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Genetic diversity and mutual relationships through principal component analysis in F_2 generation of *aus* rice (*Oryza sativa* L.)

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ABSTRACT

The present study was carried out to enumerate the extent of genetic diversity for yield components in 15 different F_2 segregating populations with their 10 parents of Aus rice using principal component analysis (PCA), clustering and linkage analysis. In the correlation matrix, 14 characters of 25 genotypes generated14 principal components without changing their relative positions and the first four PCs explained 42.1%, 15.5%, 12.2% and 7.5% of total variation towards genetic diversity. In two cluster condition, 25 genotypes were grouped into two clusters having 7 genotypes (P2 x P1, P2 x P5, P2 x P6, P1, P2, P8, P10) in cluster 1 and 18 genotypes cluster 2. In the three cluster condition, there were 4, 6, 15 individuals in cluster 1, 2 and 3, respectively. In four cluster condition, clusters consisted of 4, 14, 3, 4 genotypes, respectively. Cluster means for the days to maturity, number of non-effective tillers per plant, empty grains per plant showed the lowest values in cluster 1. On the other hand, number of tillers per plant, effective tillers per plant, panicle length, filled grains per panicle, grain length and thousand seed weight showed maximum cluster means in cluster 4. The dendrogram revealed that six genotypes (P8 x P3, P7, P3, P7 x P3, P3 x P4, P11) having more distance as compared to rest (19 genotypes) of the genotypes. In complete linkage, 25 genotypes were grouped into two major clusters. Cluster 1 containing seven genotypes and cluster 2 containing 18 genotypes; among the two clusters, there was maximum cluster distance than the sub-clusters. Cluster 1 showed two sub clusters where four genotypes (P1, P2 x P1, P2 x P5, P2) were in one cluster and three genotypes (P11, P8 and P10) in another cluster. Cluster 2 also contains two sub-clusters in which three genotypes remain in one sub-cluster and 15 genotypes in another sub-cluster. In average linkage, 25 genotypes were also grouped into two major clusters. The first cluster contains three genotypes (P7, P3 x P4, P7 x P3) and the second cluster divided into two sub-clusters which contain rest of the genotypes (22 genotypes). It can be concluded that, there were more distance where cluster number was less and the distance was less where the cluster number was more.

Key words: *Rice, multivariate analysis, correlation matrix, dendrogram, cluster distance, linkage*

INTRODUCTION

Rice belongs to the family Poaceae and is the seed of grass species *Oryza sativa* (Asian rice) or *Oryza glaberrima* (African rice). Rice, a monocot, is normally grown as an annual plant, but it can survive as a perennial in tropical areas and produce a ratoon crop for up to 30 years (Wikipedia contributors, 2024). The geographic distribution of rice extending from 50ºN to 35ºS, is expected to be the most vulnerable cultivated crop to future changing climates (Jagadish et al., 2012). Rice production has remained almost constant in the thirty years and grown on about 10.5 M ha occupying 75% of the total cultivable land (BRKB, 2020). Bangladesh produces 36.3 M tons of rice per year (BBS, 2023). Nevertheless, rice production in Bangladesh has been increasing every year, there still exists 20.7% yield gap of rice (Kabir et al., 2016). Population of Bangladesh is increasing and now a day about 170 millions of people consume rice every day (UNFPA, 2022). To fulfil this demand, rice production should be increased at a rate of 1.5 to 2.4% per year despite limited water supply, reduced cultivable lands, environmental hazards, climate change issues (Seck et al., 2012; Jamal et al., 2023). The food security of this country mostly depends on rice production (Kashem and Faroque, 2013).

Rice production must be increased to keep pace with population growth as population is increasing at an alarming rate in Asian countries. Production potential of rice in summer (Aus) and rainy (Aman) seasons remains adequately unexplored. In tropical and subtropical climate, Aus season is characterized by sufficient rainfall and so this rice is cultivated under rainfed condition (Shelley et al., 2016). Besides, most of the Aus rice grown in this season is of long duration and low yielding and cultivation of other crops after Aus rice reduces the system productivity. In Bangladesh, Hasibomi (Dhaka no. 26), Horinmura (Dhaka no. 28), Dhalasaitta (Dhaka no. 32: Genetic stock 526), Dular (Dhaka no. 22) are the local Aus varieties that can be harvested at 80 days. 'Dumahi' is a kind of short duration Saitta variety for Sylhet region and 'Larkoch' is for Dhaka region. Kalyani-2 variety can be harvested in 62 days in India (Odisha) which takes few days more in Bangladesh due to geographic region (Masuduzzaman, 2017). These genotypes of local rice can be used in breeding programs to develop high yielding short duration rice varieties. Therefore, utilization of new short duration and high yielding rice varieties would open a new era for increasing rice production in the Aus season.

At the beginning of any breeding program, breeder should have knowledge of genetic resources, nature and magnitude of genetic variability, genetic diversity and character association in a crop species (Kahani and Hittalmani, 2015). Knowledge of genetic variability among genotypes of any crop is essential for success of a breeding program (Belaj et al., 2002). The crosses between the different genotypes with maximum genetic divergence would be responsible for improvements as they are likely to yield desirable recombinants in the progeny (Kahani and Hittalmani, 2015). Hybridization is one of the major tools for the improvement of a crop which requires the analysis of genetic variability for the selection of elite types (Singh, 1980). There are several ways exist to improve rice varieties and increase yield, such as hybridization followed by selection, hybrid breeding, ideotype breeding, and enhancement of photosynthesis, exploitation of wild species, and genomic approaches (Khush, 2013). Therefore, the present study was conducted to quantify the extent of genetic variability and diversity available for yield components in the segregating population of Aus rice.

MATERIALS AND METHODS

The research work was conducted at the experimental field, Department of Genetics and Plant Breeding, Bangabandhu Sheikh Mujibur Rahman Agricultural University (BSMRAU), Gazipur during Aus season of 2017 (generation of F_2 population from F_1) and 2018 (evaluation of F_2 population). Fifteen F_2 populations along with 10 parental genotypes (Table 1) of Aus rice were evaluated in a Randomized Complete Block Design (RCBD) with three replications for 14 yield related traits.

Pre-germinated seeds of the experimental materials were sown in well prepared nursery bed for quick germination and seedling production. After field preparation, 25 days old seedlings were transplanted at the spacing of 20 cm × 20 cm. Application of fertilizers and all intercultural operations were done as per BRRI recommendation guide (Anonymous, 2018). The crop was harvested at maturity, when 90% of the seeds became golden yellow color and plants of each genotype in three different replications were separately bundled, properly tagged and then brought to the threshing floor, and were threshed separately. Data were recorded on days to panicle exertion (DPE), plant height (PHT), days to maturity (DMT), tillers per plant (TPP), effective tillers per plant (ETP), non-effective tillers per plant (NET), number of filled grains (FGP), number of empty grains (EGP), panicle length (PLT), length of

Table 1. List of parents with their sources or origin

Code no.	Genotype	Origin / Source
P1	Dhalasaitta	Bangladesh (Local)
P2	Laksmilota	Bangladesh (Local)
P3	Kataktara	Bangladesh (Local)
P4	N-ABSS	Africa
P5	BRRI dhan43	BRRI, Bangladesh
P6	BRRI dhan55	BRRI, Bangladesh
P7	BR7	BRRI, Bangladesh
P8	Japonica rice	Japan
P9	UK-01	UK
P10	Nipponbare	Japan
P11	Parija	Bangladesh (Local)

grain (GLT), breadth of grain (GBD), plant residue weight (PRW), 1000-seeds weight (TSW), yield per plant (YPP).

Analysis of variance (ANOVA) was done from the replicated data of different characters by using computer software 'STAR' (Statistical Tools for Agricultural Research). Principal Components Analysis (PCA) was done by software R. Among principal components, the first component accounts for the maximum proportion of the variance of the original image, and subsequent components account for maximum proportion of the remaining variance (Torbick and Becker, 2009). Agglomerative hierarchical clustering was done following the method given by Anderberg (1993). PCA scores for 25 genotypes of the F_2 generation were used as input for clustering. The data matrix was standardized with a column standardizing function *i.e*., CA-Q analysis. For linkage analysis, the resemblance coefficient was measured between pairs of genotypes, where 25 genotypes were taken in data matrix and the data matrix was transformed to distance matrix (resemblance matrix). Distance matrix was converted into dendrogram by using Ward's method.

