. (2018).

Table 2. Yield attributes of transplanted Rice as affected by nitrogen level and hybrid varieties. (Average two-year data)

Treatments	Panicle length (cm)	No. of filled grains panicle^{-1}	No. of unfilled grains panicle^{-1}	Test wt.(g)
Nitrogen level				
0% RDN	27.8	93.34	23.34	20.76
50% RDN	28.8	109.61	20.98	22.05
75% RDN	29.7	118.09	18.42	22.70
100% RDN	30.8	122.15	18.03	23.26
SEm ±	0.32	1.96	0.73	0.31
CD (p=0.05)	0.93	5.68	2.11	0.87
Hybrid Varieties				
Arize 6444 Gold	29.5	120.23	19.46	22.33
Ankur 7576	29.9	123.40	18.29	22.63
27 P 31	29.3	90.52	25.52	22.26
Shahi Dawat	28.5	95.76	22.67	22.15
SEm ±	0.32	1.96	0.73	0.31
CD (p=0.05)	NS	5.68	2.11	NS

No. of filled grains panicle^{-1} was affected by different nitrogen levels and varieties. 100% RDN produced significantly (P=0.05) higher number of filled grain panicle^{-1} (122.15) over 0% RDN and 50% RDN while at par with 75% RDN. Among rice varieties Ankur-7576 produced significantly more no. of filled grains panicle^{-1} (123.40) which was significantly over all the rest varieties under 100% of RDN and the lowest no. of filled grains panicle-2 (90.52) was recorded under 0% RDN for 27P31. Similar results were also reported earlier by Manzoor et al., (2006) and Laroo and Shivay (2011).

Number of unfilled grains panicle^{-1} as affected by different nitrogen levels and varieties. Different nitrogen levels had marked effect on the number of unfilled grains panicles^{-1}. Minimum unfilled grains panicle^{-1} (18.03) was found in 100% of RDN compared to rest of the nitrogen levels. Lower number of unfilled grains panicle^{-1} (18.29) was recorded in Ankur 7576 under 100 % of RDN followed by under 75% of RDN of same hybrid. Similar findings were reported by

Table 3. Yield of transplanted Rice as affected by nitrogen level and hybrid varieties. (Average two-year data).

Treatments	Grain yield (q ha^{-1})	Straw yield (q ha^{-1})
Nitrogen level		
0% RDN	36.6	69.6
50% RDN	42.4	82.9
75% RDN	47.5	88.6
100% RDN	48.5	90.2
SEm ±	1.32	1.46
CD (p=0.05)	3.83	4.23
Hybrid varieties		
Arize 6444 Gold	50.3	89.8
Ankur 7576	50.9	91.3
27 P 31	35.3	68.1
Shahi Dawat	38.4	80.8
SEm ±	1.32	1.46
CD (p=0.05)	3.83	4.23

Manzoor et al. (2006); Rahman et al. (2007); Hossain et al. (2008); Mannan et al. (2010).

Test weight of rice as affected by different nitrogen level and varieties (Table 2). Data reveal that application of 100% RDN (150 kg ha^{-1}) was recorded significant effect on test weight (23.26 g) followed by (22.7 g) with 75% RDN. The lowest test weight (20.76 g) was recorded under 0% RDN. All hybrid rice varieties have non-significant (p=0.05) effect on test weight.

Grain yield as affected by different nitrogen level and varieties. Application of 100 % RDN recorded higher grain yield (48.5 q ha^{-1}) followed by (47.5 q ha^{-1}) under 75% RDN. 0% RDN was recorded lowest grain yield (36.6 q h^{-1}). The hybrid Ankur-7576 has higher grain yield (50.9 q ha^{-1}) under 100% RDN and followed by 75% of RDN, it differs significantly. The lowest grain yield was recorded in 27P31 (35.3 q ha^{-1}) under 0% of RDN. Straw yield was significantly affected by different level and varieties. Application of 100 % RDN recorded higher straw yield (90.2 q ha-1) followed by (88.6 q ha^{-1}) under 75% RDN. 0% RDN was recorded lowest straw yield (69.6 q ha^{-1}). The hybrid Ankur-7576 has higher straw yield (91.3 q ha^{-1}) under 100% RDN and followed by 75% of RDN, it differs significantly. The lowest straw yield was recorded 27P31 (68.1 q ha^{-1}) under 0% of RDN. These findings were also confirmed by Bali et al. (1995) and Meena et al. (2003).

CONCLUSIONS

Based on two-year experimental results, it is recommended that the performance of Ankur-7576 is superior for both growth, yield attributes and yield parameters over all the varieties tested. Trial data revealed that Ankur-7576 gave highest value of growth, yield attributes and yield at 100% RDN (150 kg N ha^{-1}) which is at par with 75 % RDN (112 kg N ha^{-1}) and significantly superior over 0 % RDN and 50 % RDN (75kg N ha^{-1}). It may be concluded that, Ankur-7576 is giving good response in low level of nitrogenous fertilizer and shows good efficiency in utilization of available and applied nitrogen to the crop.

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Zinc fertilizer application improves growth, yield and profit of paddy (*Oryza sativa* L.) in a zinc deficient *Inceptisol*

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ABSTRACT

A field experiment was conducted at the Central Farm, Regional Research and Technology Transfer Station of Coastal Zone, OUAT Bhubaneswar in kharif 2021 to study the effect of zinc fertilizer application on growth, yield and income of rice in a zinc (Zn) deficient soil. The soil was sandy loam, acidic (pH 5.4), medium in organic carbon (0.61%) and available P (10.3 kg ha^{-1}) while low in available N (155.4 kg ha^{-1}) and K (82.1 kg ha^{-1}). Eight treatment combinations comprising rate, source and method of Zn application were laid out in a randomized block design with 3 replications. The results revealed that soil application of Zn 5 kg ha^{-1} as basal followed by foliar spray of Zn @ 0.1% produced highest LAI (3.87), dry matter production (25.9 g hill^{-1}), panicles m^{-2} (315.8), test weight (22.76 g), grain yield (4937 kg ha^{-1}), and zinc content in grain (39.48 mg kg^{-1}) and straw (55.87mg kg^{-1}). However, application of Zn @ 5 kg ha^{-1} alone was at par with the soil application followed by foliar spray. Hence, it is concluded that soil application of Zn 5 kg ha^{-1} at the time of final land preparation along with the soil test based NPK is optimum for higher productivity and profitability of rice in zinc deficient Inceptisol.

Key words: *Crop growth rate, dry matter, economics, normalized difference vegetation index, SPAD, yield attributes*

INTRODUCTION

Globally, rice, wheat and maize account 42% of calorie intake. For optimum yield, these crops need high rate of fertilizer application, especially N followed by P and K. Use of inorganic N, P and K in huge quantity for cereal crops has depleted the soil organic matter, deteriorated the soil health and resulted in nutrient imbalance in soil with respect to secondary and micronutrients. Low use of organic fertilizers and manures, poor residue recycling and intensive cropping causes deficiency of micronutrients (Nadeem and Farooq, 2019). In intensive cereal-based cropping systems, zinc deficiency is wide spread. About 50% of the area cultivated with cereals is deficient in zinc. Cereal grains are inherently low in Zn and growing these crops in zinc deficient soils result in further reduction of this vital micronutrient. Regular and high consumption of rice grains inherently low in available Zn (Mao et al., 2014) is related to several illnesses in human beings. In high rice consuming areas, Zn deficiency caused yield reduction and Zn malnutrition in humans (Tiong et al., 2014; Yao et al., 2012).

Among the micronutrients, deficiency of zinc in plants and soils is widely reported across the world (Alloway, 2008). Zinc deficiency is the most widespread micronutrient disorder in tropical rice, occurring in parts of China, Japan, India, Pakistan, Bangladesh, Nepal, Sri Lanka, the Philippines, the USA and Colombia under lowland conditions, and throughout the Cerrado of Brazil in upland rice. An IRRI survey (Alloway, 2008) of on-farm experiments in six countries reported that after nitrogen; phosphorus and potassium were equal second in importance while zinc and sulphur were equal third in importance. In India, it is considered as the fourth

important nutrient limiting crop yield after N, P and K (Arunachalam et al., 2013). Zinc deficiency is likely to increase from 49 to 63% by the year 2025 as most of the marginal soils brought under cultivation have such problem (Arunachalam et al., 2013).

Zinc biofortification has the potential solution of malnutrition by mineral enrichment (Waqeel and Khan, 2022). Enrichment of Zn in grain is also beneficial in crop production through better germination and seedling vigour of rice plants grown on deficient soils (Rashid et al., 2019). Avoidance of Zn deficiency or hidden hunger in rice plants for improving yield and Zn biofortification of grains can be achieved by zinc nutrition.Keeping these points in view, a field experimentwas conducted to study the effect of zinc fertilizer on growth, yield, and economicsof rice crop.

MATERIALS AND METHODS

The study was conducted in *kharif* 2021 at the E Block of Central Farm in Regional Research and Technology Transfer Station, OUAT, Bhubaneswar, India. It lies at 20°15" N latitude, 85°55" E longitude and at an altitude of 25.9 m above the mean sea level. The experimental site belongs to irrigated medium land.The soil of experiment site was acidic (pH 5.4) sandy loam (69% sand, 16% silt and 15% clay). Available N (155.4kg ha^{-1}), K (82.1 kg ha^{-1}) and Zn (0.47 mg kg^{-1}) status was low while organic carbon (0.61%) and available P status (10.3 kg ha^{-1}) was medium.

The experiment was laid out in a randomized block design with 3 replications.There were 8 treatments; T_1: Control (No Zn), T_2: Soil application of Zn 2.5 kg ha^{-1}, T_3: Soil application of Zn 2.5 kg ha^{-1} followed by foliar spray with 0.1% Zn at 25 days after transplanting (DAT), T_4: Soil application of Zn 5 kg ha^{-1}, T_5: Soil application of Zn at 5 kg ha^{-1} followed by foliar spray with 0.1% Zn at 25 DAT, T_6: One foliar spray with 0.1% Zn at 25 DAT, T_7: Two foliar spray with 0.1% Zn at 25 and 50 DAT, and T_8: Seedling root dip with 2% ZnO. The source of zinc fertilizer for soil application and foliar spray was fertilizer grade zinc sulphate heptahydrate ($ZnSO_4.7H_2O$) containing 20% Zn. For seedling root drip, fertilizer grade ZnO was used containing 80% Zn. Seedling root dip with 2% Zn was done by preparing 2.5% ZnO suspension (500 g ZnO in 20 litres water ha^{-1}) and dipping the roots of uprooted seedling for 30 minutes. For foliar spray with 0.1% Zn, solution of 0.5% $ZnSO_4.7H_2O$ was prepared by dissolving at 2500 g in 500 litre water ha^{-1}.

Rice variety 'Binadhan 11' was used in the experiment. Seedlings were raised in wet nursery beds and transplanted on 8 August using 25 days old seedlings at a spacing of 20 cm × 10 cm with 2 seedlings hill^{-1}. Considering the soil test-based fertilizer requirement, 100 kg N, 40 kg P_2O_5 and 50 kg K_2O ha^{-1} was applied uniformly using diammonium phosphate, urea and muriate of potash. The plots were irrigated as and when necessary to maintain standing water in the range of 3 to 5 cm depth. However, the field was drained to saturation before applying fertilizer and shallow irrigation was given two days after it to maintain a submerged condition.

Leaf area was measured using LI-3000C leaf area meter. Average leaf area index (LAI) was computed using the formula:

$$\text{LIA} = \frac{\text{Total leaf area}}{\text{Land area}}$$

Normalized difference vegetation index (NDVI) was measured directly in field using a handheld GreenSeeker at about 0.3 m above the crop canopy. Soil Plant Analysis Development (SPAD) value of the most recently expanded leaf was measured using SPAD-502 meter.

Crop growth rate (CGR) was recorded using the formula:

$$\text{CGR} = \frac{W2 - W1}{t2 - t1} \left(\text{g m}^{-2}\text{ d}^{-1}\right)$$

Where, W1 and W2 represent dry matter production recorded at two consecutive occasions t1 and t2, respectively. The time interval between t1 and t2 was in days (d).

Grain yield, straw yield, panicles m^{-2}, grains panicle^{-1} and other yield attributing parameters were recorded as per the standard procedure (Kumar et al., 2017). Grain and straw yields were reported in kg ha^{-1}, based on moisture content in the grain and straw at 14% and 12%, respectively.Harvest Index was calculated by dividing the grain yield by biological yield (grain + chaff + straw) and multiplying the ratio by 100.

Grain and straw samples were analysed for N content by Microkjeldhal steam distillation method (Jackson, 1973), P by diacid digestion and colorimetric determination using Bartons reagent (Piper, 1950), K by diacid digestion and flame photometric determination (Piper, 1950), and Zn by diacid digestion and atomic absorption spectrophotometric determination (Jackson, 1973).

The economics of production for different treatments were worked per hectare taking into account the cost of various inputs as well as the value of the produce as per the prevailing market price.The cost of fertilizer grade $ZnSO_4.7H_2O$ and ZnO were Rs. 75 and Rs. 125 kg^{-1}, respectively. Labor cost was Rs. 315 d^{-1}. Gross return was calculated considering Minimum Support Price for paddy for the year 2021 as Rs. 1940 q^{-1} and the local price for straw as Rs. 100 q^{-1}. The net profit was calculated by deducting the cost of cultivation from gross income (Rs. ha^{-1}). The B:C ratio was worked out as gross return divided by the cost of cultivation.

The data on harvest index was subjected to angular transformation before the statistical analysis. Analysis of variancewas applied for statistical analysis. Significance of treatments was tested by F-test (Gomez and Gomez, 1984) and the Critical Difference (CD) was calculated at 5% probability.

RESULTS AND DISCISSION

Effect of zinc fertilizer on crop growth

At harvest, highest plant height (111.7 cm), tillers $hill^{-1}$ (10.38) and dry matter production (25.9 g $hill^{-1}$) were recorded with the soil application of Zn 5 kg ha^{-1} followed by foliar spray with 0.1% Zn at 25 days after transplanting (DAT) (Table 1). Similarly, highest leaf area index (LAI) of 3.87 recorded at 80 DAT, and SPAD value of 39.5 and NDVI of 0.69 recorded at 60 DAT were noted with the same treatment. This treatment was at par with the soil application of Zn 5 kg ha^{-1}. These two treatments were superior than the remaining treatments for plant height and dry matter production. For the remaining parameters, these two treatments were at par with the soil application of zinc 2.5 kg ha^{-1} followed byfoliar spray with 0.1% Zn at 25 DAT.Soil application of zinc at 5 or 2.5 kg ha^{-1} was superior to foliar spray and seedling root dip for all the crop growth parameters. The control resulted in the lowest value for these parameters.

All the zinc fertilizer treatments were superior in increasing plant height as compared to the control. Zinc supply to crop might have helped in auxin synthesis leading to stem elongation and higher plant height. On the other hand, the test soil with deficit available zinc content 0.47 mg kg^{-1} might have resulted in limited zinc supply and auxin synthesis leading to lowest plant height in the control (University of Minnesota Extension, 2016). Zinc has a role in chlorophyll formation (Kosesakal and Unal, 2009; University of Minnesota Extension, 2016) and the latter harvests solar energy for starch synthesis. Starch is required for new tiller formation in vegetative stage. Crop in the control plot with low zinc might have suffered from inadequate starch synthesis leading to lowest tillers $hill^{-1}$. Foliage growth in terms of leaf number and leaf area are important parameters for knowing the leaf surface available for harvesting solar energy per unit land area.

Table 1. Crop growth attributes of rice at different growth stages as influenced by zinc fertilizer application.

Treatments	Plant height (cm)	Tillers $hill^{-1}$	LAI	SPAD value	NDVI	Dry matter (g $hill^{-1}$)
Control (No Zn)	102.3	8.81	3.07	33.8	0.59	21.1
Soil application of Zn 2.5 kg ha^{-1}	108.2	9.75	3.67	36.3	0.63	23.4
Soil application of Zn 2.5 kg ha^{-1} + Foliar spray with 0.1% Zn at 25 DAT	108.7	9.80	3.77	38.6	0.65	23.8
Soil application of Zn 5 kg ha^{-1}	111.5	10.35	3.85	39.3	0.68	25.6
Soil application of Zn 5 kg ha^{-1} + Foliar spray with 0.1% Zn at 25 DAT	111.7	10.38	3.87	39.5	0.69	25.9
One Foliar spray with 0.1% Zn at 25 DAT	104.8	9.21	3.43	34.3	0.61	22.4
Two Foliar spray with 0.1% Zn at 25 and 50 DAT	105.0	9.30	3.59	35.2	0.63	23.6
Seedling root dip with 2% Zn	105.0	9.34	3.50	34.4	0.62	22.7
CD (P=0.05)	2.3	0.69	0.27	2.13	0.17	1.67

LAI (Leaf area index) was recorded at 80 DAT.
SPAD (Soil plant analysis development) value and NDVI (Normalized difference vegetation index) were recorded at 60 DAT

These two parameters are jointly represented by LAI. Higher plant height and tiller number under the zinc fertilizer treatments might have helped in higher LAI. Role of zinc in chlorophyll synthesis and activation of over 300 enzymes in optimal crop growth is reflected in high NDVI and SPAD value. The sum total effect of growth parameters is reflected in terms of dry matter production.

Soil application supplied zinc at higher rate (2.5 kg ha^{-1} and 5 kg ha^{-1}) while one foliar spray provided only 0.5 kg Zn and seedling root dip supplied only 0.4 kg ha^{-1}. Higher amount of zinc supplied with the soil application might have supplied this critical nutrient continuously from the beginning of crop growth. Foliar spray was inferior to soil application due to lower rate of application and also due to the late supply at 25 DAT. Similarly, seedling root dip supplied low rate of zinc and less solubility of ZnO might be responsible for the low efficacy of this method as compared to the soil application.

The change in dry matter production per unit land area per day between two successive observations (Table 2) was estimated as crop growth rate (CGR). Highest CGR (22.08 gm^{-2} d^{-1}) was noted during 60 to 80 DAT for the treatment soil application of Zn 5 kg ha^{-1} + foliar spray with 0.1% Zn at 25 DAT closely followed by soil application of Zn 5 kg ha^{-1}. These two treatments were superior to the remaining treatments. The superiority of these two treatments was due to supply of Zn at higher rate from the beginning of crop growth.

Effect of zinc fertilizer on yield attributes and yield

Highest panicles m^{-2} (315.8), panicle length (23.34 cm), grains $panicle^{-1}$ (88.1), fertility % (94.4) and 1000 grain weight (22.76 g) were recorded with the soil application of Zn at 5 kg ha^{-1} + foliar spray with 0.1% Zn at 25 DAT (Table 3). This treatment was at par with the soil application of Zn 5 kg ha^{-1} regarding all the yield attributes. These two treatments were at par with the soil application of Zn at 2.5 kg ha^{-1} + foliar spray with 0.1% Zn at 25 DAT regarding the yield attributes except for the grains $panicle^{-1}$ in which case the latter treatment was inferior.