RESULTS AND DISCUSSION

Principal component analysis

Principal component analysis or canonical vector analysis is a sort of multivariate analysis where canonical vectors and roots representing different axes of differentiation and the amount of variation accounted for by each of such axis, respectively, are derived (Rao, 1952). It is called principal component analysis as it possesses the importance of the largest contributor to the total variation on each axis of differentiation.

In principal component analysis using correlation matrix, the standardization of columns (characters) created 14 new variables for 25 genotypes without changing their relative positions. These 14 new variables are the principal components (PC1, PC2 to PC14). Each principal component is a combination of the 14 characters of the data matrix. The loading values are standardized in such a way that the sum of square of loadings within a principal component is equal to one. The loadings are discussed as weights defining the contribution of characters in respective principal component. Kind of regression coefficients, loadings sign (+/-) are indicative of the direction of contribution. However, unlike regression, only the relative contributions are important, so all signs can be changed without affecting the analysis (Jackson, 1991). The loadings for the first principal component were selected so as to make its variance as large as possible. Loadings of the second principal component were selected such that the variance of PC2 is as large as possible, subject to the obligation that PC1 and PC2 are uncorrelated. The process was continued to create 14 principal components, but PC's having eigenvalue less than one do not have any practical significance (Legendre and Legendre, 1984; Brejda et al., 2000). The higher eigenvalues were considered as the best representative of system attributes in principal components (Ojha et al., 2017). Principal components, eigenvalues (Latent Root), percent variability, cumulative per cent variability and component loading of different characters are presented in Table 2.

The first four principal components explained 42%, 15%, 12% and 7% of total variance, respectively. These components represented 77.30% of total variation. The principal component with eigenvalues less than one was considered as non-significant (Ladumor et al., 2021). It was therefore supposed that the essential features of dataset had been represented in the first four principal components.

The first principal component (PC1) contributed the maximum towards variability (42.10%). The traits such as panicle length (0.348), total number of tillers (0.316), number of effective tillers (0.310) and filled grains per panicle (0.306) contributed more positively to PC1. The characters noneffective tillers per plant (-0.28) and days to panicle exertion (-0.27) were negatively loaded. The second principal component (PC2) contributed 15.5% of total variance and it reflected significant positive loading of the characters viz., effective tillers (0.373), total tillers (0.316) and plant height (-0.605), grain length (-0.278), empty grain (-0.260), days to maturity (-0.224) were negatively loaded.

The third principal component (PC3) contributed 12.20% to the total variability. The characters such as yield per plant (0.365), filled grains (0.356), panicle length (0.225), plant height (0.072), and plant residue weight (0.069) were positively loaded.

Table 2. Variation among rice genotypes accounted for fourteen principal components

Parameters	PC1	PC2	PC3	PC4	PC5	PC6	PC7	PC8	PC9	PC10	PC11	PC12	PC13	PC14
PHT	0.01	-0.60	0.07	-0.31	0.04	-0.22	0.00	-0.07	0.04	-0.19	0.05	-0.38	-0.52	0.04
DPE	-0.27	-0.23	-0.16	0.00	0.17	0.23	-0.72	0.24	-0.24	0.30	-0.07	-0.10	0.05	-0.01
DMT	0.31	0.37	-0.15	-0.16	0.10	-0.05	-0.10	-0.17	-0.11	0.12	-0.22	-0.34	-0.05	0.67
TPP	0.29	-0.26	-0.12	-0.07	-0.02	0.21	0.44	0.38	-0.53	0.18	-0.29	0.15	-0.06	-0.01
ETP	0.30	-0.06	0.35	-0.04	-0.12	-0.34	-0.10	-0.13	-0.19	0.60	0.43	0.13	0.02	-0.02
NET	-0.28	-0.25	-0.28	-0.27	-0.08	-0.29	0.22	-0.17	0.26	0.38	-0.33	-0.05	0.43	-0.01
PLT	0.24	-0.27	-0.12	0.00	-0.17	0.68	0.02	-0.51	0.11	0.06	0.19	-0.08	0.14	0.00
FGP	0.22	-0.22	-0.36	0.38	-0.11	-0.33	-0.22	-0.33	-0.11	-0.19	-0.24	0.42	-0.11	0.03
EGP	0.19	0.01	-0.54	-0.40	0.12	-0.04	-0.04	0.29	0.20	-0.09	0.49	0.30	0.04	0.10
GLT	0.27	-0.08	-0.18	0.51	-0.28	-0.06	0.01	0.42	0.40	0.19	0.03	-0.39	-0.03	-0.01
GBD	0.34	-0.19	0.22	-0.09	0.00	-0.15	-0.17	0.13	-0.14	-0.44	-0.02	-0.20	0.66	-0.02
TSW	0.17	-0.15	0.06	0.27	0.88	-0.00	0.14	-0.06	0.20	0.12	-0.01	0.02	0.06	-0.01
PRW	0.31	0.31	-0.24	-0.21	0.09	-0.05	-0.11	-0.14	-0.06	0.06	-0.16	-0.26	-0.12	-0.72
YPP	0.29	-0.02	0.36	-0.29	-0.08	0.16	-0.25	0.15	0.48	0.07	-0.43	0.36	-0.13	0.01
Eigenvalue	2.428	1.494	1.307	1.025	0.909	0.814	0.711	0.589	0.538	0.438	0.409	0.277	0.187	0.054
Variance proportion	0.421	0.155	0.122	0.075	0.059	0.047	0.036	0.024	0.020	0.013	0.011	0.005	0.002	0.002
Cumulative proportion	0.421	0.580	0.702	0.777	0.836	0.884	0.920	0.945	0.966	0.979	0.991	0.997	0.999	1.000

DPE - Days to panicle exertion, PHT - Plant height, DMT - Days to maturity, TPP - Tillers per plant, ETP - Effective tillers per plant, NET - Non effective tiller per plant, FGP - Filled grain, EGP - Empty grain, PLT - Panicle length, GLT - Length of grain, GBD - Breadth of grain, TSW - 1000 - Seeds weight, PRW - Plant residue weight, YPP - Yield per plant.

While noneffective tiller (-0.542), days to maturity (-0.368), grain breadth (-0.281) and total tillers per plant (-0.241) were negatively loaded. The fourth principal component (PC4) contributed 7.50% towards the total variability and it reflected significant positive loading of the characters viz., days to panicle exertion (0.511), days to maturity (0.387) and plant residue weight (0.272). While, non-effective tillers (-0.403), plant height (-0.318), yield per plant (-0.291), grain breadth (-0.279) and total tillers (-0.219) were negatively loaded.

Cluster analysis

The group identification numbers were based on the stabilization of the within group sum of square, and its increase in the number of groups contributed minimally to the decrease of the sum of squares (Table 3). The number of individuals in each group shows high genotypic variability in this germplasm set and mean values of each group are presented in Table 3. In two cluster condition, there were two clusters having 7 and 18 individuals had sum of squares of 56.40 and 179.03, respectively and their combined total percent was 29.9. The genotypes P2 x P1, P2 x P5, P2 x P6, P1, P2, P8, P10 were in the cluster 1 and rest of the genotypes are in the cluster 2.

In three cluster condition, there were 4, 6, 15 individuals in cluster 1, 2 and 3, respectively with sum of squares of 33.89, 35.93, and 111.99. The total percent for the three clusters was 45.9. In four cluster condition, the clusters contained 4, 14, 3, and 4 individuals, with corresponding sum of squares of 11.33, 94.03, 13.25, and 33.89, respectively. The percent total for four clusters was 54.6. Most of the genotypes belonged to the group 4 suggesting there was less variation among the genotypes of this group. From Table 3, it can be concluded that, there were more distance where cluster number was less and the distance was less where the cluster number was more. The total percent increased as the number of clusters increased.

It is necessary to know cluster means in breeding program because it helps in the selection of the genotypes. In Table 4, all the groups showed greater variations among the characters. For the growth parameters such as days to panicle exertion, days to maturity, number of non-effective tillers per plant, empty grains per plant showed the lowest values 57.65, 84.91, 1.1, 16.65, respectively in cluster 1.

Plant height showed the lowest value of 73.35 in cluster 3, grain breadth had the lowest value of 2.45

Table 3. Distribution of rice genotypes based on genotypic values, through the analysis of 14 agronomic traits

Clusters 2 Cluster		No. of individuals	SSW	% of total	Genotypes
	1	7	56.40	29.9	P2 x P1, P2 x P5, P1, P2, P8, P10, P11
	2	18	179.03		P1 x P4, P2 x P6, P3 x P4, P3 x P6, P3 x P7, P4 x P6, P6 x P7, P6 x P3, P7 x P3, P7 x P6, P8 x P3, P6 x P4 P7 x P11, P3, P4, P5, P6, P7
3 Cluster	1	4	33.89	45.9	P3 x P4, P3 x P7, P7 x P3, P7
	2	6	35.93		P2 x P1, P2 x P5, P1, P2, P8, P10
	3	15	111.99		P1 x P4, P2 x P6, P3 x P6, P4 x P6, P6 x P7, P6 x P3, P7 x P6, P8 x P3, P6 x P4, P7 x P11, P3, P4, P5, P6, P11
4 Cluster	1	4	11.33	51.5	P2 x P1, P2 x P5, P1, P2
	2	14	94.03		P1 x P4, P2 x P6, P3 x P6, P4 x P6, P6 x P7, P6 x P3, P7 x P6, P8 x P3, P6 x P4, P7 x P11, P3, P4, P5, P6
	3	3	13.25		P8, P10, P11
	4	4	33.89		P3 x P4, P3 x P7, P7 x P3, P7

SSW- Within cluster sum of square

in cluster 2, and plant residue weight showed minimum value of 21.61 in cluster 4. For yield contributing characters i.e. number of total tillers per plant, effective tillers per plant, panicle length, filled grains per panicle, grain length and thousand seed weight showed maximum cluster means of 19.5, 15.23, 26.24, 105.83, 9.08 and 43.62, respectively in cluster 4. Grain yield showed maximum cluster mean in cluster 2 (25.73) followed by cluster 4 (25.41).

Linkage analysis

There are so many different linkage measures that define the distance between pairs of clusters in various ways. Some measures determine the distance between two clusters based on the minimum or maximum distance found between pairs of cases (single and complete linkage, respectively) where each case is from a different cluster (Mazzocchi, 2008). Average linkage averages all distance values between pairs of cases from different clusters. Single linkage defines the distance between two clusters as the minimum distance found between one case from the first cluster and one case from the second cluster (Florek et al., 1951; Sneath, 1957). Complete linkage is similar to the single linkage measure but instead of searching for the minimum distance between pairs of cases, it looks on the furthest distance between pairs of cases (Yim and Kylee, 2015). Average linkage, also referred to as the Unweighted Pair-Group Method using Arithmetic averages. To overcome the limitations of single and complete linkage, (Yang, 2017) proposed averaging the distance values between pairs of cases. This method is supposed to represent a natural compromise between the linkage measures to provide a more appropriate evaluation of the distance between clusters. For average linkage, the distances between each case in the first cluster and every case in the second cluster are calculated and then averaged. Bratchell (1989) suggests that there is no best choice and researchers may need to employ different techniques and compare their results.

Single linkage is also known as the nearest

Table 4. Group (4 clustering) means using the multivariate method K-Means.

Group	DPE	PHT	DMT	TPP	ETP	NET	FGP	EGP	PLT	GLT	GBT	PRW	TSW	YPP
1	57.65	93.69	84.91	6.98	5.76	1.1	49.1	16.65	16.91	6.26	2.64	23.66	16.82	12.08
2	80.44	100.55	106.14	15.22	13.39	1.94	100.4	24.07	24.74	8.91	2.45	22.85	31.9	25.73
3	82.83	73.35	105.62	14.15	12.6	2.02	60.5	19.93	19.3	7.8	2.83	23.23	19.77	15.13
4	96.29	114.19	112.67	19.5	15.23	3.52	105.83	41.62	26.24	9.08	2.61	21.61	43.62	25.41

DPE - Days to panicle exertion, PHT - Plant height, DMT - Days to maturity, TPP - Tillers per plant, ETP- Effective tillers per plant, NET - Non effective tiller per plant, FGP - Filled grain, EGP - Empty grain, PLT - Panicle length, GLT- Length of grain, GBD - Breadth of grain, PRW - Plant residue weight, TSW - 1000 - Seeds weight, YPP - Yield per plant.

neighbor clustering. The distance between two groups is defined as the distance between their two closest members. The horizontal axis of the dendrogram (Fig. 1a) represents the distance or dissimilarity between clusters. The vertical axis represents the objects and clusters.

Each joining (fusion) of two clusters is represented on the graph by the splitting of a horizontal line into two horizontal lines. The horizontal position of the split, shown by the short vertical bar, gives the distance (dissimilarity) between the two clusters.

Looking at this dendrogram, it can be observed that six genotypes (P8 x P3, P7, P3, P7 x P3, P3 x P4, P11) have greater distance compare to rest (19 genotypes) of the genotypes and the clusters using these 19 genotypes occur almost at same distance. The six outliers, P8 x P3, P7, P3, P7 x P3, P3 x P4 and P11 were fused in rather arbitrarily at much higher distances.

In the complete linkage method, the similarity between the two groups is given by the individuals of each group that resemble the least.

All the data based on yield and quality traits were also analyzed by multivariate methods using cluster and principal component analysis. Dendrogram, based on Euclidean distance coefficients using 14 agronomic parameters, placed 25 genotypes into two major groups (Fig. 1b) and horizontal axis describes inter cluster distance, vertical axis describes clusters.

Genotype clusters were primarily associated with morphological differences among them and secondly with genotype. Cluster 1 contains seven genotypes and cluster 2 contains 18 genotypes; among the two clusters, there was maximum cluster distance than the sub-clusters. Cluster 1 showed two sub clusters where the genotypes P1, P2 x P1, P2 x P5, P2 were in one cluster and another cluster contained the genotypes P11, P8 and P10. Cluster 2 also contains two sub-clusters in which three genotypes remain in one sub-cluster and another sub-cluster contains 15 genotypes.

In average linkage, the distance between two groups is defined as the distance between their averages of two members. Dendrogram based on Euclidean distance coefficients using 14 agronomic parameters,