Highest grain yield (4937 kg ha^{-1}), straw yield (6025 kg ha^{-1}), harvest index (45.04%) and grain:strawratio (0.82) was recorded with the soil application of Zn 5 kg ha^{-1} + foliar spray with 0.1% Zn at 25 DAT. This treatment was at par with the soil application of Zn 5 kg ha^{-1} (4915 kg ha^{-1}). Similar results are reported (Oahiduzzaman et al., 2016; Ghoneim, 2016; Wilczewski and Warachien, 2016). These two treatments are followed by soil application of Zn 2.5 kg ha^{-1} + foliar spray with 0.1% Zn and soil application of Zn 2.5 kg ha^{-1} alone. Grain and straw yields under seedling root dip with 2% Zn, two foliar sprays, and one foliar spray were at par to each other and were inferior than the soil application. Mahmudi et al. (2015) also reported that soil application was superior than foliar spray.

Lowest grain and straw yield, harvest index and grain:strawratio was recorded with the control. Thus, in absence of soil application of zinc, foliar spray and seedling root dip improved yield attributes moderately as compared to the control. Foliage applied zinc enhances the grain yield of field crops including rice, wheat and barley (Ullah et al., 2018; Hashim et al., 2021). This was due to growth response to the

Table 2. Crop growth rate (g m^{-2} d^{-1}) of rice as influenced by scheduling of zinc fertilizer application.

Treatments	DAT			
	20-40	40-60	60-80	80-Harvest
Control (No Zn)	8.50	13.75	18.25	6.42
Soil application of Zn 2.5 kg ha^{-1}	8.92	15.08	19.75	6.75
Soil application of Zn 2.5 kg ha^{-1} + Foliar spray with 0.1% Zn at 25 DAT	9.58	15.42	19.92	6.08
Soil application of Zn 5 kg ha^{-1}	10.67	15.33	22.00	4.00
Soil application of Zn 5 kg ha^{-1} + Foliar spray with 0.1% Zn at 25 DAT	11.00	15.50	22.08	3.58
One Foliar spray with 0.1% Zn at 25 DAT	9.00	14.08	19.42	8.17
Two Foliar spray with 0.1% Zn at 25 and 50 DAT	9.17	14.00	20.00	8.17
Seedling root dip with 2% Zn	9.17	13.75	19.67	8.17
CD (P=0.05)	0.97	0.99	1.65	3.24

Table 3. Yield attributes and yield of rice as influenced by scheduling of zinc fertilizer application.

Treatments	Panicles m^{-2}	Panicle length (cm)	Grains $panicle^{-1}$	Fertility (%)	Test weight (g)	Grain yield (kg ha^{-1})	Straw yield (kg ha^{-1})	Harvest index (%)	Grain: Straw ratio
Control (No Zn)	290.4	21.63	83.5	91.2	21.61	4235	5652	42.83	0.75
Soil application of Zn 2.5 kg ha^{-1}	307.6	22.50	86.3	93.3	22.35	4670	5907	44.16	0.79
Soil application of Zn 2.5 kg ha^{-1} + Foliar spray with 0.1% Zn at 25 DAT	309.5	22.77	86.8	93.5	22.42	4763	5931	44.54	0.80
Soil application of Zn 5 kg ha^{-1}	315.1	23.17	88	94.4	22.75	4915	6002	45.02	0.82
Soil application of Zn at 5 kg ha^{-1} + Foliar spray 0.1% Zn at 25 DAT	315.8	23.34	88.1	94.4	22.76	4937	6025	45.04	0.82
One Foliar spray with 0.5% Zn at 25 DAT	301.6	22.17	84.4	92.6	22.2	4420	5782	43.33	0.76
Two Foliar spray with 0.1% Zn at 25 and 50 DAT	302.3	22.40	85.1	93.5	22.37	4537	5833	43.75	0.79
Seedling root dip with 2% Zn	303.4	22.23	84.3	91.8	22.27	4587	5832	44.03	0.79
CD (P=0.05)	10.5	0.66	1.07	1.26	0.48	168	190	0.86	0.03

critical nutrient in the test soil which in turn improved the yield attributes and finally grain and straw yield.

Effect of zinc fertilizer on N, P, K and Zn content in rice grain and straw

Highest N content in grain (1.29%) and straw (0.38%), K content in grain (0.24%) and straw (1.58%) and Zn content in grain (39.48 mg kg^{-1}) and straw (55.87 mg kg^{-1}) was recorded with the soil application of Zn 5 kg ha^{-1} + foliar spray closely followed by the soil application of Zn 5 kg ha^{-1} (Table 4). This might be due to the synergistic effect of zinc with N and K content in rice grain and straw. Improvement in Zn content in grain and straw was due to addition of zinc to the deficient soil. Dwivedi and Srivastava (2014) reported that Zn content in rice grain (10.9 mg kg^{-1}) and straw (14.5 mg kg^{-1}) in the control was improved substantially to 18.7 and 21.8 mg kg^{-1} under Zn 5 kg ha^{-1}. Similarly, Rama Lakshmi et al. (2017) reported that highest Zn content in rice grain (17.1 mg kg^{-1}) with the soil application of Zn 2.5 kg ha^{-1} + two foliar sprays vis-a-vis no zinc application (13.9 mg kg^{-1}). In our experiment, grain zinc content (34.51 mg kg^{-1}) was higher with the two foliar spray vis-à-vis one foliar spray (32.14 mg kg^{-1}) and seedling root dip (32.03 mg kg^{-1}). This was mainly due

Table 4. N, P, K and Zn content in rice grain and straw as influenced by scheduling of zinc fertilizer application.

Treatments	Nutrient Content (%)						mg kg^{-1}	
	N		P		K		Zn	
	Grain	Straw	Grain	Straw	Grain	Straw	Grain	Straw
Control (No Zn)	1.24	0.33	0.30	0.14	0.22	1.52	24.30	29.33
Soil application of Zn 2.5 kg ha^{-1}	1.27	0.37	0.28	0.13	0.23	1.55	35.37	44.83
Soil application of Zn 2.5 kg ha^{-1} + Foliar spray with 0.1% Zn at 25 DAT	1.28	0.37	0.27	0.13	0.23	1.56	36.97	47.50
Soil application of Zn 5 kg ha^{-1}	1.29	0.37	0.25	0.13	0.24	1.57	38.37	53.67
Soil application of Zn 5 kg ha^{-1} + Foliar spray with 0.1% Zn at 25 DAT	1.29	0.38	0.25	0.12	0.24	1.58	39.48	55.87
One Foliar spray with 0.1% Zn at 25 DAT	1.25	0.35	0.29	0.13	0.23	1.53	32.14	39.30
Two Foliar spray with 0.1% Zn at 25 and 50 DAT	1.26	0.35	0.28	0.12	0.23	1.54	34.51	42.85
Seedling root dip with 2% Zn	1.25	0.34	0.29	0.13	0.23	1.53	32.03	36.97
SEm (±)	0.005	0.005	0.002	0.003	0.002	0.003	0.77	1.89
CD (P=0.05)	0.01	0.02	0.01	0.01	0.01	0.001	2.33	5.68

Table 5. Comparative Economics as influenced by scheduling of zinc fertilizer application.

Treatments	Gross return (Rs ha^{-1})	Gross Expenditure (Rs ha^{-1})	Net Return (Rs ha^{-1})	B:C ratio
Control (No Zn)	87811	58000	29811	1.51
Soil application of Zn 2.5 kg ha^{-1}	96505	59253	37252	1.63
Soil application of Zn 2.5 kg ha^{-1} + Foliar spray with 0.1% Zn at 25 DAT	98333	59756	38577	1.65
Soil application of Zn 5 kg ha^{-1}	101353	60190	41163	1.68
Soil application of Zn at 5 kg ha^{-1} + Foliar spray with 0.1% Zn at 25 DAT	101803	60693	41110	1.68
One Foliar spray with 0.5% Zn at 25 DAT	91530	58503	33027	1.56
Two Foliar spray with 0.1% Zn at 25 and 50 DAT	93851	59006	34845	1.63
Seedling root dip with 2% Zn	94820	58378	36442	1.62
SEm (±)	937	-	803	-
CD (P=0.05)	2815	-	2420	-

to the timing of zinc foliar application around reproductive stage (Pandey et al., 2013). In contrast to N, K and Zn, the P content in grain and straw was highest in the control while lowest in the highest zinc application rate. The antagonistic effect between zinc and P might be responsible for this.

Effect of zinc fertilizer application on economics

Maximum net return of Rs. 41163 ha^{-1} and B:C ratio of 1.68 (Table 5) was noted with the soil application of Zn 5 kg ha^{-1} closely followed by the soil application of Zn 5 kg ha^{-1} + foliar spray with 0.1% Zn at 25 DAT (net return of Rs 41110 ha-1 and B:C ratio of 1.68). Similar results are reported by Gill and Walia (2013). Alleviation of zinc deficiency with the application of fertilizer helped in increasing yield and thereby the net return. No zinc application had the lowest net return (Rs. 29811 ha^{-1}) and B:C ratio of (1.51). Foliar spray and seedling root dip were moderately effective in improving net return.

CONCLUSION

From the above results, it is concluded that in zinc deficient Inceptisol, soil test based NPK along with application of Zn @ 5 kg ha^{-1} at the time of final land preparation resulted in higher yield, net return and B:C ratio in irrigated rice.

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Agronomic response, physiological and bio-chemical composition of transplanted rice influenced by nutrient management practices

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ABSTRACT

Field experiment was conducted at wetland farm of Tamil Nadu Agricultural University, Coimbatore during kharif season to study the effect of integrated nutrient management practices on transplanted lowland rice. The experiment was laid out in randomized block design with three replications and nine treatments. Rice CO(R) 48 was used as a test variety. Based on N equivalent basis, required quantities of dhaincha, vermicompost, farmyard manure were incorporated before transplanting of rice. The P and K requirement was supplied separately through inorganic sources as per treatment schedule. The results revealed that yield attributes (number of productive tillers m^{-2} (288), total number of grains panicle^{-1} (217), percentage of filled grains (89.4) and yield (6248 kg ha^{-1}), panicle parameters (panicle length (28.3 cm), panicle weight (4.64 g), test weight (18.1 g), physiological and bio chemical parameters (chlorophyll index (36.02), chlorophyll stability index (72.22 %), relative water content (78.43%), soluble protein content (15.58 mg g^{-1}) were significantly influenced with application of 100% NPK through inorganic fertilizers + 6.25 t dhaincha followed by application of 100% N through dhaincha + balance P & K through inorganic fertilizers. Maximum soil enzyme dynamics (urease activity (47.1 µg NH+ g^{-1} soil 24 h^{-1}), soil dehydrogenase activity (37.1 µg of TPF released g^{-1} of soil 24 h^{-1}), soil phosphatase activity (39.1 µg of p - nitrophenol released g^{-1} of soil 24 h^{-1}), nitrogen use efficiency (agronomic efficiency, physiological efficiency and apparent N recovery) and chemical composition like protein (7.41 %), carbohydrates (78.53%), amylose content (27.07 %), fat (0.58 %) and fibre (0.231 %) were high with application of 100 % N through dhaincha + balance P & K through inorganic fertilizers.

Key words: *Yield, enzyme dynamics, nitrogen use efficiency, physio- bio chemical parameters, chemical composition, rice*

INTRODUCTION

Rice (*Oryza sativa* L.) is an important and extensively cultivated food crop and feeds more than half of the world's population. The slogan "Rice is life" is most appropriate for India; as this crop plays a vital role in our national food security and is a means of livelihood for millions of rural households.

In Asia alone, more than 2 billion people obtain 60 to 70 per cent of their energy intake from rice and its derivatives. India has the largest rice area among rice growing countries and it stands second in production next to China. It produces 97.24 million tonnes of rice over an area of 43.65 million hectares with a productivity of 2.22 t ha^{-1}. As a result of green revolution, self-sufficiency in food production has been achieved through use of high external agricultural inputs. Rice is one of the major contributors to the success by contributing approximately 43 per cent of total food grain production of India (Upendra et al., 2013). In Tamil Nadu, rice occupies an area of 1.91 million hectares with an annual production of 7.45 million tonnes and productivity of 3.91 t ha^{-1} (Anonymous, 2013).

Even though the area under rice cultivation is large, the productivity is low due to various interaction factors. The imbalance in usage of fertilizers is one of the main factors responsible for the low productivity and also the continuous use of inorganic fertilizers

resulted in declining of soil fertility. To obtain the better yield, farmers have used more and more fertilizers year after year due to decline in soil fertility (Aryal et al., 2021).

High cost of fertilizer and the low purchasing capacity of the small and marginal peasants of the country, restrict the use of fertilizer inputs (Shrivastava et al., 2006). The increasing demand for rice grain production has to be achieved by using limited available resources in a sustainable manner. Importance of organics is increasingly felt these days in sustainable crop production systems. To achieve higher and sustainable rice yields, use of organic manures is a must (Ajaykumar and Krishnasamy, 2019). It is, however, difficult to meet the crop nutrient requirements with bulky organic manure alone and there is a need for integrated application of different sources of nutrients for sustaining the desired crop productivity. An integration of organic and inorganic fertilizer may be necessary to maintain the sustainability in crop production (Ajaykumar and Sivakumar, 2020). Use of organic manure, green manuring, crop residues along with inorganic fertilizers not only reduce the demand for inorganic fertilizers but also increases the efficiency of applied nutrients due to their favourable effect on physical, chemical and biological properties of soil (Prasad et al., 2002). It is envisaged that for sustainable agricultural production in the country, integrated nutrient management (INM) appears to be more promising.

An integrated approach involving organic manures and chemical fertilizers will go a long way in building up of the soil fertility on a permanent basis and the system will supply most of the nutrients in a judicious way and nutrients uptake by the crop will be enhanced. The INM concept, if properly designed, not only meets the nutrient requirement of component crops of a system, but keeps the system intact (Dutta and Bandyopadhyaya, 2003). The INM practices not only maintain soil health but also reduce input cost thereby raising the sustainability in productivity. Based on this background, research has been carried out to study the effect of organic and inorganic sources of nutrients on transplanted lowland rice.

MATERIALS AND METHODS

A field experiment was conducted during *kharif* season at wetland farm of TNAU, Coimbatore. The initial analysis of the soil of the experimental site revealed that the soil was slightly alkaline (pH = 7.85) with low in soluble salts (EC = 0.42dSm^{-1}), medium in organic carbon content (0.58 per cent), low in available N (216 kg ha^{-1}), medium in P_2O_5 (16.2 kg ha^{-1}) and high in K_2O (426 kg ha^{-1}). The irrigation water was found to be neutral in reaction (pH = 7.6) with medium level of the soluble salts (EC = 1.18 dSm^{-1}).

The maximum and minimum temperature ranged from 29.0 to 32.7°C and 19.2 to 23.7°C, respectively. With regard to relative humidity, there was a fluctuation from 77.5 to 94 per cent (0722 hours) and from 49.6 to 77.3 per cent (1422 hours). There was a total rainfall of 110 mm was received in 16 rainy days from August to January. The evaporation and bright sunshine hour's day^{-1} ranged from 2.8 to 6.6 mm and 3.1 to 7.4 hours, respectively

The study was conducted with nine treatments which are T_1 -100% N through dhaincha + balance P & K through inorganic fertilizers, T_2 - 50 % N through dhaincha + balance N, P & K through inorganic fertilizers, T_3 -100 % N through vermicompost + balance P & K through inorganic fertilizers, T_4 -50 % N through vermicompost + balance N, P & K through inorganic fertilizers, T_5 -100 % NPK (150 : 50 : 50 kg ha^{-1}) through inorganic fertilizers, T_6 -100 % NPK through inorganic fertilizers + 12.5 t farmyard manure, T_7 - 100 % NPK through inorganic fertilizers + 6.25 t dhaincha, T_8 - 100 % NPK through inorganic fertilizers + 5 t vermicompost, T_9 - Control. The experiment was laid out in RBD with three replications. The test crop were used is medium duration rice variety CO(R) 48.

Number of productive tillers m^{-2}

The tillers were counted from all the hills from tagged plants and the mean number of productive tillers m^{-2} was calculated.

Panicle length

Panicle length was measured from the point of scar to the tip of the panicle obtained from five panicles of the tagged hills and mean length of panicle was calculated and expressed in cm.

Panicle weight

Five centreanicles collected for measuring panicle

Table 1. Quantity of organic manures, inorganic fertilizers applied and nutrients added in various treatments.

Treatments	Quantity of manures/ Fertilizers used (kg ha^{-1})	Nutrient added (kg ha^{-1})					
		N		P_2O_5		K_2O	
		organic	inorg-anic	organic	inorga-nic	organic	inorganic
T_1 :100 % N through dhaincha + balance P & K through inorganic fertilizers.	24149	150	-	38	12	70	-
T_2 : 50 % N through dhaincha + balance N, P & K through inorganic fertilizers	12074	75	75	20	30	35	15
T_3 : 100 % N through vermicompost + balance P & K through inorganic fertilizers	8333	150	-	50	-	100	-
T_4 : 50 % N through vermicompost + balance N, P & K through inorganic fertilizers	4166	75	75	25	25	50	-
T_5 : 100 % NPK (150: 50:50 kg ha^{-1}) through inorganic fertilizers	Urea - 326	-	150	-	-	-	-
	SSP -312.5	-	-	-	50	-	-
	MOP - 83.3	-	-	-	-	-	50
T_6:100 % NPK (150: 50:50 kg ha^{-1}) through inorganic fertilizers + 12.5 t ha^{-1} FYM	Urea - 326	-	150	-	-	-	-
	SSP -312.5	-	-	-	50	-	-
	MOP - 83.3	-	-	-	-	-	50
	12500	75	-	50	-	77	-
T_7 :100 % NPK (150: 50:50 kg ha^{-1}) through inorganic fertilizers + 6.25 t ha^{-1} dhaincha	Urea - 326	-	150	-	-	-	-
	SSP -312.5	-	-	-	50	-	-
	MOP - 83.3	-	-	-	-	-	50
	6250	166	-	42	-	78	-
T_8 :100 % NPK (150: 50:50 kg ha^{-1}) through inorganic fertilizers + 5 t ha^{-1} vermicompost	Urea - 326	-	150	-	-	-	-
	SSP -312.5	-	-	-	50	-	-
	MOP - 83.3	-	-	-	-	-	50
	5000	90	-	32	-	60	-
T_9 : control	-	-	-	-	-	-	-

length were weighed using an electronic balance and the mean weight of the panicle was calculated and expressed in gram.

Thousand grain weight

One sample of 1000 filled grains was taken from each plot and the test weight was recorded and expressed in gram.

Number of spikelets and filled grains panicle^{-1}

The total number of spikelet from all the panicles were separated and sorted into filled and ill-filled grains and the mean values of filled grains panicle^{-1} was worked out as per the procedure (Gomez, 1972).

Filled grain percentage

The percentage of filled grain was worked out using the following formula

$$\text{Filled grain percentage} = \frac{\text{Number of filled grains per panicle}^{-1}}{\text{Number of spikelet panicle}^{-1}} \text{x} 100$$

Grain yield

The harvested produce from each net plot was threshed, sun dried, winnowed separately and the grain yield was recorded at 14 per cent moisture content and expressed in kg ha^{-1}.