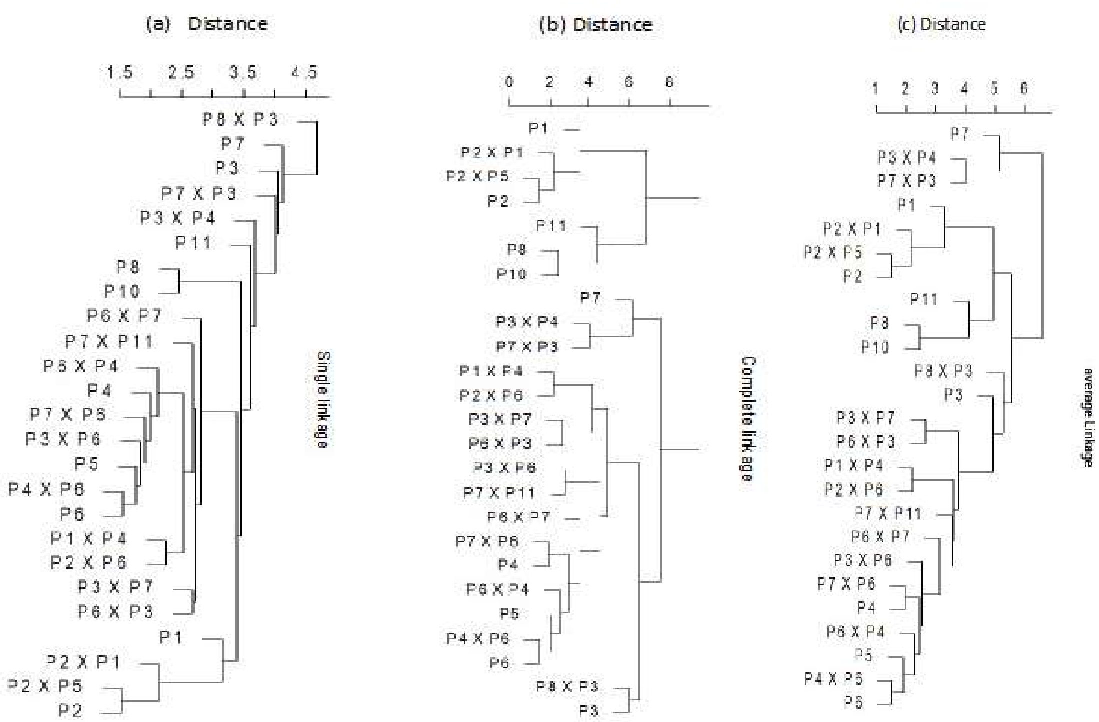

Fig. 1. Dendrogram indicating the mutual relationships in (a) single (b) complete (c) average linkage among 25 Aus rice genotypes for yield and yield contributing traits.

25 genotypes were placed into two major clusters (Fig. 1c) and horizontal axis describes inter cluster distance, vertical axis describes clusters. The first cluster had three genotypes (P7, P3 x P4, P7 x P3) and the second cluster divided into two sub-clusters which contain rest of the genotypes (22 genotypes). In this dendrogram, three main clusters can be considered as three branches that occur at about the distance and the subdivisions of these three clusters were remaining at least distance. The five outliers, P7, P1, P11, P8 x P3, and P3 are fused in rather arbitrarily at much higher distances.

As mentioned above, the linkage measure determines the calculation procedures of the distance between pairs of clusters with two or more cases. Fig. 1a-1c displays three dendrograms from three analyses, each using a different linkage measure. Although all three analyses were run on the same data, the differences between the dendrograms were easily noticeable upon visual inspection. The dendrogram in Fig. 1a clearly showed how single linkage could produce chaining because the majority of cases were grouped together into a large cluster, with minimum distance between clusters. The dendrogram in Figure 1b showed the analysis using complete linkage where the opposite problem could be observed and three clusters were derived from this analysis. Complete linkage does not necessarily merge groups that are close together due to outlying cases that may be far apart.

Average linkage represents a natural compromise between single linkage and complete linkage, as it is sensitive to the shape and size of clusters. Single linkage was sensitive to outliers, but it was impervious to differences in the density of the clusters; in contrast, complete linkage could break down large clusters although it was highly influenced by outliers (Almeida et al., 2007). As seen in the visual comparison, the average linkage was compromised between the single and complete linkage (Fig. 1a-c). However, the number of clusters obtained using average linkage was not the average between the single and complete linkage.

CONCLUSION

The present research was conducted to study the extent of genetic variability and diversity available for yield components of 15 different F_2 segregating populations and 10 parental genotypes of Aus rice. In two cluster condition, 7 genotypes were grouped in cluster 1 and 18 genotypes were grouped in cluster 2. In three cluster condition, 4, 6, 15 individuals were grouped into cluster 1, 2 and 3, respectively. In four cluster condition, clusters consisted of 4, 14, 3, 4 genotypes, respectively. The dendrogram showed that six genotypes having more distance as compare to rest of the 19 genotypes. In both complete and average linkage, 25 genotypes were grouped into two major clusters. The similarity between the two groups is given by the individuals of each group that resemble the least, in complete linkage. On the other hand, the distance between two groups is defined as the distance between their averages of two members. From the above results, it can be concluded that there was more distance where cluster number was less and the distance was less where the cluster number was more. The observed genetic variability and genetic divergence among the individuals of different segregating population would be useful for improvement of Aus rice through the selection of desirable recombinants.

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DNA fingerprinting of medicinal rice 'Njavara' using microsatellite markers

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ABSTRACT

Njavara is a unique medicinal rice cultivated in Kerala. This study presents a detailed investigation into the DNA fingerprinting of Njavara rice using Simple Sequence Repeat (SSR) markers. Using 19 selected SSR markers, fingerprinting was conducted on three Njavara accessions (Black Njavara - TGC366, White Njavara - TGC389, and Karanjavara - TGC90), Rakthasali (traditional medicinal rice), and Vaishak (KAU variety). A comprehensive fingerprint, combining all samples and markers, was created. A set of 10 markers producing unique amplicons in the three Njavara accessions were validated using 10 selected KAU varieties. Four markers (RM340, RM493, RM434, and RM202) were effective in distinguishing Njavara from KAU varieties. The dendrogram highlighted two clusters, clearly separating Njavara from non-Njavara types. These markers prove valuable in accurately discerning Njavara rice and provide insights into its genetic diversity.

Key words: *SSR, microsatellite, DNA fingerprinting, Njavara, medicinal rice*

INTRODUCTION

Rice (*Oryza sativa*) belongs to the genus *Oryza* and family Graminae. Two major rice subspecies grown in the world are indica and japonica. Rice has wide range of geographic distribution, worldwide more than 2.5 lakh germplasm collection of more than 20 species are maintained (Evenson, 1998). Rice is the staple food for half of the world population and is the major source of energy. Globally, rice is the second largest cereal produced and consumed. It provides significant number of proteins, minerals, and vitamins along with carbohydrates. Rice is cultivated in more than 100 countries all over the world and 90 per cent of the total production comes from Asia. India ranks second in terms of production of rice and accounts for 20 per cent of global rice production. The country is one of the world's largest producers of white rice and brown rice (Fukagawa and Ziska, 2019).

Many indigenous rice varieties with unique nutritional and medicinal properties are grown across various states of India. Kerala, a southern state of India, is rich in traditional rice varieties including popular aromatic varieties namely, *Gandhakasala* and *Jeerakasala*. Kerala also harbors rice varieties with high medicinal values such as, *Chennellu, Kunjinellu, Erumakkari, Karuthachembavu, Kavunginpoothala,JaathiSughi, Jeeraka hembavu, Kamaal, Kolaran, Naron, Vadakkan, Vatton*, and *Njavara*. These varieties are extensively used in ayurveda (Kumary, 2006; Chaudhari et al., 2018).

Njavara is a unique medicinal rice cultivar grown exclusively in Kerala and widely used in ayurvedic treatments. Njavara is grown in upland conditions and only needs about 60-70 days for maturity. Fibre, protein, vitamin, and mineral content of Njavara rice grains is comparatively higher than staple varieties like Jyothi (Deepa et al., 2008). According to Ayurveda, Njavara is considered as a special cereal with medicinal properties, used in the treatment of diseases like arthritis, cervical spondylitis, muscle wasting, skin diseases and certain neurological problems. *Njavara kizhi* and *Njavara theppu* are two well-known techniques in ayurveda used for the treatment of paralysis, neurological complaints, muscle degeneration, tuberculosis, arthritis, anemia, and skin diseases (Simi and Abraham, 2008).

Despite having such high medicinal value and importance, Njavara seeds available in the market are often mixtures. Farmers who cultivate Njavara are confused due to availability of different seed materials, under the same name (seed duplicity). The identity of the real Njavara variety remains an unresolved question. It is almost impossible to distinguish between crop varieties just by looking at the morphological features of the seed or the crop. Diversity analysis based on morphological markers and biochemical markers (protein marker or isozyme marker) are greatly influenced by the environment. Identification of some of these traits requires growing plants to maturity which is time consuming and resource utilizing (Chakravarthi and Naravaneni, 2006). DNA fingerprinting technique can be employed in such situations to identify the specific variety with ease and precision. Concept of DNA fingerprinting was introduced by Alec Jeffreys during 1984 based on the simple tandem repetitive regions of DNA which are dispersed in the human genome. Hybridization probes developed with this information can be used to produce fingerprints which are unique to an individual (Jeffreys et al., 1985). Presently, different molecular markers are employed for DNA fingerprinting namely, Restriction Fragment Length Polymorphism (RFLP), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR) and Simple Sequence Repeats (SSR). Among the various molecular markers available, RAPD marker analysis was used in order to characterize seven Njavara ecotypes (Kumar et al., 2008). All the Njavara ecotypes formed a single cluster at 41 percent similarity in the dendrogram, generated using Jaccard's index. Sreejayan et al. (2011) characterized 40 electromorphs of Njavara using 24 morphological characters and 11 AFLP markers. Cluster analysis grouped 36 electromorphs in a major node and four electromorphs and six out groups in a minor node. Results of AFLP analysis was similar to that of morphological grouping but AFLP resolved the cluster into subgroups. Diversity analysis of 24 Njavara cultivars collected from different parts of Kerala was carried out by Nadiya et al. (2021). On cluster analysis 24 Njavara accessions clustered separately from six check varieties used.

SSR markers have several advantages that make them suitable for the characterization and DNA fingerprinting of plant resources. Some advantages are their abundance, high degree of polymorphism in the number of repeats, co-dominant nature, high reproducibility, and ease of use.

The major Njavara groups known for their medicinal properties are Black Njavara and White Njavara, with other so-called types being variants within these groups. One such type, Karanjavara, is particularly adaptable to upland cultivation. Therefore, these three accessions were selected for the study. In this study, SSR markers were employed for the DNA fingerprinting of three Njavara accessions: Black Njavara (TGC 366), White Njavara (TGC 389), and Karanjavara (TGC 90). Additionally, the study included Rakthasali, a traditional rice variety, and Vaishak, a KAU variety. The major agro-morphological characteristics of Njavara accessions are detailed in Table 1.

MATERIALS AND METHODS

Plant material and DNA isolation

Seeds of rice varieties for DNA fingerprinting and validation of DNA fingerprint were collected from the germplasm at Department of Seed Science and Technology, College of Agriculture, Vellanikkara, Kerala Agricultural University (KAU), Thrissur, Kerala, India and Regional Agriculture Research Station (RARS), Pattambi, Palakkad, Kerala, India. Rice varieties were grown in pots and DNA was isolated from young leaves using CTAB (cetyl trimethyl ammonium bromide)-based method (Doyle and Doyle 1990). DNA concentration was measured with a spectrophotometer (Nanophotometer® NP80, Implen, Munich, Germany) and isolated DNA samples were diluted to 100 ng μL^{-1} for PCR amplification.

Amplification using SSR markers

Amplification using 19 SSR markers selected from Gramene marker database ((http://www.gramene.org) was carried out by PCR over the DNA of Black Njavara, White Njavara, Karanjavara, Rakthasaali and Vaishak. PCR amplification was carried out using reaction mixes of final volume 20 µL containing template DNA (100 ng μL^{-1}, 1 µL), Taq DNA polymerase (3U μL^{-1}, 0.3 µL) (GeNei, Bangalore, India), Taq Buffer A with MgCl2 (10X, 2 µL) (GeNei, Bangalore, India),

Table 1. Major agro-morphological characteristics of Njavara accessions.

Sl. no.	ACCESSION & CODE NO.	Karanavara (TGC 90)	Black Njavara (TGC 366)	White Njavara (TGC 389)
1	Leaf: Pubescence of Blade Surface	Weak	Strong	Strong
2	Leaf: Average Length of Blade (cm)	42.30	46.80	31.80
3	Leaf: Average Width of Blade (cm)	1.80	1.30	1.20
4	Culm: Attitude	Open	Open	Erect
5	Lemma: Color of Stigma	White	White	Yellow
6	Flagleaf: Attitude of Blade (Late Observation)	Erect	Semi Erect	Semi Erect
7	Panicle: Curvature of Main Axis	Semi Straight	Semi Straight	Deflexed
8	Spikelts per panicle (No.)	50	90	55
9	Spikelet: Color of Tip of Lemma	Black	Black	Yellow
10	Lemma and Palea: Color	Black	Brown	Gold and Gold furrows on straw background
11	Panicle: Secondary Branching	Strong	Strong	Clustered
12	Panicle: Attitude of Branching	Erect	Erect	Semi erect
13	Grain: Weight of 1000 fully developed grains (g)	25.44	20.72	17.64

(KAU, 2023)

dNTP mix (10 mM each, 1 L) (GeNei, Bangalore, India), forward primer (10 pM, 1 L), reverse primer (10 pM, 1 L) and nuclease free sterile water (13.7 L). Amplification was performed in thermal cycler (Biorad, USA) by following initial denaturation step at 94° C for 5 mins, then 40 cycles of cyclic denaturation (94° C for 1 min), annealing (55-60° C for 55 seconds) and cyclic extension (72°C for 1 min) followed by final extension (72° C for 5 mins). PCR products along with 100 bp ladder (100 bp StepUp, GeNei, Bangalore) were separated by agarose gel electrophoresis (2.5% agarose gel, Agarose low EEO for molecular biology, SRL, Mumbai, India) at uniform voltage of 85 Volts for 30 to 45 mins. SSR markers were selected for DNA fingerprinting based on polymorphism, specificity of amplification and presence of intact bands.

DNA fingerprinting of Njavara accessions, Rakthsali and Vaishak

Nineteen SSR markers selected were used in DNA fingerprinting of Njavara accessions (Black Njavara, White Njavara and Karanjavara). PCR amplification and agarose gel electrophoresis was performed same way as in screening. DNA fingerprint profile was developed and represented using colour charts.

Validation of SSR markers unique to Njavara accessions

SSR markers produced unique banding pattern for three Njavara accession were selected for validation with 10 KAU released varieties *viz*., Manuratna, Harsha, Samyuktha, Aiswarya, Uma, Kanchana, Pournami, Akshaya, Jyothi and Supriya by PCR amplification.

Data analysis

Amplicon sizes were calculated using PyElph 1.4 (Pavel and Vasile, 2012). Marker alleles were scored separately as '1' for presence and '0' for absence of bands and scoring matrix was generated. Cluster analysis was carried out and separate dendrograms for DNA fingerprinting and validation was constructed using UPGMA based on Jaccard's similarity coefficient with the help of NTSYS-pc2.10z

Polymorphism information content was calculated using the formula (Smith et al., 1997);

$$PIC = 1- \sum_{i-1}^{n} Pi^2$$

Where, Pi is the frequency of the i^{th} allele

Effective number of alleles was calculated using the formula (Aranzana et al., 2003);

$$Ae = \frac{1}{\sum_{i=1}^{1} Pi^2}$$

Where, Pi is the frequency of the i_{th} allele

Shannon's Information Index was calculated using the formula (Weaver and Shannon, 1964)

$$I = - \sum_{i=1}^{n} Pi \ln Pi$$

Where, Pi is the frequency of the i^{th} allele

Colour Chart was generated based on the amplicons produced by a particular marker. In colour chart, if two varieties show same colour for a particular marker it means both varieties produced amplicon of same size with respect to that marker. If colours are different, it means both varieties produced amplicon of different size with respect to that marker. So, the colour chart will give over all idea on which markers are unique to each accession/ variety.