Agronomic efficiency

The agronomic efficiency *i.e.*, the response in yield per unit input as indicated by the following formula (Yoshida, 1981).

$$AE = \frac{\text{Grain yield in fertilized plot } \left(\text{kg ha}^{-1}\right) - \text{Grain yield in unfertilized plot } \left(\text{kg ha}^{-1}\right)}{\text{Quantityof fertilizer N applied } \left(\text{kg ha}^{-1}\right)}$$

Physiological efficiency

The physiological efficiency also known as efficiency of utilization as indicated by kg of grain per kg of absorbed N (Yoshida, 1981) was computed as follows

$$PE = \frac{\text{Grain yield in fertilized plot } \left(\text{kg ha}^{-1}\right) - \text{Grain yield in unfertilized plot } \left(\text{kg ha}^{-1}\right)}{\text{N uptake in fertilized plot } \left(\text{kg ha}^{-1}\right) - \text{N uptake in unfertilized plot } \left(\text{kg ha}^{-1}\right)}$$

Apparent N recovery

Apparent N recovery, also known as recovery fraction was computed as per the formula suggested by Pillai and Vamadevan (1978).

$$ANR = \frac{Y_t - Y_o}{N_t} \times 100$$

Where,

Y_t - Uptake of N in particular treatment (kg ha^{-1}),

Y_0 - Uptake of N in unfertilized plot (kg ha^{-1}), and

N_t - Quantity of N applied for the treatment (kg ha^{-1})

Physiological and biochemical parameters

Chlorophyll index

Chlorophyll content of leaves were recorded as described by Peng et al. (1996) using the chlorophyll meter (SPAD - 502, Soil Plant Analysis Development Section, Minolta Camera Co. Ltd., Japan). The readings were recorded on the upper most fully expanded leaves in five randomly chosen plants at four growth stages. The average values were worked out and expressed as chlorophyll index.

Chlorophyll stability index

The chlorophyll stability index (CSI) was estimated by using the method of Murthy and Majumdar (1962) and expressed in per cent.

$$CSI = \frac{\text{Total chlorophyll content (Treated)}}{\text{Total chlorophyll content (Control)}} \times 100$$

Relative water content

The relative water content (RWC) was estimated using the formula given by Barrs and Weatherly (1962). Samples were collected for analysis and mean values were arrived. To determine the plant relative water content, 25 leaf discs were collected from the third leaf from the top of the plant in each treatment and the fresh weights were recorded immediately. The weighed leaf discs were then soaked in a petri-dish in distilled water for 4 hours at 25ºC and then the turgid weight was measured. The samples were then dried in a hot air oven at 72ºC for 48 hours, to obtain their dry weights. RWC was calculated by using the following formula and expressed in percentage.

$$RWC = \frac{\text{(Fresh weight – Dry weight)}}{\text{(Turgid weight - Dry weight)}} \times 100$$

Soluble protein content

Soluble protein content in the leaf was estimated at 660 nm by using Folin-Ciocalteau reagent by following the procedure described by Lowry et al. (1950).

Leaf samples of 250 mg were macerated with 10 ml of phosphate buffer and the contents were centrifuged at 3000 rpm for about 10 minutes. The supernatant were collected and made up to 25 ml. From this, 1 ml of the supernatant and 5 ml of alkaline copper tartarate reagent were mixed with 0.5 ml of folin-ciocalteau reagent and the optical density values were measured at 660 nm in the spectrophotometer. The soluble protein content was expressed as mg g^{-1} of fresh weight by using bovine serum albumin as the standard.

Chemical parameters

Rice samples of each treatment were cleaned by removing stones and other foreign particles. Good grains were powdered and used for chemical analysis.

Fat content

Fat was estimated as crude ether extract of the dry material. Fat content in per cent was calculated by the following formula (A.O.A.C., 1980).

$$\text{Fat content in percentage} = \frac{\text{Weight of ether extract}}{\text{Weight of the sample}}$$

Protein

The protein content of rice samples was estimated as per the method suggested by Lowry et al. (1951). The estimation of protein was based on the development of blue color by the hydroxyl groups present in the amino acids with the folin-Cocteau phenol reagent. The protein content of the sample was expressed as a percentage.

Carbohydrate

Carbohydrate content was estimated from the samples of each treatment by anthrone method as suggested by

Table 2. Standard methods followed for soil enzyme analysis of the pre experimental field.

Enzyme	Value	Substrate	Method	Reference
Dehydrogenase (µg of TPF released g^{-1} of soil 24 h^{-1})	8.22	2,3,5- TriphenylTetrazolium chloride	Spectrophotometer at 485 nm	Casida et al. (1964)
Urease (µg NH_4^+ g^{-1} of soil 24 h^{-1})	27.68	10 per cent urea solution	Spectrophotometer at 630 nm	Tabatabai, and Bremner (1969)
Phosphatase (µg of p-nitrophenol released g^{-1} of soil h^{-1})	12.02	p-nitrophenol phosphate	Spectrophotometer at 420 nm	Halstead (1964)

Hedge and Hofreiter (1962) and expressed as percentage.

Amylose content and fibre

The method suggested by Sadasivam and Manickam (1996) was followed in determining amylose and fibre content.

Assessment of enzyme activity

The enzyme activity was determined at initial and postharvest stages of rice. The substrates and methods followed for enzyme assays were presented in Table 2.

Statistical analysis

The data on various characters studied during the course of investigation were subjected to an analysis of variance (F-test) as per the methods suggested by Gomez and Gomez (2010). Wherever statistical significance was observed, critical difference (CD) at 0.05 level of probability was worked out for comparison. Non-significant comparisons were indicated as NS.

RESULTS AND DISCUSSION

Yield attributes and yield

Significant differences were noticed in respect of number of productive tillers m^{-2}, total number of grains $panicle^{-1}$, percentage of filled grains and yield (Table 3).

Application of 100 per cent NPK through inorganic fertilizers + 6.25t dhaincha attained its statistical supremacy by recording more number of productive tillers m^{-2} (288), total number of grains $panicle^{-1}$ (217), percentage of filled grains (89.4) and grain yield (6248 kg ha^{-1}) which was on par with 100 per cent NPK through inorganic fertilizers + 5t vermicompost and 100 per cent NPK through inorganic fertilizers + 12.5 t FYM.

Among the other treatments, 100 percent N through dhaincha + balanced P & K through inorganic fertilizers recorded significantly higher number of productive tillers m^{-2} (235), total number of grains $panicle^{-1}$ (197), percentage of filled grains (86.3) and grain yield (5778 kg ha^{-1}). 100 per cent RDN through green manure incorporation (Sesbania aculeata) might have released the N - NH_4^+ N into the soil solution on decomposition, which is ready usable by rice plants. Lee (2012) reported that green manures, particularly the legumes like *Sesbania aculeata* and *Sesbania* rostrata which are comparatively high in N and low in C: N ratio behave almost like chemical fertilizers in respect to the improvement in productive tillers and yield of rice.

The least number of productive tillers m^{-2} (166), total number of grains $panicle^{-1}$ (131), percentage of filled grains (79.2) and grain yield (3050 kg ha^{-1}) were registered with the treatment of control. In this study,

Table 3. Effect of organic and inorganic sources of nutrients on yield attributes and grain yield of transplanted lowland rice.

Treatments	No. of productive tillers m^{-2}	Total no. of grains $panicle^{-1}$	No. of filled grains $panicle^{-1}$	Filled grain (%)	Grain yield (kg ha^{-1})
T_1	235	197	170	86.3	5778
T_2	196	182	154	84.4	5234
T_3	204	183	155	84.9	5340
T_4	190	181	153	84.3	5111
T_5	187	181	151	83.5	5009
T_6	274	212	188	88.7	6210
T_7	288	217	194	89.4	6248
T_8	285	213	190	89.2	6220
T_9	166	165	131	79.2	3050
SEd	9	6	5	2	199
CD (P=0.05)	19	13	10	4	416

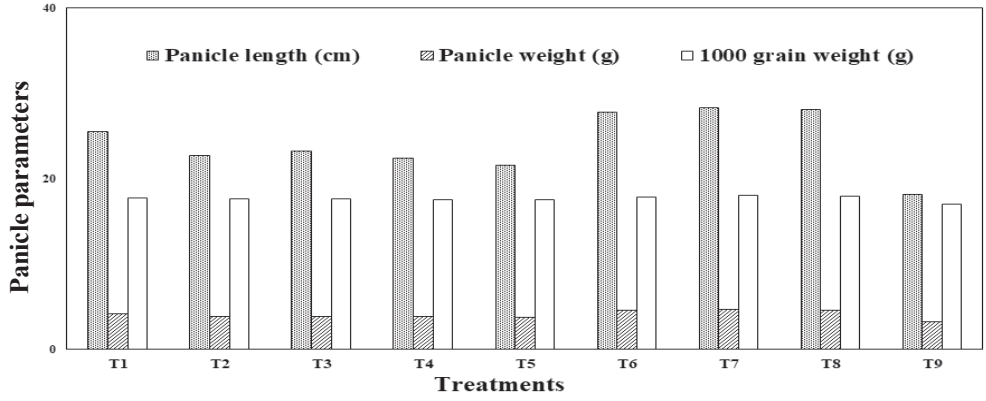

Fig.1. Effect of organic and inorganic sources of nutrients on panicle parameters and 1000 grain weight (g) of transplanted lowland rice.

the non-production of N from any source led to a negative rice performance and the lowest yield parameters were seen in absolute control at the lowest value. If N was not supplied through organic or inorganic source, rice has to obviously depend upon initial N present in the soil, which was not sufficient to produce even reasonable yield (Amitkumar et al., 2013).

Panicle parameters

The organic and inorganic sources of nutrient management practices significantly influenced the panicle parameters of transplanted rice (Fig. 1). Application of 100 per cent NPK through inorganic fertilizers + 6.25 t dhaincha recorded higher panicle weight (4.64 g), panicle length (28.3 cm) and thousand grain weight (18.1 g) which was comparable with 100 per cent NPK through inorganic fertilizers + 5 t vermicompost and 100 per cent NPK through inorganic fertilizers + 12.5 t FYM. This might be due to continuous supply of nutrients by the enriched organics led to better tiller production, enhanced panicle length, weight and filled grain of rice. Among the other treatments, 100 per cent N through dhaincha + balanced P & K through inorganic fertilizers significantly recorded higher panicle weight (4.16 g), panicle length (25.5 cm) and thousand grain weight (17.7 g). The least panicle parameters were recorded with control. Rao (1988) indicated that further filling of grains with photosynthates is likely to occur due to steady and continuous supply of N throughout the entire crop growth period due to gradual transformation and mineralization of organics, solubilization of water insoluble P compounds by organic acids released during decomposition of organics resulting in greater P availability to crop coupled with higher native K availability might have played a key role in ensuring superior yield attributes by organics in combination with inorganic N like in INM practice. This was in agreement with the findings of several workers who reported an increase in yield contributing characters due to addition of mineral N along with organics like Sesbania aculeata (Geethalakshmi, 1996), FYM (Kenchaiah, 1997) and Pressmud (Jain and Tiwari, 1995).

Physiological and biochemical parameters

The organic and inorganic sources of nutrients had influenced physiological and biochemical parameters

Table 4. Effect of organic and inorganic sources of nutrients on physiological and biochemical parameters of transplanted lowland rice.

Treatments	Chlorop-hyll Index	Chlorophyll stability index (%)	Relative water content (%)	Soluble protein content (mg g^{-1})
T_1	34.51	69.26	75.15	14.13
T_2	33.66	67.56	73.23	13.15
T_3	34.01	67.80	74.01	13.21
T_4	33.21	66.07	72.91	13.01
T_5	33.05	65.92	72.03	12.86
T_6	35.76	71.47	77.28	15.02
T_7	36.02	72.22	78.43	15.58
T_8	35.89	71.98	77.76	15.29
T_9	32.08	64.01	70.23	12.08
SEd	0.52	0.75	0.86	0.35
CD (P=0.05)	1.24	1.65	1.81	0.82

(Chlorophyll Index, Chlorophyll Stability Index (%), Relative water content (%), Soluble protein content (mg g^{-1}) of transplanted lowland rice (Table 4). Application of 100 per cent NPK through inorganic fertilizers + 6.25 t dhaincha recorded higher Chlorophyll Index (36.02), Chlorophyll Stability Index (72.22 %), relative water content (78.43%) and Soluble protein content (15.58 mg g^{-1}), which was comparable with 100 per cent NPK through inorganic fertilizers + 5 t vermicompost and 100 per cent NPK through inorganic fertilizers + 12.5t FYM. Sesbania aculatae induced an increase in chlorophyll content in plants and this could be due to increase in enzyme protein (Fujii et al., 1991).

Among the other treatments, application of 100 percent N through dhaincha + balance P & K through inorganic fertilizers significantly recorded highest Chlorophyll Index (34.51), Chlorophyll Stability Index (69.26 %), Relative water content (75.15%) and Soluble protein content (14.13 mg g^{-1}). The ameliorative effect of organic amendments might be linked to increased photosynthetic apparatus which in turn considerably increased the biosynthesis of osmotic solutes Li et al. (2009) in tomato. Increased RWC indicated better growth and development, which in turn depended on leaf area. Rapid early growth and maintenance of RWC at reasonably higher level during reproductive phase greatly influenced the yield. The lowest physiological and biochemical parameters were recorded in control.

Table 5. Effect of organic and inorganic sources of nutrients on chemical compositions of transplanted lowland rice.

Treatments	Protein (%)	Carbohy -drate (%)	Amylose (%)	Fat (%)	Fibre (%)
T_1	7.41	78.53	27.07	0.58	0.231
T_2	7.29	75.65	24.72	0.53	0.207
T_3	7.32	76.85	24.78	0.52	0.209
T_4	7.27	75.27	22.68	0.53	0.200
T_5	7.00	74.75	21.92	0.52	0.195
T_6	7.34	77.05	24.90	0.51	0.211
T_7	7.39	77.81	25.79	0.56	0.220
T_8	7.35	77.33	25.49	0.55	0.212
T_9	6.27	73.75	19.50	0.50	0.184
SEd	0.61	7.31	2.22	0.05	0.018
CD (p=0.05)	1.30	NS	4.52	NS	0.038

Nitrogen use efficiency

Agronomic efficiency and apparent N recovery

The agronomic efficiency which indicated the quantity of rice production per unit quantity of N applied is often expressed as the product of efficiency of absorption and efficiency of utilization. The agronomic efficiency (AE) varied from 13.0 to 18.1 (Fig. 2.). Among the different treatments, application of 100 per cent N through dhaincha + balance P & K through inorganic fertilizers recorded higher AE of 18.1 and100 per cent NPK through inorganic fertilizers + 6.25t dhaincha (17.2). Higher physiological efficiency (57.6) was

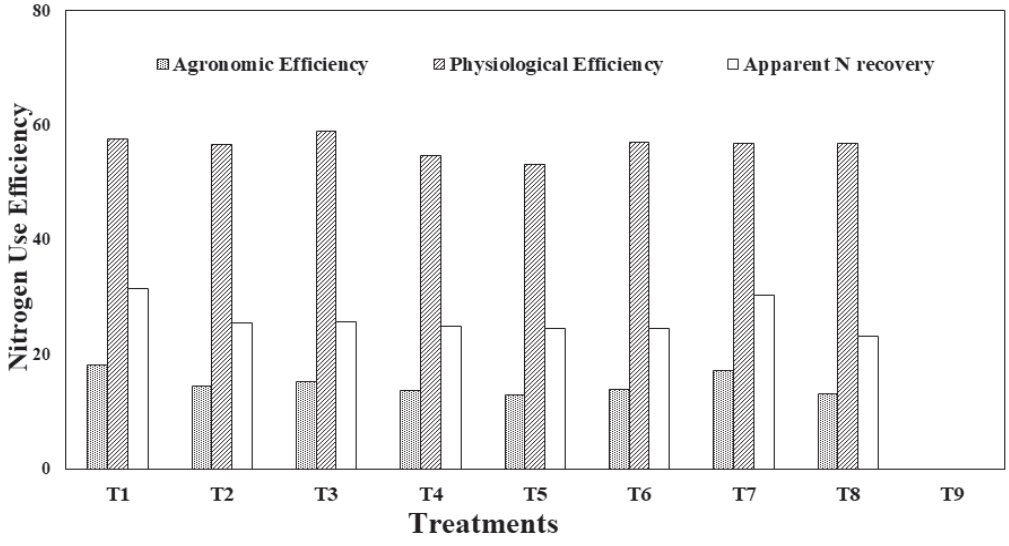

Fig. 2. Effect of organic and inorganic sources of nutrients on nitrogen use efficiency of transplanted lowland rice.

registered under the application of 100 per cent N through vermicompost + balance P & K through inorganic fertilizers and 100 per cent N through dhaincha + balance P & K through inorganic fertilizers registered higher physiological efficiency (57.6). The apparent N recovery indicates the efficiency of absorption of applied N. Agronomic efficiency and apparent N recovery were improved with application 100 per cent N through dhaincha + balance P & K through inorganic fertilizers (31.5) followed by 100 per cent NPK through inorganic fertilizers + 6.25 t Dhaincha (30.3). This might have contributed considerable quantity of N mineralization making more N available to plants leading to increased N use efficiency. Similar results were also reported by Seshadri et al. (2005).

Chemical composition

The chemical composition like protein, carbohydrates, amylose content, fat and fibre are presented in table 5. Application of 100 per cent N through dhaincha + balance P & K through inorganic fertilizers registered with higher values of protein (7.41 %), carbohydrates (78.53%), amylose content (27.07 %), fat (0.58 %) and fibre (0.231 %) which was followed by 100 per cent NPK through inorganic fertilizers + 6.25 t dhaincha and it was comparable with 100 per cent NPK through inorganic fertilizers + 5 t Vermicompost and 100 per cent NPK through inorganic fertilizers + 12.5 t farmyard manure. All these characters were lower in absolute control. Nitrogen being an important element and constituent of the amino acids and protein probably, the increased uptake of N might have resulted in the increment of the crude protein content. This might have led to accumulation of higher quantities of seed components like calcium carbonate and increased the lipid metabolism which helps in increasing the protein content in seed. These results are in accordance by the findings of Roy and Singh (2006). Higher and proper nutrition through the organic matter with ensured supply of nutrients might have led to increase in total amylase content and crude protein (Natarajan, 2007). Lamphin (1990) also stated that organic farming have resulted in higher protein content in the cereals.

Soil enzyme activity

The soil enzyme activity was influenced by the INM practice, organic manures and recommended NPK fertilizers application (Fig. 3).