RESULTS AND DISCUSSION

DNA fingerprinting of Njavara and non-Njavara types

Nineteen SSR markers yielded a total of 98 amplicons of size ranging from 90 bp to 315 bp. Six markers (RM10346, RM231, RM274, RM340, RM3805 and RM501) produced unique bands for Black Njavara. RM493 also yielded unique banding pattern with two amplicons of 202 bp and 233 bp. SSR markers are codominant and they produce two or more band, if the locus is heterozygous. For a self-pollinated crop like rice, it is unusual to generate two bands, since most of the loci will be in homozygous condition. The reasons for generation of two bands can either be due to retaining of the locus in heterozygous condition, even after generations or due to the presence of another locus with the same annealing sites. Five SSR markers *viz*., RM10346, RM6089, RM295, RM434 and RM247 produced unique amplicons for White Njavara. In White Njavara also RM493 generated codominant banding pattern with amplicons of size 208 bp and 233 bp. Five SSR markers *viz*., RM493, RM231, RM249, RM434 and RM258 produced unique bands in Karanjavara. In Rakthasali, five SSR markers *viz*., RM493, RM517, RM340, RM295 and RM247 yielded distinct amplicons. RM209 produced two bands, with amplicons of size 128 bp and 158 bp. Twelve out of the 19 SSR markers namely; RM10346, RM493, RM517, RM231, RM273, RM249, RM340, RM3805, RM434, RM258, RM202 and RM21 produced unique amplicons in Vaishak (Table 2). Using the amplification profile of each marker, DNA fingerprint profile was generated and represented using colour chart (Fig. 1).

Cluster analysis was carried out using UPGMA based on Jaccard's similarity coefficient and dendrogram generated. Jaccard's index/ similarity coefficient can range from zero to one. A coefficient of zero indicates no similarity and one corresponds to 100 per cent similarity (Chung et al., 2019). Njavara accessions and Rakthasali were clustered together and Vaishak was out grouped at 0.08 similarity coefficient. This indicated that medicinal rice varieties have a higher degree of genetic relatedness among themselves. White Njavara was separated from Black Njavara, Karanjavara and Rakthasali at 0.2 similarity coefficient. Rakthasali was separated from the cluster of Black Navara and Karanjavara at 0.25 similarity coefficient. Black Njavara and Karanjavara showed a maximum of 30 per cent similarity between them (Fig. 2). Similar studies on DNA fingerprinting of Njavara accessions/ cultivars along with non-Njavara types have been carried out by earlier workers also. Kumar et al. (2008) reported that seven Njavara accessions were clustered separately from two local varieties when RAPD was used as marker. Forty Njavara electromorphs along with six non-Njavara types were characterized by Sreejayan et al. (2011), using AFLP markers. Cluster analysis outgrouped the six non-Njavara types from Njavara electromorphs. Similarly, Nadiya et al. (2021) carried out the genetic diversity assessment of 24 Njavara

Table 2. Details of amplicons produced by selected SSR markers on PCR.

Sl. no.	SSR marker	Molecular weight of amplicon (bp)				
		Black Njavara	White Njavara	Karan-javara	Rakt-hasali	Vais-hak
1	RM10346	256	262	294	294	315
2	RM493	233, 202	233, 208	202	227	233
3	RM517	278	278	278	270	246
4	RM231	176	182	192	182	166
5	RM6089	166	156	150	166	150
6	RM273	202	202	202	202	206
7	RM274	152	160	148	148	160
8	RM249	134	134	130	134	122
9	RM340	112	115	115	180	156
10	RM3805	94	92	92	92	90
11	RM501	185	152	160	160	152
12	RM295	182	172	182	178	182
13	RM434	160	148	156	160	144
14	RM278	144	141	144	144	141
15	RM258	144	144	146	144	140
16	RM202	174	174	174	174	184
17	RM21	134	134	134	134	166
18	RM209	150	135	150	128, 158	135
19	RM247	178	196	178	172	178

Amplicon size (bp)	Black Njavara	White Njavara	Karanjavara	Rakthasali	Vaishak
315					RM10346
294			RM10346	RM10346	
278	RM517	RM517	RM517		
270				RM517	
262		RM10346			
256	RM10346				
246					RM517
233	RM493	RM493			RM493
227				RM493	
208		RM493			
206					RM273
202	RM493 RM273	RM273	RM493 RM273	RM273	
196		RM247			
192			RM231		
185	RM501				
184					RM202
182	RM295	RM231	RM295	RM231	RM295
180				RM340	
178	RM247		RM247	RM295	RM247
176	RM231				
174	RM202	RM202	RM202	RM202	
172		RM295		RM247	
166	RM6089			RM6089	RM231 RM21
160	RM434	RM274	RM501	RM501 RM434	RM274
158				RM209	
156		RM6089	RM434		RM340
152	RM274	RM501			RM501
150	RM209		RM6089 RM209		RM6089
148		RM434	RM274	RM274	
146			RM258		
144	RM278 RM258	RM258	RM278	RM278 RM258	RM434
141		RM278			RM278
140					RM258
135		RM209			RM209
134	RM274 RM21	RM274 RM21	RM21	RM274 RM21	
130			RM274		
128				RM209	
122					RM274
115		RM340	RM340		
112	RM340				
94	RM3805				
92		RM3805	RM3805	RM3805	
90					RM3805

Colour codes for bands.

Fig. 1. DNA fingerprint profile represented using colour chart.

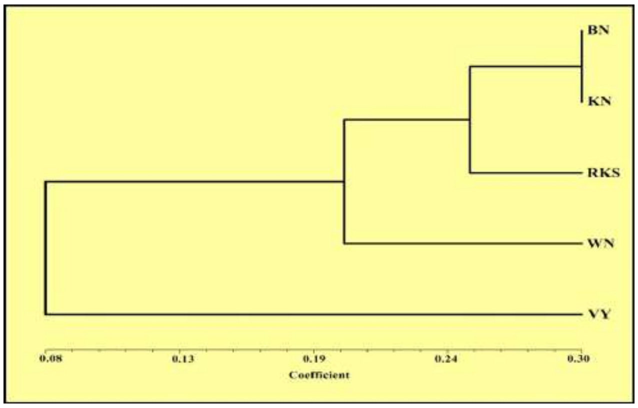

Fig. 2. Dendrogram of amplification profile of DNA fingerprinting (NTSYS-pc 2.10z).
BN- Black Njavara, WN- White Njavara, KN- Karanjavara, RKS- Rakthasali, VY- Vaishak

cultivars from different parts of Kerala using ISSR marker. Seven improved varieties were used as check. Cluster analysis grouped Njavara cultivars in a major node and improved varieties in a minor node. Our results are in agreement with earlier reports on cluster analysis using RAPD, AFLP and ISSR markers.

The polymorphism information content (PIC) values across 19 SSR markers were observed to range from 0.32 to 0.72. RM202, RM21, and RM273 exhibited the lowest PIC value of 0.32, while RM231, RM434, RM340, RM10346, and RM209 displayed the highest PIC value of 0.72. Simultaneously, the Shannon's information index (I) values showed variation, with RM209 having the highest (1.38), and RM202, RM21, and RM273 having the lowest (0.50) values, as detailed in Table 3. This comprehensive analysis offers valuable insights into the genetic diversity and informativeness of the SSR markers within the studied population. PIC and Shannon's Index are crucial metrics in molecular marker studies, providing a measure of information content within genetic markers. These metrics play a crucial role in population genetics, breeding programs, forensics, and evolutionary biology, offering a holistic perspective on genetic marker studies. A higher PIC value indicates greater variation, while an elevated Shannon's Index reflects increased genetic diversity. These metrics serve as guiding factors in making informed decisions for population genetics, breeding programs, forensics, and evolutionary studies, helping to formulate strategies based on observed genetic traits (Bonchev and Rouvray, 2003).

Table 3. Polymorphism Information Content, Effective number of alleles and Shannon's Information Index of SSR markers.

Sl. no.	Markers	PIC	Ae	I
1	RM517	0.56	2.27	0.95
2	RM274	0.64	2.78	1.05
3	RM501	0.64	2.78	1.05
4	RM231	0.72	3.57	1.33
5	RM434	0.72	3.57	1.33
6	RM278	0.48	1.92	0.67
7	RM340	0.72	3.57	1.33
8	RM10346	0.72	3.57	1.33
9	RM3805	0.56	2.27	0.95
10	RM202	0.32	1.47	0.50
11	RM6089	0.64	2.78	1.05
12	RM295	0.56	2.27	0.95
13	RM258	0.56	2.27	0.95
14	RM21	0.32	1.47	0.50
15	RM247	0.56	2.27	0.95
16	RM273	0.32	1.47	0.50
17	RM249	0.56	2.27	0.95
18	RM493	0.69	1.67	1.32
19	RM209	0.72	2.50	1.38

PIC- Polymorphism Information Content, Ae- Effective number of alleles, I- Shannon's Information Index.

Validation of SSR markers unique for Njavara accessions

Ten SSR markers (RM340, RM517, RM21, RM493, RM249, RM10346, RM434, RM231, RM202 and RM274) which were found to yield unique banding pattern between Njavara accessions as well as compared to Vaishak, were used for validation of the results. In order to validate the ability of these markers to produce Njavara-specific banding pattern, PCR amplification was carried out with the three Njavara accessions along with ten varieties, released by Kerala Agricultural University (KAU). Four SSR markers (RM202, RM434, RM340 and RM493) generated distinct banding pattern for the three Njavara accessions, compared with KAU varieties (Fig. 3).

Cluster analysis was carried out on the amplification profile. Two major clusters were observed in the dendrogram: one with all the three Njavara accessions and the other with ten KAU varieties tested. Njavara accessions showed only four per cent similarity with KAU varieties, with respect to the ten SSR markers used (Fig. 4). The results of cluster analysis

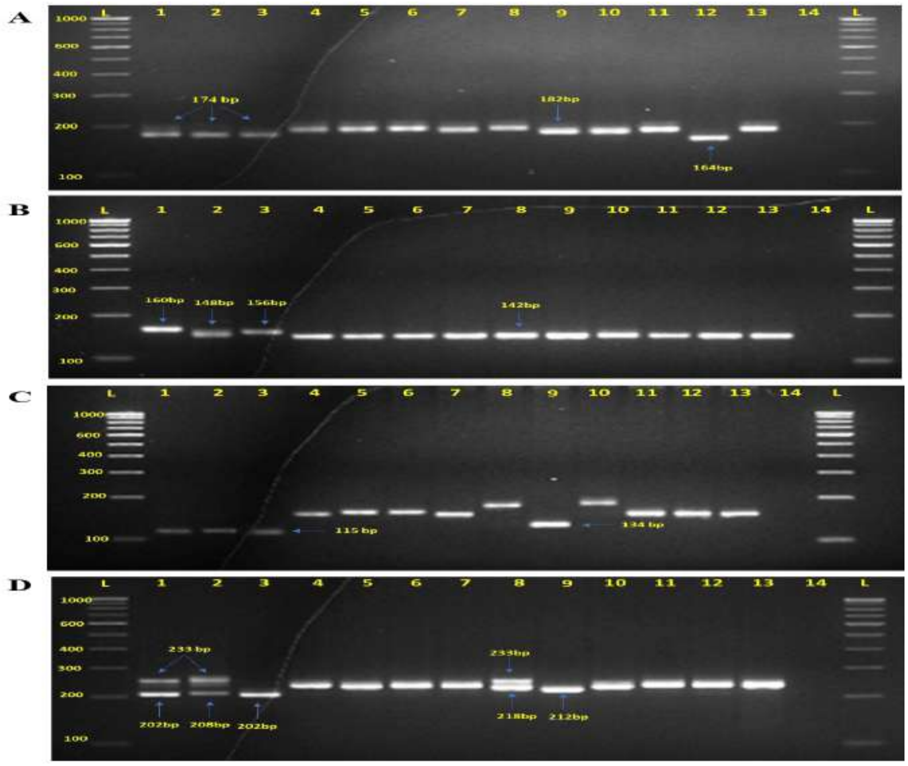

Fig. 3. Amplification profile of markers produced distinct banding pattern for Njavara accessions. **(A)** RM202, **(B)** RM434, **(C)** RM340 and **(D)** RM493. L- 100 bp ladder, 1- Black Njavara, 2- White Njavara, 3- Karanjavara, 4- Manuratna, 5- Harsha, 6- Samyuktha, 7- Aiswarya, 8- Uma, 9- Kanchana, 10- Pournami, 11- Akshaya, 12- Jyothi, 13-Supriya, 14- Negative control.

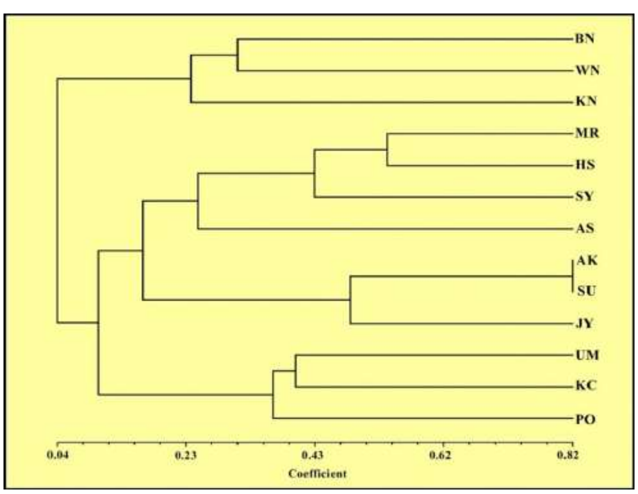

Fig. 4. Dendrogram of amplification profile of validation (NTSYS-pc 2.10z). BN- Black Njavara, WN- White Njavara, KN- Karanjavara, MR- Manuratna, HS- Harsha, SY- Samyuktha, AS- Aiswarya, UM- Uma, KC- Kanchana, PO- Pournami, AK- Akshaya, JY- Jyothi, SU-Supriya

clearly suggested that these markers could be used to identify Njavara accessions.

In conclusion, this study delves into the DNA fingerprinting of Njavara accessions through the utilization of SSR markers. The study was able to successfully generate DNA fingerprint profile of Njavara accessions and confirmed the importance of using DNA fingerprinting with SSR markers in genotype characterization and assessment of genetic similarity among Njavara accessions. Upon validation, four SSR markers *viz*., RM202, RM434, RM340 and RM493 produced unique banding pattern for Njavara accessions. These markers can be used for discriminating Njavara from other rice varieties and minimize seed duplicity.

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Genetic diversity analysis of North-East Indian rice landraces: An integrated morphological and molecular perspective

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ABSTRACT

The research aims to address the insufficiency of studies on grain yield and yield traits of North East Indian rice landraces. Focusing on genetic diversity through integrated morphological and molecular analysis, the goal is to identify and preserve diverse rice varieties. It aims to conserve ideal parental genotypes and expand the genetic foundation to meet food demand. The investigation was carried out during Kharif-2021 season, fifty-two rice accessions were evaluated for nine yield traits in randomized complete block design with five replications at the Agricultural Research Station (ARS), Bapatla of Acharya N G Ranga Agricultural University. D2 analysis, revealed high genetic diversity present among the genotypes and grouped them into six clusters using yield traits. Clusters I, II, III and VI comprised 26, 16, 6 and 2 rice genotypes, respectively. The test weight played high role in the total genetic divergence (83.71%). The genetic diversity (GD) ranged from 0.0000 to 0.7301, with an average of 0.4909. While the polymorphic information content (PIC) ranged was from 0.00 to 0.68, with an average of 0.42. The number of alleles identified ranged from 1.00 to 5.00, with an average of 3.05 alleles per marker.

Key words:

INTRODUCTION

Rice (*Oryza sativa* L.) serves as a primary food source for more than 3.5 billion people globally and is grown in at least 114 countries (FAOSTAT, 2022). In India, rice is grown across 43.79 million hectares, yielding a milled rice production of 112.91 million tonnes in the 2017-18 (Anonymous, 2020). Rice positions as the key cereal crop and primary nutritional staple in the North East region of India, encompassing 75% of the cultivated land in the area. (4.58 million hectares) (GOI, 2021).

Many ethnic groups and tribes in North-East India grow their own rice varieties, called rice landraces. North-East India is home of diverse rice landraces, cultivated by various tribes for taste, quality and cultural significance. While this region displays rich agroecological heterogeneity, there are few studies investigating the grain yield and yield traits of these landraces. This research gap presents a challenge in developing improved rice varieties tailored to the needs of the region (Avasthe, 2009). Therefore, it becomes crucial to prioritize the preservation and utilization of these valuable landraces. By leveraging advancements in genetic diversity analysis and selection strategies, researchers can identify local cultivars harboring superior yield potential (Surname et al., 2019).