1. Urease (µg NH_4^+ g^{-1} of soil 24 h^{-1})
2. Dehydrogenase (µg of TPF released g-1 of soil 24 h^{-1})
3. Phosphatase (µg of p-nitrophenol released g-1 of soil 24 h^{-1})

Higher urease activity (47.1 µg NH + g^{-1} soil 24 h^{-1}), soil dehydrogenase activity (37.1 µg of TPF released g^{-1} of soil 24 h^{-1}), soil phosphatase activity (39.1 µg of p - nitrophenol released g^{-1} of soil 24 h^{-1}) were observed with 100 per cent N through dhaincha +

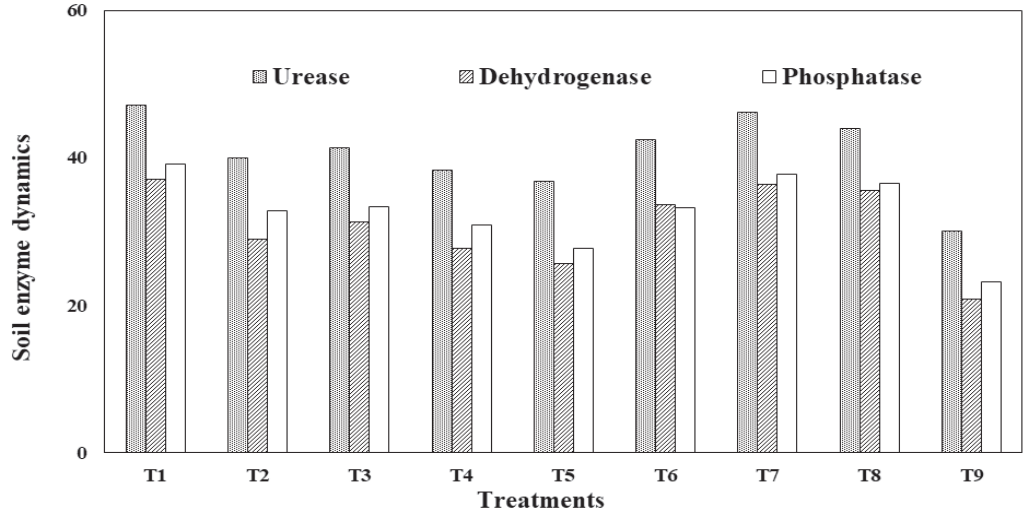

Fig.3. Effect of organic and inorganic sources of nutrients on soil enzyme dynamics of transplanted lowland rice.

balance P and K through inorganic fertilizers followed by 100 % NPK through inorganic fertilizers + 6.25 t dhaincha. The lowest soil enzyme activities (urease activity @ 30 µg $NH+ g^{-1}$ soil 24 h^{-1}, soil dehydrogenase activity @ 20.8 µg of TPF released g^{-1} of soil 24 h^{-1}, soil phosphatase activity @ 23.2 µg of p - nitrophenol released g^{-1} of soil 24 h^{-1}) were registered with absolute control. The organic manures particularly Sesbania aculatae improves the soil unease and dehydrogenase activities and which might have improved soil phosphatase activities too. High organic carbon content in soil applied with dhaincha stimulated the soil microorganisms by serving as source of carbon, energy and other nutrients essential for their growth and multiplication (Stursova and Baldrian, 2010). Higher phosphatase activity was observed with enriched organic manures treated with rock phosphate (Taylor et al., 2002).

CONCLUSION

From the experimental results, it can be concluded that application of 100 percent N through dhaincha and balanced P and K through inorganic fertilizers is recommended for getting maximum yield attributes of rice including physiological, biochemical parameters, chemical composition along with soil enzyme activities followed by application of 100 per cent NPK through inorganic fertilizers + 6.25t dhaincha. These nutrient management practices seem to be better option for rice growers of Tamil Nadu.

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Effect of different nutrient management practices on nutrient availability and uptake in Vaikom kari soils of Kuttanad, Kerala

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ABSTRACT

A field experiment was laid out in RBD with 16 treatments in three replications with rice variety Uma. The treatments were dolomite, lime + $MgSO_4$ or Rice Husk Ash (RHA) + $MgSO_4$ along with 100% package of practice recommendations of Kerala Agricultural University (POP) alone or with 100% POP + foliar spray of 13:0:45 (1%) or borax (0.5%) or 13:0:45 + borax at PI stage. Lime + $MgSO_4$ + 75% POP + 13:0:45 + borax as well as lime without $MgSO_4$ + 100% POP combined with 13:0:45 or borax or both were also included as treatments. The treatment dolomite + POP + 13:0:45 produced the highest grain yield of 5.42 and 5.57 t ha^{-1} during 2015 and 2016 respectively. This treatment was followed by dolomite + POP + 13:0:45 + borax and lime + POP + $MgSO_4$ + 13:0:45 during both the years. Lower yields were produced by the treatments involving RHA and 75% POP. The pooled analysis of two years' data also proved the significance of the treatments involving dolomite + POP or lime + POP + $MgSO_4$ on grain yield. The highest yield of 5.49 t ha^{-1} was recorded by dolomite + POP + 13:0:45 followed by dolomite + POP + 13:0:45 + borax and lime + $MgSO_4$ + POP + 13:0:45. The treatments involving RHA and 75% POP registered significantly lower grain yield in the pooled data. The treatments involving dolomite registered lower status of soil available Fe and higher status of available Mn and B. Higher status of available Zn was registered by the treatments involving dolomite or lime + $MgSO_4$. The treatments involving dolomite, lime + $MgSO_4$ or RHA + $MgSO_4$ along with POP registered higher available Cu in the soil. Dolomite treatments recorded lower status of Na and exchangeable Al in the soil. Dolomite or lime + $MgSO_4$ along with POP + 13:0:45 with or without borax registered higher uptake of Fe, Mn and Zn while dolomite + POP + 13:0:45 with or without borax recorded higher uptake of Cu and B. The treatments involving RHA and 75% POP recorded lower uptake of micronutrients during both the years. Uptake of Na was the highest with RHA + POP + $MgSO_4$ + 13:0:45 during first year and with dolomite + POP during second year. Higher Al uptake was observed with lime + POP + 13:0:45 with or without $MgSO_4$. The grain yield was significantly and positively correlated with the uptake of Mn, Zn, Cu and B and significantly and negatively correlated with Fe during the first year. During the second year, the yield was significantly and positively correlated with uptake of nutrients except Na and Al. The results indicated that amelioration of soil acidity is a crucial management practice for improving the availability and uptake of nutrients resulting in higher yield.

Key words: *Soil acidity, nutrient management,micronutrient availability, nutrient uptake, Vaikom kari soils*

INTRODUCTION

Rice (*Oryza sativa* L.)constitutes the staple food of about two-third of the global population.Kuttanad is distributed in and around the Vembanad Lake whichis the rice bowl of Kerala with 16% of the total rice area and 30% of production in the State (GoK, 2014). Kuttanad soils areaffected by severe acidity and periodic saline water inundation with consequent accumulation of soluble salts. The soils are sub divided and named according to morphological conditions into *kayal*, *karappadam* and *kari*. In these soils, free sulphuric acid is formed by oxidation of sulphur (S) compounds of organic residues or that accumulated in the soil from sea water by repeated inundation. Besides, they contain toxic concentrations of iron (Fe), aluminium

(Al) and unidentified toxic organic compounds (Chattopadhyay and Sidharthan, 1985). In kari soils, the shallow water table with poor drainage andlow aeration enhances the problem of Fe and Al toxicity damaging the roots and hampering the nutrient uptake by plants which necessitates foliar nutrition at critical growth stages.In spite of the high organic carbon content, the soils are low in available nutrient status including available N. Wide spread deficiencies of magnesium (Mg) and boron (B) are also reported. Poorgrain filling and grain discolouration are found to be associated with reducednutrient uptake. Besides, heavy chemical fertilizer application by farmers is causing nutrient imbalance in the soil. Liming is an important practice adopted in many parts of the worldto ameliorate soil acidity. It enhances the physical, chemical and biological properties of acid soils (Bolan, et al., 2003). Burnt lime shell (calcium oxide) is the most common liming material used in Kerala. However, due to ecological constraints, its collection and extraction are restricted in many places and its availability is also limited leading to high cost. Dolomite (calcium magnesium carbonate), which is comparatively a cheaper liming material, imported from the neighbouring states, is also being used. Rice husk ash (RHA), a waste product from rice mills is another potential liming material, which is cheap and environment friendly. Hence lime, dolomite and RHA were evaluated as soil acidity ameliorants in kari soils for enhancing rice yield. Judicious application of NPK, foliar nutrition of N and K at critical growth stage of panicle initiation through water soluble KNO_3 and alleviation of Mg deficiency through application of dolomite/ $MgSO_4$ and B deficiency through foliar nutritionof borax were experimented for improving the nutrient availabilityfor rice in Vaikomkari soils.

Rice, Vaikomkari, acidity amelioration, micro nutrients, nutrient management, lime, dolomite, rice husk ash, foliar nutrition, Fe toxicity

MATERIALS AND METHODS

The experiment was conducted in farmer's fields in Vaikomkari soils in Kottayam district from August to November in 2015 and 2016. The treatments were dolomite, lime + $MgSO_4$ or RHA + $MgSO_4$ along with 100% POP alone or with 100% POP + foliar spray of 13-0-45 (1%) or borax (0.5%) or 13-0-45 + borax at PI stage. Lime + $MgSO_4$ + 75% POP + 13-0-45 + borax as well as lime without $MgSO_4$ + 100% POP combined with 13-0-45 or borax or both were also included as treatments. High yielding medium duration rice variety 'Uma (MO-16)' was used for the study. Fertilizers @ 90:45:45 kg NPK ha^{-1} were applied in all the treatments except T_{13}, where 75% of the recommended dose was applied. $MgSO_4$ @ 80 kg ha^{-1} was applied in soil as basal dose in respective treatments. Potassium nitrate (13:0:45) and borax were given as 1% and 0.5% foliar spray respectively, at PI stage as per treatments. Lime@ 600 kg ha^{-1}, dolomite and RHA each @ 500 kg ha^{-1} were applied in two splits as basal dose and at 30 days after sowing. The analytical data on initial soil physico-chemical properties are presented in Table 1. Soil samples were also collected before each fertilizer application at 20 DAS, 35 DAS, PI stage and harvest. Analysis of plant samples were carried out before PI stage and samples of grain and straw at harvest for micronutrients *viz*., Fe, Mn, Zn, Cu and B. Available Na and heavy metal Al in the soil were also analysed. Uptake of micronutrients was computed by multiplying nutrient content of each part with respective dry weight expressed in kg ha^{-1}. The total uptake was also worked out and expressed in kg ha^{-1}. The procedures followed for analysis of micronutrients and Na and Al are mentioned in Table 3.

RESULTS AND DISCUSSION

The grain yield was significantly influenced by nutrient management practices during both the years (Table 1). The highest grain yield of 5.42 and 5.57 t ha^{-1} during I and II year respectively were produced by dolomite + POP + 13:0:45 (T_2). The treatment involving lime + $MgSO_4$ + 100% POP (T_8) was superior to that involving 75% POP (T_{13}) during both the years. Lower yields were produced by the treatments involving RHA (T_9 to T_{12}). The pooled analysis of two years` data (Fig. 1) also proved the significance on grain yield by the treatments involving dolomite + POP or lime + $MgSO_4$ + POP along with a foliar spray of 13:0:45 or a combined spray of 13:0:45 and borax. The supply of Mg in addition to the correction of acidity in dolomite or lime + $MgSO_4$ application might have resulted in higher yield as per the reports of Koruth et al. (2013) and Biswas (2013), where application of Mg as basal dose lead to significant increase in grain and straw yield

Table 1. Effect of acidity and nutrient management practices on yield and harvest index.

Treatments	2015 grain yield (t ha^{-1})	Straw yield (t ha^{-1})	HI	2016 Grain yield (t ha^{-1})	Straw yield (t ha^{-1})	HI	Pooled Grain yield (t ha^{-1})
T_1	5.00	6.10	0.45	5.13	5.84	0.47	5.06
T_2	5.42	6.58	0.45	5.57	6.21	0.47	5.49
T_3	4.95	6.33	0.44	5.25	6.62	0.44	5.10
T_4	5.33	6.08	0.47	5.48	6.48	0.46	5.41
T_5	4.33	5.30	0.45	5.01	5.83	0.46	4.67
T_6	5.10	6.00	0.46	5.32	6.73	0.44	5.21
T_7	4.80	5.75	0.46	5.15	6.32	0.45	4.98
T_8	4.92	5.83	0.46	5.37	6.20	0.46	5.14
T_9	3.22	4.58	0.41	3.97	5.12	0.44	3.59
T_{10}	3.62	4.42	0.45	4.79	5.57	0.46	4.20
T_{11}	3.42	4.92	0.41	4.45	5.47	0.45	3.93
T_{12}	3.78	4.85	0.44	4.58	5.42	0.46	4.18
T_{13}	4.17	6.28	0.40	4.88	6.11	0.44	4.53
T_{14}	4.48	6.10	0.42	4.95	6.19	0.45	4.72
T_{15}	4.22	5.83	0.42	5.03	6.07	0.45	4.63
T_{16}	4.63	6.10	0.43	5.20	6.58	0.44	4.92
SEm (±)	0.12	0.22	0.01	0.14	0.32	0.01	0.13
CD(0.05)	0.333	0.627	0.030	0.410	0.663	-	0.386

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

of rice in Mg deficient soils. Dolomiteapplication to lowland rice fields, affected by Fe^{2+} toxicity, could improve plant height, shoot and root dry weight and grain yield by decreasing plant Fe content and increasing P and K contents (Suriyagoda et al. 2016). The treatments involving RHA registered significantly lower grain yield in the pooled datadue to its lower efficiency compared to dolomite or lime to ameliorate acidity in extremely acidic soil condition. The treatment involving 75% POP (T_{13}) registered lower grain yield compared to similar treatment with 100% POP (T_8) which might be due to insufficient supply of primary nutrients. Higher yield of treatments involving foliar spray of 13:0:45 or combined spray of 13:0:45 and borax might be due to the timely foliar nutrition of N, K and B and effective absorption and utilization of the nutrients. Application of N and K through foliar spray is especially important in kari soils which is deficient in available N and high in Fe and Ca status that are antagonistic to K. The antagonistic effect of Ca on K was reported by Tisdale et al., 1993. The foliar nutrition is particularly beneficial for the rice crop, with damaged roots, especially from MT to PI stage when nutritional demand for the crop is at peak. The root damage is caused by several factors such as Fe toxicity (Bridgit and Potty, 2002), Al toxicity (Famoso et al., 2010) or excess H_2S accumulation on root surface that decrease root respiration and causes reduced nutrient uptake resulting in deficiencies of K, P, Ca, or Mg in soil (Ramasamy, 2014). Son et al. (2012) had reported the beneficial effect of foliar applied K as KNO_3 in improving grain yield when K uptake via the root zone is limited. Hussain et al. (2012) noticed substantial improvement in rice growth and yield due to application of B at transplanting, tillering, flowering and grain formation stages either by foliar or soil application. Acidity and nutrient management practices had profound influence on straw yield (Table 1). During first year, dolomite + POP + 13:0:45 registered the highest straw yield of 6.58 t ha^{-1}. However, no conspicuous variation in straw yield was observed between this treatment and other dolomite treatments or treatment involving 75% POP or lime treatments without $MgSO_4$ (lime + POP + 13:0:45 and lime + POP + 13:0:45 + borax). During second year, the highest straw yield was produced by lime + $MgSO_4$ + POP + 13:0:45 followed by treatments involving dolomite and lime + $MgSO_4$ along with 100% POP and foliar sprays. The treatments involving RHA recorded markedly lower straw yield during both the years. Significant influence of the treatments on HI was observed only during first year (Table 1). Higher harvest indices were obtained for treatments involving dolomite + POP, lime + $MgSO_4$ + POP and RHA + $MgSO_4$ + POP + 13:0:45 or 13:0:45 + borax. The treatments involving 75% POP and lime without $MgSO_4$ recorded lower HI, the lowest being registered by 75%

Table 2. Physico-chemical properties of the soil of experimental site before the experiment.

Soil parameters	2015		2016		Rating		
	Content	Rating	Content	Rating	Deficiency	Sufficiency	Toxicity
pH	4.23	Extremely acidic	4.29	Extremely acidic	-	-	
EC (dsm^{-1})	0.66	Low	0.39	Low	-	-	>2 dsm^{-1}
Organic carbon (%)	5.69	High	4.85	High	<0.5	>0.5	-
Available N kg ha^{-1}	125.44	Low	172.48	Low	<280	280-560	-
Available P kg ha^{-1}	5.47	Low	6.10	Low	<10	10-25	-
Available K kg ha^{-1}	138.88	Medium	226.24	Medium	<110	110-270	-
Available Ca mg kg^{-1}	382.50	High	605.50	High	<300	>300	-
Available Mg mg kg^{-1}	87.50	Low	49.53	Low	<120	>120	-
Available S mg kg^{-1}	673.90	High	620.04	High	<5	5-10	-
Available Fe mg kg^{-1}	1432	Above toxic limit	1630	Above toxic limit	<5	>5	>300
Available Mn mg kg^{-1}	1.41	High	8.01	High	<1	>1	-
Available Zn mg kg^{-1}	1.79	High	6.21	High	<1	>1	-
Available Cu mg kg^{-1}	0.64	Low	0.13	Low	<1	>1	-
Available B mg kg^{-1}	0.24	Low	0.21	Low	<0.5	>0.5	-
Available Na mg kg^{-1}	152.30	High	230.10	Above toxic limit	<80	80-120	>160
Exchangeable Al mg kg^{-1}	71.00	Below toxic limit	78.08	Below toxic limit	-	-	>120
Dehydrogenase activity	88.75	-	103.6	-	-	-	-

Source of rating: Venugopal et al. (2013).

POP. These treatments also registered lower grain yield during both the years as well as in the pooled data.

Initially, the soil was extremely acidic in nature (Table 2) as per classification of soil acidity (KAU, 2011). During both the years, the soil acidity was reduced below the initial status by the application of ameliorants throughout the cropping period except at harvest (Table 3). Soil pH showed an increasing trend up to tillering stage which decreased afterwards irrespective of treatments during both the years. Considerable reduction in soil pH was observed at harvest during both the years with all the treatments which might be due to drying of soil as well asdiminished effect of liming at harvest. It is evident from the data that the effect of liming materials applied as basal and at 30 DAS did not last after the crop and hence is necessary to follow liming during every crop season. Significant influence of the treatments on soil pH was observed during both the years (Table 3). Among the treatments, dolomite or lime with or without $MgSO_4$ performed better in ameliorating acidity than RHA treatments at all stages during both the years. Rice can grow well in a pH of 5.5 to 6.5 (Singh, 1999) but the soil pH was below 5.0 at all stages in the case of RHA treatments during both the years. Only a slight rise in soil pH could be brought about by the application of RHA compared to dolomite and lime. Hence RHA proved ineffective in ameliorating soil acidity in extremely acidic soil (pH 3.5 to 4.5) such as the kari soil in this study for improving growth and yield of rice. It may be effective in ameliorating moderately (pH 5.5 to 6) or slightly acid (pH 6 to 6.5) soils.

Among the soil available nutrients, the availability of Fe was initially very high (Table 2) which was much above the toxic limit. Though the availability of Fe was brought down by nutrient management practices during the cropping period (Table 4), it was always well above the toxic limit. A decreasing trend of available Fe content from seedling to tillering stage and an increasing trend up to PI stage during first year and up to harvest stage during second year were observed. Drying of soil at harvest and diminishing effect of soil ameliorants leading to lowering of soil pH might have increased the availability of Fe in the soil. The decrease in Fe content at harvest during first year might be due to the rainfall towards end of crop season. Among the treatments, those involving dolomite and lime + $MgSO_4$ registered lower content of available Fe. Higher availability of Fe was noticed with RHA treatments at all stages of the crop which could be attributed to comparatively high soil acidity in RHA applied plots. The initial available Mn status in soil was sufficient which was comparatively higher during

Table 3. Effect of acidity and nutrient management practices on soil pH.

Treatments	pH							
	2015				2016			
	Seedling	Tillering	PI	Harvest	Seedling	Tillering	PI	Harvest
T_1	5.51	5.75	5.32	3.83	4.90	5.60	5.14	3.66
T_2	5.60	5.68	5.32	4.25	5.18	5.59	5.07	3.05
T_3	5.73	5.80	5.27	3.80	5.11	5.29	5.12	3.06
T_4	5.53	5.83	5.33	3.91	4.84	5.34	5.07	3.36
T_5	5.70	6.01	5.42	3.91	5.07	5.42	5.19	3.19
T_6	5.44	5.66	5.28	4.32	5.05	5.49	5.34	3.04
T_7	5.40	5.97	5.26	3.80	4.94	5.69	5.11	3.42
T_8	5.45	5.90	5.31	4.09	5.02	5.28	5.22	3.15
T_9	4.48	4.52	4.23	3.52	4.38	4.27	4.22	2.95
T_{10}	4.57	4.58	4.39	3.64	4.20	4.69	4.19	2.89
T_{11}	4.42	4.43	4.24	3.50	4.09	4.29	4.32	2.79
T_{12}	4.48	4.56	4.29	3.42	4.15	3.95	4.39	2.69
T_{13}	5.57	5.72	5.32	4.14	5.03	5.34	5.26	3.00
T_{14}	5.66	5.99	5.31	3.75	5.16	5.41	5.17	3.22
T_{15}	5.55	5.90	5.37	3.75	5.01	5.23	5.26	3.19
T_{16}	5.54	5.90	5.26	3.89	5.04	5.31	5.24	3.17
SEm (±)	0.06	0.10	0.06	0.11	0.08	0.15	0.13	0.14
CD(0.05)	0.184	0.278	0.173	0.330	0.224	0.422	0.388	0.414

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

second year (Table 2). Available Mn content increased from the initial status during first year up to PI stage and decreased at harvest (Table 5). The leaching loss of Mn due to higher rainfall during the period might have reduced the content of available Mn. During second year, available Mn content decreased from the initial value, declined at PI stage to deficiency level for many treatments even below the detectable limit but increased sharply at harvest. The reduction in available Mn status during the PI stage could be due to very high Ca and Fe contents in the soil showing antagonism with Mn (Tisdale et al., 1993). The sharp increase in Mn availability at harvest might be due to higher soil acidity induced by the dry soil condition at harvest. In general, higher status of available Mn was registered by dolomite treatments. Similar to available Mn, the initial available Zn was above sufficiency level during both the years but was much higher during second year (Table 2). During the cropping period, the soil available Zn content increased up to tillering stage decreased at PI stage and further increased at harvest for most of the treatments during first year (Table 6). During second year, an increasing trend up to PI stage and a decline at harvest were observed. In general, dolomite and lime + $MgSO_4$ treatments registered higher available Zn in the soil.

The soil was deficient in available Cu during both the years initially (Table 2) which could be due to the rich organic matter content in kari soil which binds Cu and makes it less available. Acute Cu deficiency due to chelation with insoluble organic matter that reduces the nutrient availability in peat soils has been reported by Sanyal and Majumdar (2009). Cu is more strongly bound to the organic matter and slow rate of decomposition of organic matter in acid soils decreases the release of Cu that cause deficiency (Jeffery and Uren, 1983). The high S content of the soil (Table 2) could also reduce the availability of Cu by forming CuS

Table 4. Effect of acidity and nutrient management practices on available Fe status in the soil, mg kg^{-1}.

Treatments	Available Fe							
	2015				2016			
	Seedling	Tillering	PI	Harvest	Seedling	Tillering	PI	Harvest
T_1	818.67	625.33	888.67	613.27	1073.67	723.33	920.67	1089.67
T_2	965.83	761.23	788.13	594.57	1192.33	783.37	1075.00	917.20
T_3	504.77	464.77	608.77	618.50	948.40	808.60	1126.00	1428.33
T_4	862.07	717.87	644.07	606.90	1056.87	846.27	1162.00	1288.33
T_5	698.77	635.40	928.23	845.10	1083.67	809.77	1122.33	1428.33
T_6	720.00	540.70	1034.60	893.60	1203.67	742.80	1186.00	1723.67
T_7	747.50	714.13	930.83	870.93	1227.33	920.40	655.73	1227.67
T_8	631.40	766.33	730.57	749.40	1120.00	842.43	1015.07	1274.33
T_9	1020.00	1012.67	1197.00	1066.03	1466.33	1177.43	1235.67	1665.00
T_{10}	1064.67	992.33	1231.00	881.53	1208.00	983.60	1235.67	1654.67
T_{11}	1061.33	1114.00	1200.80	937.10	1284.33	1044.97	1464.00	1769.33
T_{12}	1134.77	1176.33	1103.33	881.67	1257.33	999.33	1442.00	1682.33
T_{13}	831.03	960.03	800.37	762.57	1199.33	858.37	1312.33	1611.67
T_{14}	663.13	908.80	781.10	669.73	1103.67	982.23	1218.67	1650.33
T_{15}	629.47	943.10	857.37	796.27	1199.33	1077.00	1114.67	1568.33
T_{16}	759.40	845.00	802.77	749.53	1240.67	942.00	1153.67	1641.33
SEm (±)	106.17	87.58	44.90	40.12	39.70	58.20	51.69	70.15
CD(0.05)	306.639	252.951	129.692	115.865	114.672	168.082	149.286	202.617

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

which is less soluble. Upon flooding, as the redox potential decreased, insoluble or unavailable sulfides of Cu might have formed which upon draining increased Cu availability to rice plant (Harmsen and Vlek, 1985).During first year, the Cu availability was raised to sufficiency level at PI and thereafter increased further at harvest stage (Table 7) which might be due to the mineralization of organic matter towards the later stages of crop. The Cu status increased at seedling stage from initial status but went even below detectable level at tillering, PI and harvest stages for many treatments during second year. This shows the highly dynamic nature of these soils.Generally, the treatments involving dolomite, lime + $MgSO_4$ or RHA + $MgSO_4$ along with 100% POP registered higher available Cu in the soil. Initially, the soil was deficient in available B during both the years (Table 2). Deficiency of B (0.21 to 0.3 mg kg^{-1}) in kari soils has also been reported by Sasidharan and Ambikadevi (2013). The deficiency can be corrected with the soil application of borax @ 10 kg ha^{-1} or foliar spray with 0.5% boric acid (KAU, 2011). During first year, available B content was higher than the initial status at all stages but remained deficient throughout the cropping period (Table 8). However, during second year available B content increased sharply from the initial status at seedling stage irrespective of treatments, maintained the increase up to tillering stage. Afterwards, the availability of B decreased below detectable limit for most of the treatments. In general, higher available B status was recorded by dolomitetreatments.

Comparatively higher status of available Na was found initially in the soil (Table 2) which is due to the proximity of the field to the saline Vembanad Lake which makes it more prone to saline water inundation

Table 5. Effect of acidity and nutrient management practices on available Mn status in the soil,mg kg^{-1}.

Treatments	Available Mn							
	2015				2016			
	Seedling	Tillering	PI	Harvest	Seedling	Tillering	PI	Harvest
T_1	3.23	3.63	3.26	1.90	3.12	3.25	0.41	9.76
T_2	3.83	4.93	3.91	2.96	4.40	3.48	0.62	10.06
T_3	3.94	4.38	3.18	2.37	2.55	3.20	0.79	9.73
T_4	3.97	4.45	3.53	2.76	5.67	1.60	2.16	10.55
T_5	4.02	2.16	3.94	1.46	5.66	2.21	1.08	8.43
T_6	3.80	3.04	4.02	2.55	3.18	2.01	-	8.13
T_7	5.69	4.77	2.24	2.68	3.51	1.50	-	9.63
T_8	5.65	3.87	2.61	1.14	4.95	3.34	1.37	6.87
T_9	5.04	3.73	5.64	2.42	2.62	2.60	0.42	7.96
T_{10}	6.12	3.43	4.86	2.56	3.76	1.95	1.52	7.09
T_{11}	6.11	3.90	3.28	2.67	2.85	1.86	-	8.15
T_{12}	5.49	3.37	4.03	2.84	2.19	1.31	-	7.93
T_{13}	4.43	3.10	3.35	1.79	3.08	1.73	0.72	8.44
T_{14}	5.75	3.04	3.08	1.55	4.14	2.49	1.01	7.32
T_{15}	5.17	4.27	4.18	1.35	4.22	2.87	1.48	7.94
T_{16}	6.49	3.31	4.11	1.32	2.44	2.08	0.50	8.67
SEm (±)	0.40	0.37	0.39	0.27	0.22	0.18	0.34	0.45
CD(0.05)	1.164	1.075	1.140	0.768	0.634	0.517	0.986	1.302

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

Table 6. Effect of acidity and nutrient management practices on available Zn status in soil,mg kg^{-1}.

Treatments	Available Zn							
	2015				2016			
	Seedling	Tillering	PI	Harvest	Seedling	Tillering	PI	Harvest
T_1	1.74	3.33	2.10	5.58	1.79	2.69	3.49	1.92
T_2	2.42	2.54	2.84	6.75	1.51	2.63	4.04	2.05
T_3	2.16	3.64	3.90	5.28	1.37	2.80	3.20	1.97
T_4	2.26	4.26	3.58	5.77	1.52	2.54	4.18	1.36
T_5	1.91	3.85	5.95	3.89	1.29	2.99	4.04	1.57
T_6	2.43	3.13	2.64	2.87	1.28	3.82	3.47	1.60
T_7	2.49	3.06	1.91	3.15	1.92	2.85	3.11	1.15
T_8	2.78	3.62	1.77	4.04	1.70	3.14	3.38	2.06
T_9	2.77	2.87	2.47	1.87	1.37	2.30	4.11	1.93
T_{10}	1.81	2.90	2.00	1.48	1.67	2.98	4.05	2.08
T_{11}	2.57	3.36	3.33	3.24	1.86	2.99	3.38	2.17
T_{12}	2.31	4.54	1.82	2.80	2.00	2.73	2.73	2.01
T_{13}	3.08	3.46	2.47	2.12	1.58	2.52	3.46	1.60
T_{14}	2.08	2.96	1.64	1.85	1.12	2.78	4.01	2.33
T_{15}	2.50	3.40	2.75	1.79	1.29	3.10	3.86	2.62
T_{16}	1.98	4.70	2.19	1.51	1.64	3.23	3.97	1.69
SEm (±)	0.21	0.30	0.28	0.28	0.12	0.20	0.18	0.10
CD(0.05)	0.617	0.868	0.820	0.806	0.341	0.570	0.532	0.280

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

during summer. There was plot to plot variation in available Na during both the years where second year status was higher and above the level of toxicity. In general, the Na status was reduced below the initial value during both the years and the reduction being more pronounced during second year (Table 9). A sharp decrease of available Na status observed at tillering and PI stages of second year followed by a rise in the Na level at harvest which could be due to the capillary rise of subsoil Na upon drying of the soil at harvest. This rise in Na status was not observed during first year which might be due to higher rainfall towards the end of the crop season that prevented further rise of Na from the subsurface soil.Though the soil was high in exchangeable Al status initially (Table 2), it was below the critical limit of toxicity. This could be due to the

Table 7. Effect of acidity and nutrient management practices on available Cu status in soil, mg kg^{-1}.

Treatments	Available Cu							
	2015				2016			
	Seedling	Tillering	PI	Harvest	Seedling	Tillering	PI	Harvest
T_1	0.97	0.81	1.82	5.18	0.72	0.17	0.58	0.20
T_2	0.72	0.53	1.73	6.77	0.56	-	-	0.10
T_3	0.71	0.96	1.28	8.82	0.60	-	-	0.23
T_4	0.95	0.53	1.57	5.88	0.69	-	-	0.20
T_5	0.80	0.93	1.12	3.07	0.68	0.20	-	-
T_6	0.55	0.59	2.45	4.07	0.68	-	-	0.08
T_7	0.48	0.62	2.42	3.26	0.77	0.27	-	-
T_8	0.80	1.02	3.07	1.90	0.59	-	-	0.52
T_9	0.50	0.13	2.64	1.47	0.80	0.42	0.08	0.13
T_{10}	0.62	0.46	3.46	5.03	0.66	0.31	0.10	0.14
T_{11}	0.59	0.79	5.31	3.32	0.93	0.44	0.58	-
T_{12}	0.36	1.02	2.27	2.41	0.80	0.52	0.14	0.26
T_{13}	0.60	0.24	3.94	2.92	0.81	0.39	0.12	-
T_{14}	0.64	0.26	2.51	3.33	0.72	0.30	0.14	-
T_{15}	0.67	0.59	4.08	3.13	0.63	0.42	-	-
T_{16}	0.69	0.75	2.05	2.45	0.76	0.49	-	-
SEm (±)	0.081	0.08	0.08	0.21	0.07	0.04	0.02	0.03
CD(0.05)	0.235	0.243	0.228	0.597	0.190	0.131	0.076	0.085

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

Table 8. Effect of acidity and nutrient management practices on available B status in soil, mg kg^{-1}.

Treatments	Available B							
	2015				2016			
	Seedling	Tillering	PI	Harvest	Seedling	Tillering	PI	Harvest
T_1	0.36	0.42	0.42	0.41	1.14	1.16	0.26	-
T_2	0.40	0.42	0.41	0.34	1.36	0.69	0.30	-
T_3	0.40	0.40	0.35	0.39	1.17	0.95	0.29	0.03
T_4	0.45	0.48	0.43	0.41	0.89	0.84	0.28	-
T_5	0.43	0.40	0.36	0.32	0.54	0.85	0.21	-
T_6	0.38	0.41	0.37	0.33	0.84	0.53	0.10	-
T_7	0.44	0.43	0.39	0.32	1.08	0.51	0.23	0.07
T_8	0.42	0.43	0.33	0.40	1.24	0.25	0.16	-
T_9	0.46	0.48	0.35	0.36	0.82	0.95	0.11	0.48
T_{10}	0.44	0.44	0.41	0.36	0.76	0.94	0.15	0.68
T_{11}	0.44	0.42	0.36	0.41	0.64	1.03	0.15	0.57
T_{12}	0.44	0.39	0.36	0.38	0.58	1.27	0.15	0.71
T_{13}	0.44	0.42	0.39	0.31	1.23	1.35	0.10	-
T_{14}	0.47	0.44	0.36	0.32	0.54	0.45	0.22	-
T_{15}	0.47	0.36	0.37	0.33	0.78	1.05	0.22	-
T_{16}	0.42	0.44	0.40	0.38	0.37	1.09	0.16	-
SEm (±)	0.01	0.01	0.01	0.01	0.15	0.11	0.02	0.09
CD(0.05)	0.014	0.018	0.020	0.033	0.444	0.315	0.067	0.261

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

high available Fe content in the soil which is antagonistic to Al. The Al content decreased from the initial value during the cropping period at all stages during both the years (Table 10). Merino et al. (2010) has reported that Ca plays a fundamental role in the amelioration of pH and Al toxicity and improving physiological and biochemical processes in plants through Al-Ca interactions. Ca deficiency triggers Al toxicity in plants whereas addition of Ca alleviates Al toxicity (Rengel and Zhang, 2003). During first year, the Al content was reduced at harvest whereas during second year, it was increased.This could be due to higher rainfall towardscrop harvest during first year that resulted in a dilution effect of Al whereas the dry condition at harvest

Table 9. Effect of acidity and nutrient management practices on available Na status in the soil, mg kg^{-1}.

Treatments	Available Na							
	2015				2016			
	Seedling	Tillering	PI	Harvest	Seedling	Tillering	PI	Harvest
T_1	138.83	128.17	129.50	125.00	125.00	86.50	88.00	180.50
T_2	142.33	127.00	131.67	124.00	109.00	100.83	84.50	198.17
T_3	138.17	126.33	134.33	123.37	100.83	92.17	87.00	186.17
T_4	153.17	126.67	129.17	124.50	104.83	107.17	90.33	189.83
T_5	148.17	132.17	125.33	121.33	99.17	98.17	86.17	197.67
T_6	146.33	125.50	131.33	121.67	113.33	96.17	86.67	195.00
T_7	149.33	130.33	128.67	127.67	122.17	98.33	86.00	206.17
T_8	148.17	126.50	130.20	119.33	155.17	102.83	84.83	176.17
T_9	144.73	130.17	130.67	119.33	147.50	100.17	86.17	204.67
T_{10}	164.17	135.67	132.00	122.00	133.00	105.83	86.67	205.00
T_{11}	154.77	138.00	131.67	120.67	139.50	99.67	87.33	207.00
T_{12}	158.67	136.17	132.20	120.67	138.83	110.50	87.50	198.00
T_{13}	148.07	137.83	128.40	119.50	111.17	96.33	86.83	197.17
T_{14}	137.50	131.67	126.50	119.17	130.50	97.50	85.17	177.83
T_{15}	141.67	135.33	124.83	119.17	124.17	108.17	91.17	168.00
T_{16}	146.50	138.00	125.67	119.83	138.67	96.83	83.33	169.33
SEm (±)	2.22	0.66	1.61	1.01	10.97	2.77	1.44	11.41
CD(0.05)	6.405	1.903	4.653	2.907	31.690	8.007	-	-

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

during second year increased acidity and exchangeable Al.

Nutrient management practices had profound influence on the uptake of micronutrients, Na and Al by the crop during both the years (Table 11, Table 12 and Table 13). Dolomite or lime + $MgSO_4$ along with POP + 13:0:45 with or without borax registered higher uptake of Fe, Mn and Zn. Uptake of Na was the highest with RHA + POP + $MgSO_4$ + 13:0:45 during first year and with dolomite+POP during second year. Higher Al uptake was observed with lime + POP + 13:0:45 with or without $MgSO_4$. Higher uptake of Cu and B were observed with dolomite + POP + 13:0:45 with or without borax. In the case of Fe, the uptake was drastically reduced during second year. The reason might be the higher availability and higher uptake of Ca by the crop as reported by Tisdale et al. (1993). In general, higher uptake of micronutrients was observed with dolomite or lime +$MgSO_4$ treatments during both the years. This could be attributed to higher efficiency of these soil ameliorants in correcting soil acidity. The treatments involving RHA and 75% POP recorded lower uptake of micronutrients during both the years and it was reflected in lower grain yield with these treatments. In general, the uptake of nutrients except Fe was comparatively higher during second year especially with respect to B uptake.

Significant but positive correlation of grain yield with uptake of Mn, Zn, Cu and B and negative correlation with Fe was observed during first year (Table 13). However, the yield was positively and significantly correlated with the uptake of all nutrients except Na and Al during second year. The results indicated that amelioration of soil acidity is a crucial management practice for improving the availability and uptake of nutrients resulting in higher yield.

Table 10. Effect of acidity and nutrient management practices on exchangeable Al in soil, mg kg^{-1}.

Treatments	Exchangeable Al							
	2015				2016			
	Seedling	Tillering	PI	Harvest	Seedling	Tillering	PI	Harvest
T_1	16.58	12.97	20.67	6.19	31.18	19.48	48.92	84.40
T_2	18.78	14.86	18.16	8.11	26.15	18.58	51.11	73.20
T_3	21.06	20.33	21.47	6.37	25.52	13.09	47.82	79.38
T_4	19.24	19.22	25.44	10.15	28.51	18.30	48.38	86.67
T_5	20.29	15.79	9.97	11.73	21.88	22.05	59.44	84.27
T_6	15.77	12.32	20.18	8.61	31.80	20.08	46.89	81.29
T_7	51.36	13.19	17.26	8.35	35.14	15.04	52.34	96.33
T_8	51.39	14.73	19.78	6.83	37.49	25.95	49.41	68.67
T_9	47.95	42.89	23.68	33.67	43.95	42.76	56.33	79.55
T_{10}	50.22	53.81	41.29	33.30	37.58	32.46	72.82	70.92
T_{11}	48.80	53.26	45.84	29.76	56.73	34.76	72.89	81.50
T_{12}	47.92	49.46	38.36	34.77	56.56	32.02	80.41	79.27
T_{13}	19.24	22.48	19.81	13.79	42.15	34.37	54.36	97.57
T_{14}	19.77	19.38	15.45	7.46	41.44	24.88	61.21	100.60
T_{15}	13.34	18.49	15.83	10.58	30.76	17.34	61.04	97.30
T_{16}	22.19	28.45	17.72	6.93	24.37	20.81	61.34	105.47
SEm (±)	3.97	3.03	2.95	2.89	2.09	2.99	3.23	4.51
CD(0.05)	11.470	8.757	8.522	8.355	6.036	8.634	9.334	13.022

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

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Table 11. Effect of acidity and nutrient management practices on uptake of Ca, Fe and Mn, kg ha$^{-1.}$

Treatments	Ca uptake						Fe uptake						Mn uptake					
	2015			2016			2015			2015			2015			2015		
	Grain	Straw	Total	Grain	Straw	Total	Grain	straw	Total	Grain	Straw	Total	Grain	Grain	Total	Grain	Straw	Total
T_1	21.59	46.45	68.04	29.52	108.07	137.59	1.52	2.66	4.18	1.02	1.01	2.03	1.33	1.67	3.00	0.10	0.11	0.20
T_2	33.43	55.44	88.86	30.83	111.55	142.38	1.94	2.78	4.72	1.07	1.49	2.56	0.95	2.30	3.25	0.07	0.23	0.30
T_3	31.58	58.38	89.97	28.42	99.62	128.04	1.62	3.05	4.67	0.86	1.24	2.10	0.97	1.36	2.33	0.07	0.18	0.25
T_4	40.45	60.38	100.83	30.17	104.91	135.08	1.05	3.67	4.72	0.83	1.31	2.14	0.88	0.91	1.79	0.08	0.10	0.18
T_5	27.99	49.67	77.66	25.68	92.59	118.27	3.06	3.19	6.24	0.76	1.21	1.97	0.65	0.96	1.61	0.09	0.07	0.15
T_6	34.08	63.46	97.55	29.97	106.89	136.86	3.06	3.73	6.79	1.01	1.25	2.26	0.96	2.10	3.06	0.06	0.13	0.19
T_7	32.07	59.44	91.51	26.97	103.16	130.13	2.46	3.61	6.07	0.90	1.33	2.23	0.51	0.84	1.35	0.07	0.09	0.16
T_8	26.26	63.06	89.32	32.08	108.43	140.52	4.31	1.82	6.13	0.90	1.39	2.28	2.16	1.07	3.24	0.09	0.07	0.16
T_9	17.25	53.32	70.57	19.82	68.98	88.79	3.13	3.80	6.92	0.61	1.20	1.81	1.66	0.81	2.47	0.06	0.04	0.10
T_{10}	18.49	57.47	75.97	20.56	67.57	88.13	3.30	3.30	6.59	0.77	0.88	1.65	1.20	0.78	1.98	0.04	0.09	0.13
T_{11}	18.63	52.59	71.22	18.17	64.11	82.28	2.35	3.57	5.93	0.73	0.72	1.45	0.76	1.02	1.78	0.04	0.10	0.14
T_{12}	18.70	56.63	75.33	20.69	71.22	91.92	2.12	4.12	6.23	0.83	1.03	1.85	0.75	0.86	1.61	0.06	0.20	0.25
T_{13}	18.85	47.90	66.75	25.11	86.06	111.17	2.46	3.36	5.82	0.72	1.58	2.31	0.83	0.84	1.67	0.06	0.08	0.14
T_{14}	23.22	70.53	93.75	28.46	96.88	125.33	2.26	3.88	6.14	0.70	1.38	2.08	0.85	1.50	2.35	0.06	0.21	0.27
T_{15}	19.56	52.50	72.05	27.44	93.76	121.20	2.46	3.27	5.72	0.68	0.79	1.47	0.82	1.81	2.63	0.06	0.18	0.24
T_{16}	24.14	54.50	78.64	26.98	99.43	126.41	2.70	3.28	5.98	0.86	1.22	2.08	0.75	1.70	2.46	0.07	0.20	0.27
SEm (±)	1.01	2.99	3.46	1.67	3.31	4.00	0.32	0.404	0.48	0.05	0.09	0.13	0.13	0.15	0.16	0.01	0.01	0.012
CD(0.05)	2.903	8.635	10.005	4.822	9.562	11.552	0.94	-	1.384	0.143	0.271	0.365	0.362	0.446	0.461	0.018	0.027	0.034

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

Table 12 Effect of acidity and nutrient management practices on uptake of Zn, Cu and B, kg ha^{-1}

Treatments	Zn uptake						Cu uptake						B uptake					
	2015			2016			2015			2016			2015			2016		
	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total
T_1	0.14	0.34	0.48	0.12	0.19	0.31	0.53	0.51	1.04	0.13	0.14	0.27	0.055	0.065	0.12	0.042	0.49	0.53
T_2	0.22	0.39	0.61	0.18	0.14	0.31	0.48	0.76	1.24	0.11	0.08	0.19	0.074	0.077	0.15	0.058	0.63	0.69
T_3	0.15	0.23	0.38	0.18	0.15	0.33	0.51	0.60	1.11	0.11	0.10	0.21	0.067	0.087	0.15	0.065	0.67	0.74
T_4	0.15	0.19	0.34	0.19	0.15	0.34	0.64	0.93	1.57	0.08	0.15	0.22	0.072	0.085	0.16	0.071	0.74	0.81
T_5	0.19	0.29	0.48	0.14	0.15	0.29	0.48	0.63	1.11	0.11	0.11	0.21	0.059	0.065	0.12	0.039	0.37	0.41
T_6	0.18	0.24	0.41	0.19	0.19	0.38	0.61	0.59	1.20	0.08	0.09	0.17	0.061	0.071	0.13	0.061	0.41	0.47
T_7	0.17	0.16	0.33	0.15	0.18	0.32	0.50	0.46	0.96	0.13	0.05	0.19	0.060	0.072	0.13	0.055	0.63	0.69
T_8	0.20	0.14	0.34	0.17	0.18	0.35	0.24	0.31	0.55	0.11	0.08	0.19	0.061	0.087	0.15	0.083	0.56	0.64
T_9	0.15	0.15	0.30	0.11	0.13	0.24	0.21	0.22	0.43	0.06	0.04	0.10	0.043	0.063	0.11	0.033	0.25	0.28
T_{10}	0.20	0.16	0.36	0.12	0.14	0.26	0.16	0.56	0.72	0.05	0.09	0.14	0.051	0.062	0.11	0.045	0.29	0.33
T_{11}	0.12	0.12	0.24	0.10	0.12	0.22	0.19	0.28	0.47	0.08	0.05	0.13	0.050	0.062	0.11	0.034	0.30	0.33
T_{12}	0.12	0.14	0.26	0.09	0.17	0.26	0.16	0.52	0.68	0.06	0.07	0.13	0.047	0.069	0.12	0.052	0.38	0.43
T_{13}	0.14	0.18	0.32	0.11	0.11	0.22	0.55	0.44	0.99	0.07	0.07	0.13	0.049	0.063	0.11	0.043	0.34	0.38
T_{14}	0.21	0.15	0.36	0.16	0.09	0.25	0.65	0.61	1.25	0.07	0.10	0.17	0.057	0.067	0.12	0.044	0.24	0.29
T_{15}	0.15	0.19	0.35	0.16	0.09	0.25	0.48	0.45	0.93	0.08	0.09	0.17	0.052	0.065	0.12	0.057	0.25	0.31
T_{16}	0.19	0.33	0.53	0.13	0.10	0.23	0.54	0.44	0.98	0.10	0.06	0.16	0.057	0.068	0.13	0.075	0.29	0.37
SEm (±)	0.021	0.06	0.07	0.01	0.01	0.02	0.07	0.09	0.12	0.01	0.01	0.01	0.002	0.002	0.003	0.008	0.05	0.05
CD(0.05)	0.0619	0.163	0.193	0.038	0.037	0.056	0.200	0.271	0.340	0.025	0.034	0.041	0.0057	0.0066	0.010	0.0229	0.137	0.133

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

Table 13. Effect of acidity and nutrient management practices on uptake of Na and Al, kg ha^{-1}.

Treatments	Na uptake						Al uptake					
	2015			2016			2015			2016		
	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total	Grain	Straw	Total
T_1	0.95	7.63	8.58	2.79	106.86	109.65	0.61	0.43	1.04	0.80	0.54	1.34
T_2	1.09	7.73	9.14	2.76	85.79	88.55	0.82	0.93	1.75	0.55	0.51	1.06
T_3	1.24	9.28	10.52	2.69	86.66	89.35	0.85	1.65	2.49	0.62	0.52	1.14
T_4	1.45	7.93	9.38	2.87	90.26	93.13	0.68	1.15	1.83	0.62	0.55	1.17
T_5	1.11	6.86	7.96	2.38	88.78	91.17	0.66	0.96	1.61	0.92	0.49	1.41
T_6	1.20	7.54	8.74	2.76	97.04	99.80	0.55	2.01	2.56	1.09	0.65	1.73
T_7	1.10	9.66	10.77	2.75	93.35	96.10	1.21	1.07	2.27	0.79	0.59	1.38
T_8	1.07	7.73	8.80	3.09	101.36	104.45	0.90	1.24	2.14	0.70	0.69	1.38
T_9	1.04	14.00	8.77	2.17	82.10	84.27	0.90	1.06	1.97	0.75	0.53	1.28
T_{10}	1.04	6.04	11.57	2.03	79.42	81.45	0.99	1.64	2.63	0.69	0.59	1.28
T_{11}	0.86	8.36	9.23	1.75	79.65	81.40	0.75	1.15	1.90	0.61	0.45	1.06
T_{12}	1.14	8.04	9.19	2.05	86.63	88.68	0.83	1.73	2.56	0.72	0.54	1.26
T_{13}	1.04	6.20	7.24	1.98	73.11	75.09	0.45	1.75	2.20	0.62	0.55	1.18
T_{14}	1.12	9.55	10.67	2.79	95.28	98.07	1.18	1.74	2.92	0.63	0.54	1.17
T_{15}	0.83	7.40	8.23	2.64	98.91	101.55	1.37	0.71	2.07	0.75	0.59	1.34
T_{16}	0.97	8.78	9.75	2.68	103.32	105.99	0.82	0.55	1.37	0.49	0.58	1.07
SEm (±)	0.07	0.63	0.65	0.15	6.74	6.78	0.08	0.20	0.23	0.05	0.03	0.07
CD(0.05)	0.206	1.829	1.889	0.425	19.465	19.567	0.242	0.572	0.657	0.132	0.093	0.190

T_1 - Dolomite + POP*, T_2 - Dolomite + POP + 13:0:45 as foliar spray (1%) at panicle initiation (PI) stage, T_3 - Dolomite + POP + Borax as foliar spray (0.5%) at PI stage, T_4 - Dolomite + POP + 13:0:45 as foliar spray + Borax as foliar spray, T_5 - Lime + POP + $MgSO_4$ (soil application 80 kg ha^{-1}), T_6 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray (1%) at PI stage, T_7 - Lime + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_8 - Lime + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_9 - Rice Husk Ash (RHA) + POP + $MgSO_4$ (soil application 80 kg ha-1), T_{10} -RHA + POP + $MgSO_4$ +13:0:45 as foliar spray (1%) at PI stage, T_{11} - RHA + POP + $MgSO_4$ + Borax as foliar spray (0.5%) at PI stage, T_{12} - RHA + POP + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{13} -75% POP + Lime + $MgSO_4$ + 13:0:45 as foliar spray + Borax as foliar spray, T_{14} - Lime + POP + 13:0:45 as foliar spray (1%) at PI stage, T_{15} - Lime + POP + Borax as foliar spray (0.5%) at PI stage, T_{16} - Lime + POP +13:0:45 as foliar spray + Borax as foliar spray, *POP recommendation - 90:45:45 kg NPK ha^{-1} (KAU, 2011).

Table 14. Correlation analysis of grain yield versus nutrient uptake at harvest.

Variables correlated with grain yield	Correlation coefficient	
	2015	2016
N uptake at harvest	0.179	0.532**
P uptake	0.781**	0.517**
K uptake	0.584**	0.713**
Ca uptake	0.714**	0.752**
Mg uptake	0.711**	0.667**
S uptake	0.334*	0.596**
Fe uptake	-0.412**	0.385**
Mn uptake	0.371*	0.382**
Zn uptake	0.370*	0.470**
Cu uptake	0.644**	0.520**
B uptake	0.768**	0.587**
Na uptake	0.104	0.275
Al uptake	-0.120	0.032

* significant at 0.05 level, ** significant at 0.01 level.

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Evaluating sequential application of pre and post emergence herbicides in direct-seeded rice

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ABSTRACT

A field experiment was conducted at Agriculture Research Farm, Bihar Agricultural University, Sabour, Bhagalpur, Bihar during kharif season of 2018-19 to study the effect of different herbicides on weed species, growth attributes and yields of direct seeded rice. The experiment comprised of 11 treatments based on weed management practices. The 11 treatments were lone application of pendimethalin and pyrazosulfuron as pre-emergence herbicides while others were as post-emergence at 20 days after sowing i.e. pyrazosulfuron @ 25 g/ha PE, pendimethalin @1000 g/ha PE, pyrazosulfuron @ 25 g /ha PE fb 2,4-DEE @ 750 g/ha POE, pyrazosulfuron @ 25 g /ha PE fb bispyriba-Na @ 25 g/ha POE, pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE, bispyribac-Na @ 25 g/ha POE, bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE, ethoxysulfuron @ 15 g/ha + pyrazosulfuron @ 20 g /ha POE, halosulfuron @ 67.5 g/ha + azimsulfuron @ 30 g/ha POE, hand weeding thrice and weed check. Among all the herbicidal treatments, the minimum weed density (5.13 m^{-2}), dry weight (4.82 g m^{-2}), highest grain yield (5.41 t/ha) and benefit cost ratio (1.9) were recorded with application of pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE.

Key words: *Direct seeded rice, post-emergence herbicides, weed dynamics, rice yield*

INTRODUCTION

Rice (*Oryza sativa* L.) is the main food crop of the universe and above 90 per cent of rice is grown globally and consumed in Asia. Rice is mainly cultivated by transplanting in the puddled field, which creates the development of hard pan and also reimbursement soil structure, because of retention water on the rice fields for a long time it naturally controls the weeds, but all these are not sufficient in the practice. With the resource safeguarding strategies, direct seeding rice (DSR) crop is being lifted as a virtual substitute for transplanting rice. Agriculturalists are ready to implement DSR in order to reduce water and labor cost by circumventing puddling of soil, nursery supervision and transplanting procedures. It helps in maintaining soil structure, diminishes greenhouse gas output enables appropriate sowing of subsequent wheat and divergence of crop by early maturation about one week (Verma et al., 2017).

Being an adoptable technology, the area under DSR is enhancing day by day even though weed management is a difficult task. Weed problem is severe in DSR which is mainly because of lack of standing water. Weeds interfere with the growth and development of rice and may aggregate failures of thecrop (Jayadeva et al., 2011). Although hand weeding is highly effective, it is very difficult to practice because of unavailability of agriculture labour and surge in labour prices. Herbicide application is proved to be effective in managing the weeds and it is cost effective also. Due to a complex form of weed flora in DSR, single herbicidal application may not control the weed population effectively (Mahajan and Chauhan, 2013) which pressed the need for development of herbicide combinations. Herbicidal combinations are essential for effective weed management in DSR because of

complex weed flora. Herbicidal combinations control the majority of weed population in no time apart from their availability for affordable prices. The present study was carried out to assess the effect of alone and sequential application of pre and post-emergence herbicides and their combinations on weed dynamics, crop growth and yield of DSR.

MATERIALS AND METHODS

A field experiment was conducted at Agriculture Research Farm, Bihar Agricultural University, Sabour, Bhagalpur, Bihar (longitude 87°2'42" east and latitude 25°15'40" North at altitude of 46 m a MSL as the heart of Indo-Gangetic Plains) during *kharif* season of 2018-19. The soil of the experimental site was loamy sand in texture having neutral soil reaction (pH 7.27) with electrical conductivity (0.27 dSm^{-1}), low in organic carbon (0.46%) and available N (180.61 kg/ha), medium in available P (22.65 kg /ha) and K (206.88 kg/ha). The experiment comprised of 11 weed management practices, *viz*., alone application of pendimethalin and pyrazosulfuron as pre-emergence (PE) while other herbicides were as post-emergence (POE) at 20 days after sowing (DAS) including i.e. pyrazosulfuron @ 25 g/ha PE, Pendimethalin @1000 g/ha PE, Pyrazosulfuron @ 25 g /ha PE fb 2,4-DEE @ 750 g/ha POE, Pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 25g/ha POE, Pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE), Bispyribac-Na @ 25 g/ha POE, Bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE, Ethoxysulfuron @ 15 g/ha + pyrazosulfuron @ 20 g/ha POE, Halosulfuron @ 67.5 g/ha + azimsulfuron @ 30 g/ha POE, Hand Weeding thrice at 15, 30 & 45 DAS and weed check. The experiment was laid out in randomized block design (RBD) with three replications. Rice variety Sabour Sampanna dhan (BRR0059) was sown on 16th June 2018 using for line sowing by hand plough with seed rate of 30 kg/ha in rows spaced at 20 cm apart. The recommended dose of fertilizers (120 N kg/ha, 60 kg P_2O_5/ha and 40 kg K_2O/ha) and plant protection measures for insect-pest and disease were applied. The data on plant height (cm), dry matter accumulation (g m^{-2}), crop growth rate (g m^{-2} day^{-1}), leaf area index and number of tillers (m^{-2}) were measured at 90 DAS and maturity stage. The required amounts of herbicides were sprayed using 375 l/ha of water with knapsack sprayer fitted with a flat fan nozzle. Weed observation was done 60 days after sowing (DAS), a quadrate of 0.25 m-2 was placed at two places in each plot to determine the density of different weeds. The data on weed density were subjected to square root transformation. Yield and B:C ratio were also calculated for each treatment separately. The data were statistically analyzed further through Analysis of Variance (ANOVA) technique.

RESULTS AND DISCUSSION

Weed flora

Weed flora of experimental plots were comprised of broad-leaved weeds (BLWs) like caesulia axillaris, Phyllanthus niruri, Euphorbia hirta, Eclipta alba, Trianthema portulacastrum, Commelina benghalensis, Physalis minima and Ageratum conyzoides, grasses like *Echinochloa crusgalli*, *Echinochloa colona*, *Cynodon dactylon*, *Eleusine indica*, *Paspalum distichum* and *Digitaria sanguinalis* and sedges like *Cyperus rotundus*, *Cyperus iria*, *Cyperus compressus*, *Fimbristylis miliacea* and *Fimbristylis dichotoma* were very frequent.

Weed density and dry matter

Among all the treatments, minimum weed density/m2 was recorded in hand weeding plot and the maximum weed density/m^2 was recorded in weed check plot at 45 and 60 DAS (Table 1 and Table 2). Hand weeding plot has recorded less weed dry matter at 15, 30, 45 and 60 DAS at all growth stages of crop and weed check plot has recorded significantly highest weed dry matter (Table 3). It is probably because of elimination of weeds at the time of intercultural which lead to the reduction in weed dry matter. Similar findings were also reported by Dayaram et al. (2016).

Among the all herbicidal treatments, the minimum weed density/m^2 was recorded in pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE at 45 DAS which was statistically at par with pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium @ 25 g/ha POE at 45 and 60 DAS. The maximum weed density/m^2 was recorded in treatment pendimethalin @ 1000 g/ha PE. The application of herbicides can be attributed to their efficacy to control wide spectrum of weeds (Parihar et al., 2020). The minimum weed dry weight was recorded in pyrazosulfuron @ 25 g/ha PE which was

Table 1. Effect of chemical weed management on weed density/m^2 at 45 days after sowing in direct seeded rice.

Treatments	*Cyperus rotundus*	*Echinochloa* spp	*Eleusine indica*	*Caesulia axillaris*	Other weeds	Total weeds
Pyrazosulfuron @ 25 g /ha PE	5.66 (31.53)	4.33 (18.30)	2.66 (6.67)	3.24 (10.03)	3.92 (14.92)	8.48 (71.42)
Pendimethalin @ 1000 g/ha PE	6.13 (37.14)	4.53 (20.08)	2.85 (7.72)	3.41 (11.14)	4.06 (16.00)	9.02 (80.94)
Pyrazosulfuron @ 25 g/ha PE fb 2,4-DEE @ 750 g/ha POE	5.20 (26.62)	4.96 (24.15)	2.77 (7.17)	2.10 (3.93)	3.58 (12.37)	8.41 (70.30)
Pyrazosulfuron @ 25 g/ha PE fb Bispyribac-sodium@ 25 g/ha POE	4.42 (19.07)	3.29 (10.42)	2.28 (4.70)	2.00 (3.57)	3.12 (9.25)	6.63 (43.42)
Pyrazosulfuron @ 25 g/ha PE fbBispyribac-sodium @ 20 g/ha + Pyrazosulfuron @ 20 g /ha POE	4.13 (16.63)	3.08 (9.03)	2.08 (3.87)	1.97 (3.40)	3.00 (8.52)	6.20 (38.05)
Bispyribac-sodium@ 25 g/ha POE	5.10 (25.50)	4.79 (22.53)	2.71 (6.83)	2.58 (6.15)	3.54 (12.08)	8.21 (66.95)
Bispyribac sodium@ 20 g/ha. + Pyrazosulfuron @ 20 g /ha POE	4.97 (24.17)	3.96 (15.23)	2.67 (6.68)	2.59 (6.23)	3.51 (11.85)	7.64 (57.91)
Ethoxsulfuron @ 15 g /ha + Pyrazosulfuron @ 20 g /ha POE	4.74 (21.98)	4.46 (19.44)	2.85 (7.66)	2.76 (7.18)	3.67 (13.00)	7.91 (62.09)
Halosulfuron @ 67.5 g /ha + Azimsulfuron@ 30 g /ha POE	4.56 (20.37)	3.76 (13.63)	2.42 (5.40)	2.38 (5.17)	3.36 (10.82)	7.11 (50.22)
Hand weeding (15,30 and 45 DAS)	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)
Weed check	7.46 (55.20)	7.60 (57.25)	4.12 (16.47)	3.98 (15.39)	4.57 (20.37)	12.24 (149.29)
SEm ±	0.11	0.17	0.14	0.10	0.11	0.17
LSD (P=0.05)	0.33	0.50	0.40	0.31	0.33	0.51

Data in parenthesis were transformed to $\sqrt{x}$ + 0.5 before analysis. The figures in parentheses are the original values.

statistically at par with pendimethalin @ 1000 g/ha PE, pyrazosulfuron @ 25 g/ha PE fb 2,4-DEE @ 750 g/ha POE, pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium @ 25 g/ha POE, pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium @ 20 g/ha + pyrazosulfuron @ 20 g /ha POE and the maximum dry weight of weed was obtained with halosulfuron @ 67.5 g/ha + azimsulfuron@ 30 g /ha POE at 15 DAS. In rest of the growth stages (30, 45 and 60 DAS), the minimum total dry weight was recorded in pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE which was statistically at par with pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium@ 25 g/ha POE and the maximum dry weight of total weeds was noticed in pendimethalin @ 1000 g/ha PE.

The application of herbicides can be attributed to their efficacy to control wide spectrum of weeds. The integration of herbicides which resulted in broad spectrum weed control over the other treatments as pre emergence herbicides eliminated the early emerged weeds and post emergence herbicides controlled the later germinated weeds which resulted in lowest weed population as well as weed dry weight. These results are in conformity with the findings of Patel et al. (2018) who noticed that application of pyrazosulfuron-ethyl @ 25 g/ha fb bispyribac-sodium salt @ 50 g/ha at 30 DAS suppressed the weed population and dry weight over control.

Effect on growth attributes

There was no significant difference in plant height between treatments at 30, 60, 90 DAS and at maturity (Table 4). Crop grown under hand weeding plot had tallest plants during 30 to 90 days of crop stages and extending up to at maturity. The minimum plant height was recorded in the weedy check at all growth stage. The higher dry matter accumulation were recorded under hand weeding thrice condition at 90 DAS and at maturity, whereas lowest dry matter accumulation was recorded in weedy check (Table 4). Amongst herbicides, maximum dry matter accumulation was obtained with

Table 2. Effect of chemical weed management on weed density/m^2 at 60 days after sowing in direct seeded rice.

Treatments	*Cyperus rotundus*	*Echinochloa crusgulli*	*Eleusine indica*	*Caesulia axillaries*	*Cyperus iri, Phyllanthus niruri, Paspalum disttichum* and *Cynodon dactylon*	Total weeds density
Pyrazosulfuron @ 25 g/ha PE	5.00 (24.50)	4.15 (16.73)	2.50 (5.83)	3.11 (9.19)	3.67(12.98)	7.78 (69.23)
Pendimethalin @ 1000 g/ha PE	5.51 (29.83)	4.33 (18.30)	2.61 (6.40)	3.24 (10.02)	3.81(14.05)	8.31 (78.60)
Pyrazosulfuron @ 25 g/ha PE fb 2,4-DEE @ 750 g/ha POE	4.44 (19.30)	4.78(22.37)	2.61 (6.32)	2.01 (3.57)	3.39(11.00)	7.71 (54.95)
Pyrazosulfuron @ 25 g/ha PE fb Bispyribac-Na @ 25 g/ha POE	3.31 (10.50)	2.94(8.17)	1.95 (3.33)	1.97 (3.40)	2.71(6.93)	5.42 (32.33)
Pyrazosulfuron @ 25 g/ha PE fb Bispyribac-Na @ 20 g/ha + Pyrazosulfuron @ 20 g /ha POE	3.09 (9.07)	2.82(7.50)	1.91 (3.20)	1.78 (2.70)	2.57(6.13)	5.13 (28.60)
Bispyribac-Na @ 25 g/ha POE	4.41 (18.97)	4.35(18.48)	2.98 (8.41)	2.49 (5.72)	3.18(9.71)	7.48 (61.29)
Bispyribac-Na @ 20 g/ha + Pyrazosulfuron @ 20 g/ha POE	4.13 (16.65)	3.88(14.73)	2.57 (6.13)	2.45 (5.53)	3.00(8.53)	6.81 (51.57)
Ethoxsulfuron @ 15 g/ha + Pyrazosulfuron @ 20 g/ha POE	3.89 (14.73)	4.32(18.13)	2.59 (6.40)	2.62 (6.38)	3.25(10.07)	7.06 (55.71)
Halosulfuron @ 67.5 g/ha + Azimsulfuron@ 30 g/ha POE	3.64(12.87)	3.57(12.33)	2.30 (4.84)	2.13 (4.17)	2.81(7.42)	6.14 (41.63)
Hand weeding (15, 30 and 45 DAS)	0.71(0.00)	0.71(0.00)	0.71 (0.00)	0.71 (0.00)	0.71(0.00)	0.71 (0.00)
Weed check	7.03(49.00)	7.36(53.74)	3.82 (14.07)	3.86 (14.40)	4.13(16.60)	11.57(147.81)
SEm ±	0.15	0.16	0.17	0.10	0.13	0.18
LSD (P=0.05)	0.45	0.47	0.49	0.31	0.37	0.54

Data in parenthesis are subjected to square root transformation $\sqrt{x}$ + 0.5 before analysis.

pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE at 90 DAS and maturity minimum dry matter accumulation was obtained with pendimethalin @ 1000 g/ha PE. Herbicides (pre and post) in combinations were produced maximum dry matter as compared to single

Table 3. Effect of chemical weed management practices on total weed dry matter (g/m^2) at various crop growth stages of direct seeded rice.

Treatments	15 DAS	30 DAS	45 DAS	60 DAS
Pyrazosulfuron @ 25 g /ha PE	4.91 (23.62)	6.17 (37.56)	6.74 (44.87)	6.90 (47.05)
Pendimethalin @ 1000 g/ha PE	5.21 (26.61)	6.54 (42.32)	7.00 (48.57)	7.04 (49.02)
Pyrazosulfuron @ 25 g/ha PE fb 2,4-DEE @ 750 g/ha POE	5.15 (26.13)	5.63 (31.31)	6.17 (40.94)	6.32 (39.50)
Pyrazosulfuron @ 25 g/ha PE fb Bispyribac-sodium@ 25 g/ha POE	5.08 (25.37)	4.14 (16.70)	4.67 (21.56)	4.82 (24.88)
Pyrazosulfuron @ 25 g/ha PE fbBispyribac-sodium @ 20 g/ha + Pyrazosulfuron @ 20 g/ha POE	5.13 (25.91)	3.82 (14.17)	4.44 (19.29)	4.57 (20.42)
Bispyribac-sodium@ 25 g/ha POE	7.12 (50.18)	5.24 (26.93)	5.78 (32.92)	5.93 (34.70)
Bispyribac sodium@ 20 g/ha. + Pyrazosulfuron @ 20 g /ha POE	6.81 (45.83)	4.92 (24.04)	5.62 (30.98)	5.86 (33.90)
Ethoxsulfuron @ 15 g/ha + Pyrazosulfuron @ 20 g/ha POE	7.10 (49.98)	5.53 (30.31)	6.09 (36.65)	6.22 (38.22)
Halosulfuron @ 67.5 g/ha + Azimsulfuron @ 30 g/ha POE	7.35 (53.59)	4.56 (19.94)	5.28 (27.37)	5.40 (28.72)
Hand weeding (15,30 and 45 DAS)	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)	0.71 (0.00)
Weed check	8.47 (71.31)	11.23 (104.00)	11.32 (127.62)	12.68 158.13)
SEm ±	0.11	0.14	0.08	0.09
LSD (P=0.05)	0.32	0.40	0.24	0.27

Data in parenthesis were transformed to $\sqrt{x}$ + 0.5 before analysis. The figures in parentheses are the original values.

Table 4. Effect of chemical weed management on growth attributes at 90 DAS and maturity of direct seeded rice.

Treatments	Plant height (cm)		Dry matter accumulation (g m^{-2})		Crop growth rate (g m^{-2} day^{-1})		Leaf area index		No. of tillers (m^{-2})	
	90 DAS	At maturity	90 DAS	At maturity	60-90 DAS	90- At maturity	90 DAS	At maturity	90 DAS	At maturity
Pyrazosulfuron @ 25 g/ha PE	77.7	100.5	631.0	878.7	6.11	4.19	4.41	3.53	273.5	251.7
Pendimethalin @ 1000 g/ha PE	77.3	99.9	625.0	876.3	6.10	4.1	4.36	3.45	260.3	236.7
Pyrazosulfuron @ 25 g/ha PE fb 2,4-DEE @ 750 g/ha POE	79.3	101.2	640.3	907.7	5.83	4.46	4.38	3.50	281.8	258.3
Pyrazosulfuron @ 25 g/ha PE fb Bispyribac-Na @ 25 g/ha POE	84.0	105.5	681.7	1002.0	6.26	5.34	4.73	3.73	312.8	281.3
Pyrazosulfuron @ 25 g/ha PE fb Bispyribac-Na @ 20 g/ha + Pyrazosulfuron @ 20 g/ha POE	84.2	108.3	696.7	1025.7	6.68	5.70	4.75	3.75	326.4	293.7
Bispyribac-Na @ 25 g/ha POE	79.7	103.3	652.0	990.0	6.59	5.63	4.50	3.53	295.5	268.7
Bispyribac-Na @ 20 g/ha + Pyrazosulfuron @ 20 g/ha POE	81.1	104.3	660.0	992.7	6.64	5.54	4.69	3.65	303.6	273.0
Ethoxsulfuron @ 15 g/ha + Pyrazosulfuron @ 20 g/ha POE	78.3	102.2	642.0	928.3	6.62	4.77	4.39	3.64	278.2	252.0
Halosulfuron @ 67.5 g/ha + Azimsulfuron@ 30 g/ha POE	82.1	104.8	675.7	998.7	6.10	5.38	4.66	3.67	309.5	278.7
Hand weeding (15,30 and 45 DAS)	87.6	109.0	715.0	1058.0	6.83	5.72	4.84	3.77	344.7	307.3
Weed check	77.0	99.0	455.3	873.0	3.73	5.29	3.82	2.50	214.1	194.7
SEm ±	2.5	3.0	11.2	4.5	0.42	0.22	0.11	0.03	12.4	11.3
LSD (P=0.05)	NS	NS	33.0	13.3	1.24	0.66	0.32	0.10	36.6	33.3

herbicide. Application of pyrazosulfuron as a pre-emergence herbicide and in combination with bispyribac-sodium as post emergence resulted in greater reduction of weed density in suppers of lowest plant-weed competition compared to rest of the treatments. This finding was in conformity with Ramachandiran and Balasubramanian (2012).

Hand weeded thrice has obtained higher crop growth rate (CGR) which is significantly superior to rest of the treatments (Table 2). In case of herbicides, the highest CGR was under pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE whereas the lowest was recorded in pendimethalin @ 1000 g/ha PE at 90 DAS maturity.

Maximum leaf area index (LAI) was observed in the hand weeding thrice and in contrast weedy check arrested significantly minimum LAI at at 90 DAS (Table 4). The maximum LAI was observed under pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE which was at par with pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 25 g/ha POE at 90 DAS and maturity. Among herbicidal treatments better weed control efficiency has led to higher growth and development and ultimately improved the LAI.

Irrespective of growth stages, the maximum number of tillers of rice was observed in hand weeding thrice and minimum in weedy check (Table 4). Among the all herbicidal treatments, the maximum number of effective tillers were recorded significantly in pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE at 90 DAS and maturity stage being at par with pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 25 g/ha POE and halosulfuron @ 67.5 g/ha + azimsulfuron @ 30 g/ha POE. The minimum number of effective tillers were recorded in pendimethalin @ 1000 g/ha PE. The higher tillers in particular treatment due to weed density and weed dry weight along with higher weed control efficiency from initial stages to the critical period of

Table 5. Effect of chemical weed management practices on yield attributes of direct seeded rice.

Treatments	No. of panicles/ m^2	No. of grains/ panicle	Test weight (g)	Length of panicle (cm)
Pyrazosulfuron @ 25 g/ha PE	251.7	115.5	23.1	26.7
Pendimethalin @ 1000 g/ha PE	236.7	114.6	23.0	26.4
Pyrazosulfuron @ 25 g/ha PE fb 2,4-DEE @ 750 g/ha POE	261.3	102.7	22.9	26.3
Pyrazosulfuron @ 25 g/ha PE fb Bispyribac-sodium@ 25 g/ha POE	281.3	136.3	24.1	30.7
Pyrazosulfuron @ 25 g/ha PE fbBispyribac-sodium @ 20 g/ha + Pyrazosulfuron @ 20 g/ha POE	293.7	141.1	24.3	30.8
Bispyribac-sodium@ 25 g/ha POE	268.7	122.4	23.7	28.6
Bispyribac sodium@ 20 g/ha. + Pyrazosulfuron @ 20 g/ha POE	276.0	125.2	23.4	28.9
Ethoxsulfuron @ 15 g/ha + Pyrazosulfuron @ 20 g/ha POE	269.0	117.3	24.0	26.4
Halosulfuron @ 67.5 g/ha + Azimsulfuron@ 30 g/ha POE	278.7	133.7	23.2	29.1
Hand weeding (15,30 and 45 DAS)	307.3	145.5	24.7	31.0
Weed check	194.7	97.1	22.9	24.8
SEm ±	11.3	4.4	0.4	1.1
LSD (P=0.05)	33.3	13.1	NS	3.3

crop weed competition led to better field of crop. Similar findings were also observed by Kumar et al. (2015).

Yield attributes

Hand weeded plot has obtained highest yield attributes viz., panicle length, number of effective tillers/m^2, number of grains/panicle and test weight (Table 5). The increased yield attributing characters is because of positive impact of hand weeding at 15, 30 and 45 DAS on tillering and low weed density leads to better uptake of nutrients. Among the herbicide applied treatments, pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE has recorded higher yield attributes *viz*., panicle length, number of effective tillers/m^2, number of grains/panicle and test weight which were at par with the pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium@ 25 g/ha POE.

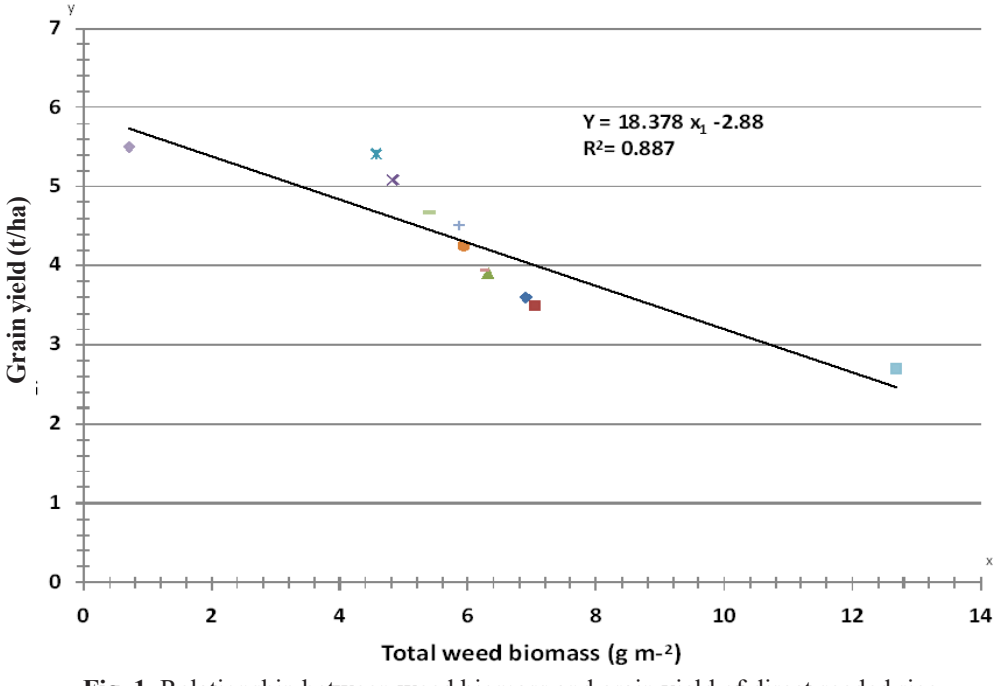

Fig. 1. Relationship between weed biomass and grain yield of direct seeded rice.

Table 6. Effect of chemical weed management practices on grain, straw yield and Harvest index of direct seeded rice.

Treatments	Grain yield (t /ha)	Straw yield (t /ha)	Harvest index (%)	B:C ratio
Pyrazosulfuron @ 25 g/ha PE	3.60	4.99	42.0	1.1
Pendimethalin @ 1000 g/ha PE	3.50	5.01	41.2	1.0
Pyrazosulfuron @ 25 g/ha PE fb 2,4-DEE @ 750 g /ha POE	3.90	5.26	42.4	1.2
Pyrazosulfuron @ 25 g/ha PE fb Bispyribac-sodium@ 25 g/ha POE	5.08	6.35	44.4	1.8
Pyrazosulfuron @ 25 g/ha PE fbBispyribac-sodium @ 20 g/ha + Pyrazosulfuron @ 20 g /ha POE	5.41	6.67	44.7	1.9
Bispyribac-sodium@ 25 g/ha POE	4.25	5.90	42.0	1.4
Bispyribac sodium@ 20 g/ha. + Pyrazosulfuron @ 20 g /ha POE	4.51	6.06	42.8	1.5
Ethoxsulfuron @ 15 g/ha + Pyrazosulfuron @ 20 g /ha POE	3.94	5.37	42.1	1.2
Halosulfuron @ 67.5 g/ha + Azimsulfuron@ 30 g /ha POE	4.67	6.20	43.2	1.5
Hand weeding (15, 30 and 45 DAS)	5.50	6.69	45.5	1.5
Weed check	2.70	3.97	40.7	0.6
SEm ±	0.28	0.44	2.4	0.2
LSD (P=0.05)	0.82	1.31	NS	0.5

The higher yield attributes are because of low weed density, weed dry weight and higher weed control efficiency as it controlled the weeds effectively starting from initial stages to the critical weed competition stage leading to better growth of crop. Similar findings were also observed by Kumar et al. (2015) who reported that yield attributing parameters *viz*., number of effective tillers/hill, panicle length, filled grains/panicle and test weight were found significantly higher with pyrazosulfuron ethyl @ 30 g/ha. The minimum yield attributes in weedy check is because of weed competition by uncontrolled weed growth.

Relationship between weed biomass and rice grain yield

The above-ground biomass of weeds and rice were negatively correlated with each other, as weeds biomass increased, the analogous decline in rice grain yield was recorded. Result depicted that each gram increment in weed biomass reflected as decrease of gain yield. Results clearly show that if initial flush of weed is controlled either by chemically or manual boosted yield (Fig. 1) (Parihar et al., 2020). The figure depicts that rice yield 88.7 % depends on weed flora crop and maximum reduction may be expected under direct seed rice.

Effect on yield and economics

The hand weeding thrice has recorded significantly higher grain and straw yield among all the treatments and found at par with pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE, pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 25 g/ha POE and halosulfuron @ 67.5 g/ha + azimsulfuron @ 30 g/ha POE (Table 6). The weedy check treatment produced significantly lower grain yield as compared to remaining treatments. The significantly higher grain yield was noticed in pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE and found comparable with pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 25 g/ha POE and halosulfuron @ 67.5 g/ha + azimsulfuron@ 30 g/ha POE. The increased grain yield is result of better suppression of weeds and yield attributes like number of effective tillers/m^2, panicles length, number of grains/ panicle (Pratap et al., 2017). The treatment pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE obtained significantly higher straw yield of 6.69 t /ha which was at par with pyrazosulfuron @ 25 g/ha PE fb bispyribac-sodium@ 25 g/ha POE, bispyribac-sodium@ 25 g/ha POE, bispyribac-sodium@ 20 g/ha + pyrazosulfuron @ 20 g/ha POE, ethoxsulfuron @ 15 g/ha + pyrazosulfuron @ 20 g /ha POE, halosulfuron @ 67.5 g/ha + azimsulfuron@ 30 g/ha POE. There is no significant difference observed among all the weed management treatments. Harvest index followed the same trend as that of grain/straw yield and recorded higher value in hand weeded plot.

The highest B:C ratio was observed in pyrazosulfuron @ 25 g/ha PE fb bispyribac-Na @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE and the lowest

B:C ratio was observed in weed check plot.

CONCLUSION

Weed management is one of the major in direct seeded rice which can be taken care with the application of herbicides. This reduced yield drastically as seen in weedy check. Application of pyrazosulfuron @ 25 g/ha PE fb bispyribac sodium @ 20 g/ha + pyrazosulfuron @ 20 g/ha POE controlled the weeds and enhanced the growth. Hence, the treatment is being suggested for better yields.

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SHORT COMMUNICATION

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First report of bacterial leaf blight of rice caused by *Sphingomonas* sp. in Southern Karnataka, India

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ABSTRACT

Bacterial leaf blight disease is a major biotic constraint of rice cultivation distributed widely in Asia including India. During the survey (Kharif 2019) in southern Karnataka bacterial leaf blight severity ranged from 30-55% was recorded from maximum tillering onwards till the dough stage. The symptoms observed were water-soaked stripes from the tip of the leaves to white wavy margins along one or both the edges turning yellowish white and later became straw-colored and finally dries out. Symptomatic blight disease samples were collected from the field and the causal organism was isolated on nutrient agar medium following colony morphology and biochemical characteristics was studied. The isolates were isolated and tested for pathogenicity on variety Jyothi under glass house condition. DNA fingerprinting of 16SrDNA and nucleotide blast analysis of the seventeen isolates sequenced, five showed 97-100% sequence identity with Sphingomonas panni, Sphingomonas paucimobilis, and Sphingomonas sp. and rest of twelve matched to Xanthomonas oryzae and Pantoea sp.

Key words: *Rice, bacterial leaf blight, Sphingomonas sp.,16SrDNA*

Rice is an important cereal crop in Cauvery command area of southern Karnataka. During the survey (*kharif*, 2019) in southern Karnataka bacterial leaf blight severity ranged from 30-55% was recorded from maximum tillering onwards till the dough stage. At tillering stage, the disease initiated as water-soaked stripes from the tip of the leaves and as white wavy margins along one or both the edges turning yellowish white and later became straw-colored (creamish yellow). During the dough stage, white and straw coloured stripes were observed on one or both sides and along the midribs of the flag leaf and top leaves. The stripes increased in length and breadth, coalesced, becomes necrotic, twisted, scroll, and dries out (Fig. 1). The symptoms observed were slightly different from leaf blight caused by *Xanthomonas oryzae* pv *oryzae* producing lesions which were water soaked, yellow in color with irregular wavy margin and progresses down the leaves. The lesions initially developed at the leaf margin near its tip. In the early morning hours, bacterial ooze consisting of small, yellowish, spherical masses was seen on the margins or veins of the freshly infected leaves under moist conditions. (Nino-Liu et al., 2006). Symptomatic blight disease samples were collected from the field to isolate the putative causal agent. Molecular characterization was carried out by DNA fingerprinting.

The symptomatic tissue pieces (1 mm length and 0.5 mm width) were surface sterilized with 70% ethanol rinsed thrice with sterile distilled water and transferred to 1 ml Eppendorf tubes having 500 µl sterile water and kept for 15 min. The suspension was diluted sevenfold and streaked on nutrient agar plates and incubated at 28°C for 48h. Isolated colonies were bright yellow color, convex, opaque, mucoid circular with a smooth margin. Predominant single colonies were selected and transferred into nutrient agar slant and stored at 4°C whereas in 15% glycerol at 20°C for long term storage. The isolates were isolated and tested for pathogenicity on variety Jyothi. The plants were

(A) (B) (C)

Fig. 1. Symptoms of leaf blight disease of Rice caused by Sphingomonas sp. in the field. A. Symptoms in field at tillering stage B. Close-up view of initial symptoms on leaves C. Close up view of the diseased leaves at final stage.

raised using seeds treated in 500 ppm streptocycline solution for 12 h to avoid seed transmission. The pure culture of isolates was grown in nutrient broth at 28°C for 48 h and the density of the culture was estimated using an SL 210 UV VIS Spectrophotometer (at OD 600 nm) which was found to be approx. 10^7 CFU/ml. Inoculation was carried out using the pure culture of isolates grown in nutrient broth at 28°C for 48 h with density of approx. 10^7 CFU/ml at OD 600 nm using SL 210 UV VIS Spectrophotometer. Inoculation was done by clipping the tip of the fully expanded uppermost leaf of 45-day-old plants and placed in a greenhouse at 27 ±3°C and 90% relative humidity. Inoculated plants were observed for the development of symptoms after 15

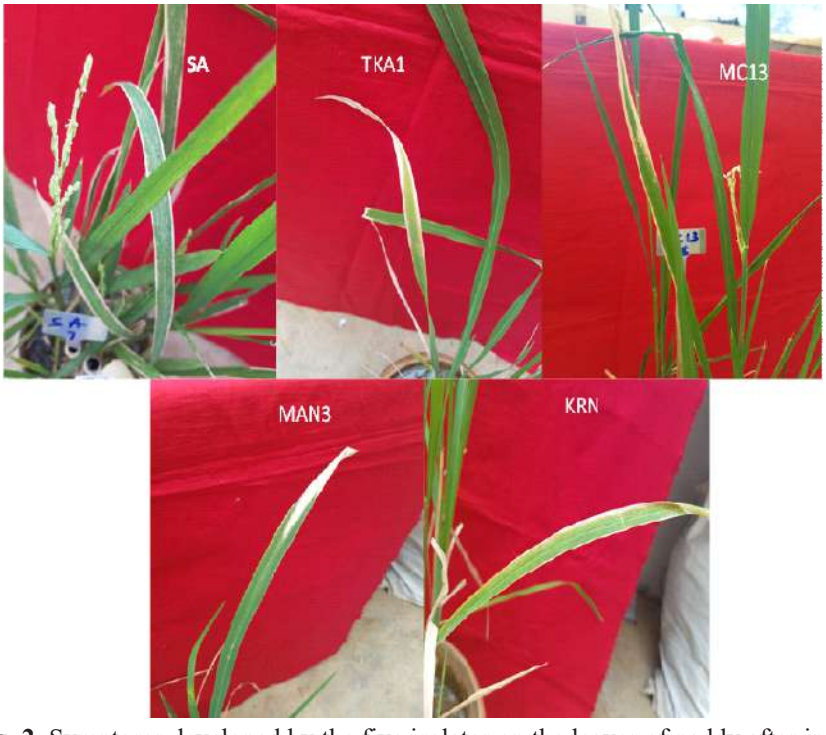

Fig. 2. Symptoms developed by the five isolates on the leaves of paddy after inoculation.

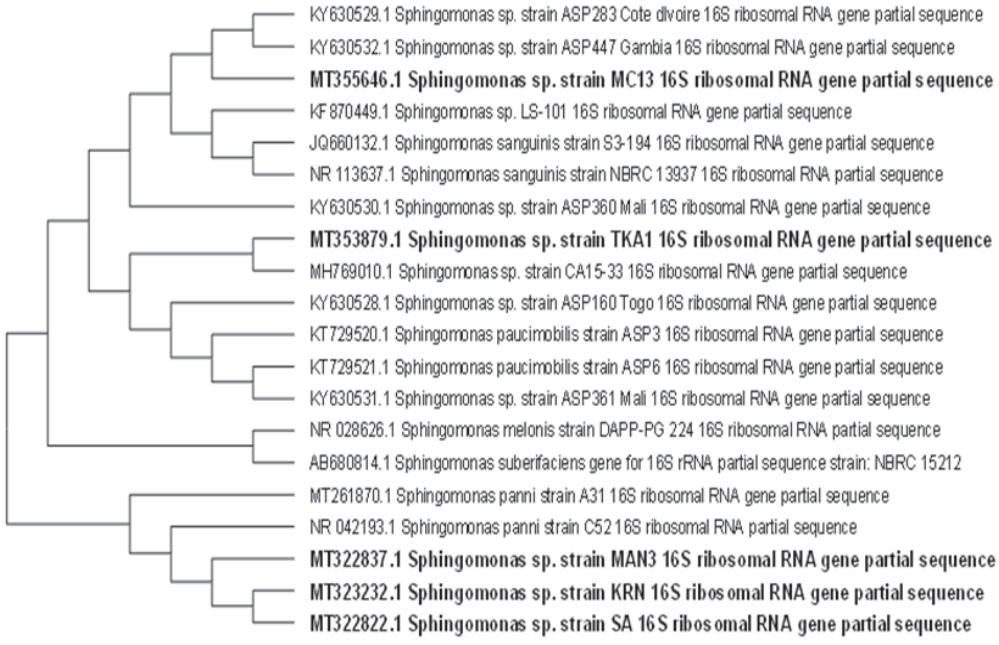

Fig. 3. Phylogenetic tree of *Sphingomonas* sp. with the type strains was inferred using the Maximum Likelihood method in MEGA X. Test of phylogeny of 20 nucleotide sequences was computed by bootstrap method (500 replicates) using the Tamura 3-parameter method.

DAI. Observation on lesion length was recorded at weekly intervals till the dough stage. The symptoms developed (15 DAI) as water-soaked lesions from the tip in one or both the margins of the leaf all along the leaf blade in a wavy pattern (Fig. 2). Symptoms appeared were similar to those observed on naturally infected plants in the field while the water inoculated control plants didn't developed any symptoms. The bacterium re-isolated from the inoculated plants had cultural and biochemical characteristics similar to that of original culture thus fulfilling Koch's postulates. The Sphingomonas sp. isolates *viz*., KRN, MAN3, MC13, SA, and TKA1 were Gram-negative, aerobic rods. They showed positive reactions for oxidase fermentation, potassium hydroxide, solubility test, carbohydrate fermentation, citrate utilization, nitrate reduction, catalase activity, salt tolerance, and starch hydrolysis while negative for malate utilization and variable reaction to gelatin liquefaction. Among the *Sphingomonas* sp. isolates, KRN and SA were positive while MAN3, MC13, and TKA1 were negative for gelatin liquefaction. All the Sphingomonas sp. isolates utilized glucose, sucrose, fructose, lactose, and maltose while none of the isolates utilized sorbitol and cellobiose.

A total of seventeen representative isolates were selected and were subjected to molecular characterization.

Molecular characterization was carried out by DNA fingerprinting. The bacterial genomic DNA was isolated following CTAB method and amplified by polymerase chain reaction using universal primers pair of 16SrRNA Fdp1 (AGAGTTTGATCCTGGCTCAG) and Rvp2 (ACGGCTACCTTGTTACGACTT). Amplification of the 16SrDNA generated amplicon of ~1400bp which was sequenced using Sanger sequencer. The forward and reverse sequence was alligned in NCBI and subjected to blastn analysis. Of the seventeen isolates sequenced five showed 97-100% sequence identity with *Sphingomonas panni*, *Sphingomonas paucimobilis*, and *Sphingomonas* sp. and rest of twelve mathched to *Xanthomonas oryzae* and *Pantoea* sp. The sequences of five *Sphingomonas* strains were deposited in GenBank database under the accession numbers: MT323232.1(KRN, Mysuru), MT355646.1 (MC13, Mandya), MT322837.1 (MAN3, Mandya), MT322822.1 (SA, Mandya), MT353879.1 (TKA1, Mysuru).

Phylogenetic analysis of five type strains of Sphingomonas and 15 reference strains (obtained from NCBI) was inferred using the Maximum Likelihood method in MEGAX (Fig. 3). The phylogenetic analyses clustered the isolates in two clusters I and II. Cluster I was further divided into cluster I a and cluster Ib wherein Ia comprised of five reference strains viz., KY630529 (ASP283), KY630532 (ASP447), KF870449 (LS101), JQ660132 (S3194), NR113637 (NBRC), KY630530 (ASP360) and one type strain MT355646 (MC13). Cluster Ib had one type strain MT353879 (TKA1) and reference strain MH 769010 (CA15-33), KY630528.1 (ASP160), KT729520 (ASP3), KT 729521 (ASP6) KY630531.1 (ASP361). Cluster IIa included pathogenic strain S. melonis NR028626 (DAPP-PG224) and reference strain AB680814 S suberifaciens (NBRC15212) while cluster II b contain MT261870 (A31), NR042193.1 (CS2) and three type strain MT322837(MAN3), MT323232 (KRN) and MT322822 (SA). The strain identified in the present study showed similarity with the reference strains which are reported as causal agent of bacterial leaf blight disease of rice from Benin, Burkina Faso, The Gambia, Ivory Coast, Mali, Nigeria, Tanzania and Togo (Kini et al., 2017) (Fig. 3).

Sphingomonas species have frequently been isolated from rice seed (Midha et al., 2016) and have been found on leaves of 26 plant species of 11 families, but few are recorded as plant pathogens. A phytopathogenic Sphingomonas species has been reported to cause a brown spot on yellow Spanish melon fruit (Buonaurio et al., 2001) and bacterial leaf blight of Paliuris spina-christi (Deldavleh et al., 2013). Kini et al., 2017 reported the *Sphingomonas* sp. causing bacterial leaf blight of rice in Benin, Burkina Faso, the Gambia, Ivory Coast, Mali, Nigeria, Tanzania and Togo. To our knowledge this is the first report of a leaf blight disease of rice caused by *Sphingomonas* sp. in India.

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Abstract: Maximum 250 words conveying the objectives and the most important results. It should state briefly the purpose of the research, the principal results and major conclusions

Key words: Immediately after the abstract, provide a maximum of 3-5 key words in italics should be provided for indexing purpose.

Introduction: It should be concise and include the scope of the work in relation to the state of art in the same field along with specific objectives.

Materials and Methods: A full technical description of the methods followed for the experiment(s) should be given, providing enough information. This section should provide sufficient details to allow the work to be reproduced by an independent researcher. Detailed methodology should be given when the methods are new while for standard methods, only references may be cited.

Results and Discussion: In this section, results of the experiment(s) should be reported in a clear and concise manner. Data should be properly analyzed. The discussion should deal with interpretation of results and relate the author's findings with the past work on the same subject. Result and Discussion may also be submitted as separate section.

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