To understand species diversity and their genetic makeup, genetic diversity studies are essential. They help construct systematic agriculture, and to discover and conserve ideal parental genotypes, and establish genetic linkages. Understanding genetic diversity is crucial for preservation methods and expanding the genetic foundation to meet global and local food demand (Roy et al.,2023).

Plant genetic diversity has been evaluated through morphological and molecular markers. Mahalanobis distance (D2) statistics provide a robust method for identifying clustering patterns, helping to establish links between genetic and geographical variations. It also aids in exploring the influence of various quantitative traits in achieving maximum divergence (Murty and Arunachalam, 1966). Evaluating plant genetic diversity through morphological traits can be unreliable due to the influence of environmental factors. The introduction of PCR-based molecular marker technology offers a highly effective and dependable means to assess genetic diversity in crop germplasm (Chitwood et al., 2016). Among various molecular markers, simple sequence repeats (SSR) stand out as a marker of choice due to several advantages. This makes SSRs highly suitable for characterizing rice because of their reproducibility, simplicity, ease of scoring, multi-allelic nature, high variability, co-dominant inheritance, and broad genome coverage (Roy et al., 2014).

This study aimed to evaluate the genetic structure and diversity among a group of northeast Indian rice landraces, utilizing both phenotypic traits and SSR markers. The findings will provide a foundation for developing a protocol to assess genetic diversity and select suitable parental lines for creating new rice varieties.

MATERIALS AND METHODS

Experiment locationand design

In the study, fifty-two rice accessions (Table 1) were evaluated at the Agricultural Research Station (ARS), Bapatla of Acharya N. G. Ranga Agricultural University (ANGRAU) during *kharif*, 2021. Each genotype was grown in a single row, three meters long, spaced at 20X15 cm. The study was conducted using five replications and the data were analysed using Randomized Block Design.

Morphological observations

Five plants were randomly selected from each block of genotypes for yield and yield attributing traits data collection. In this experiment, nine quantitative characters were recorded for five desired plants of each genotype. The recorded morphological traits include Days to 50% flowering (DF50%), Plant height (PH), Number of tillers per hill (NTPH), Number of effective tillers per hill (NETPH), Panicle length (PL), Number of grains per panicle (NGPP), Panicle fertility percentage (PFP), Test Weight (TW), and Grain yield per plant (GYPP).

Table 1. List of landraces used in the study.

S. no.	Designation	S. no.	Designation
G1	Ambemohar	G27	Kudral-7
G2	Apputhakal	G28	Kulakar
G3	Arakuloya	G29	Machakanta
G4	Asandi	G30	Madhumurangi
G5	Bahurupi	G31	Madhuraj-55
G6	Badshabhog	G32	Mangala Mahsuri
G7	Burmablack	G33	Mappilai samba
G8	Chattisgarh local	G34	Matha triveni
G9	China biceinee	G35	Mysore malliga
G10	Chinni krushnudu	G36	Narayanakamini
G11	Chintalurisannalu	G37	Navara
G12	Chittiga	G38	Pancharatna
G13	Chittimuthyalu	G39	Parimala sanna
G14	Doddiga	G40	Pathariya
G15	Halla Bhatta	G41	Poongar
G16	Illappu samba	G42	Ramyagali
G17	Kakirekkalu	G43	Ranikanaka
G18	Kalabhatt	G44	Ranikanda
G19	Kalachampa	G45	Samuelbhog
G20	Kalajeera	G46	Seeraga samba
G21	Kalanamak	G47	Selam sanna
G22	Kandasagar	G48	Sonakshika
G23	Karuppukavuni	G49	Kalasur
G24	Karigajavalli	G50	Kuzipatali
G25	Karisala	G51	Raktasali
G26	Kudral-6	G52	Krishna Vreehi

SSR genotyping

We assessed the genetic diversity of 52 rice accessions by employing 136 SSR (Supplimentary Table 1) markers, which covered all 12 chromosomes. These specific SSR markers were selected for genetic diversity analysis due to their polymorphic nature and distinct banding patterns that could be observed among different genotypes. The sequences of these SSR markers, along with their annealing temperature and chromosomal locations, were obtained from the GRAMENE database. The genomic DNA from each genotype in the current study was extracted according to the provided protocol. (Plaschkeet al., 1995). The amplification conditions were determined following the specified procedure of (Panaud et al.,1995).

Gel electrophoresis, photography and allele scoring

Amplified products were separated by size through 3% agarose gel electrophoresis and 1x TBE buffer in a horizontal electrophoresis tank. The gel photograph was digitally captured using a Gel Documentation System (UVP, UK). The molecular weight of specific bands or amplified fragments was determined in base pairs by comparing them with the band sizes of a 100 bp ladder (GeNeI Company), with IR-36 serving as a molecular weight reference (Temnykh et al., 2000).

Data analysis

The analysis of variance (ANOVA) was conducted using the OPSTAT open-source software to assess the data (opstat.pythonanywhere.com). Mahalanobis distance, cluster groupings and the intra-cluster and inter-cluster distances are calculated by following the outlined approach of Singh and Choudhary (2010). Percent contribution towards total divergence was calculated by Mahalanobis distance (D2) statistic (Mahalanobis, 1936). D2 analysis is done by INDOSTAST software. We used Power Marker version 3.25 to calculate summary statistics, which encompassed the number of alleles per locus, the frequency of major alleles, and the polymorphism information content (PIC) values. The PIC values for each marker were determined following the method suggested by (Botstein et al., 1980).

RESULTS AND DISCUSSION

Analysis of variance

Table 2, presents the collective analysis of variance for nine quantitative traits. The results reveal significant differences among genotypes for all traits under study these significant differences suggest that there is a substantial amount of genetic variation among the evaluated genotypes. Similar results reported by Demeke et al. (2023) in rice.

Genetic diversity by Mahalanobis D^2 statistic

In the world of plant breeding, the genetic diversity of genotypes is often measured by Mahalanobis D^2 method (Agalya et al., 2024). This study aimed to identify suitable parents for hybridization by analyzing the genetic diversity of 52 rice accessions.

The results of the D^2 analysis confirmed the presence of high genetic diversity among the genotypes. Tocher's method-based dendrogram analysis grouped the 52 rice accessions into six clusters using yield traits (Table 3 and Fig. 1). Clusters I, II, III and VI comprised 26, 16, 6 and 2 rice genotypes, respectively. Clusters IV and V composed the solitary genotypes 'Apputhakal' and 'Madhuraj-55', which were the best genotypes among 52 rice accessions in terms of yield. Cluster VI comprised kudral-7 and Selam sanna, which showed the highest number of grains per panicle. Cluster I have the largest group comprising 26 genotypes. They have a relatively low day to flowering. The cluster III genotypes have a relatively high panicle length. Cluster II genotypes exhibit optimal performance.

The intra-cluster distances among 52 rice landraces varied widely, ranging from 0.00 to 14.36 (Table 4). The maximum intra-cluster distance was recorded for cluster I (14.36), which consisted of 26 genotypes, followed by cluster II (13.15) and cluster III (11.09). Additionally, three mono solitary clusters, namely cluster-IV, V, and VI (2 genotypes) showed an intra-cluster distance of 0.00, indicating that the genotypes within these clusters were highly similar and formed separate groups. Inter-cluster distances ranged from 5.94 to 73.77, with the maximum distance observed

Table 2. The mean squares and significant level of yield, and its related traits revealed by ANOVA.

S. no.	Character	Replication (DF=4)	Treatment (DF= 51)	Error (DF=204)	C.V.
1	Days to 50 per cent flowering	0.47	168.66**	0.46	0.78
2	Plant height (cm)	170.22	602.33**	120.6	9.17
3	tiller number	8.09	67.27**	6.58	19.41
4	Productive tiller number	6.18	65.10**	5.71	21.28
5	Panicle length (cm)	6.54	4.91**	3.13	7.84
6	number of seeds per panicle	3661.27	5231.54**	3465.07	26.98
7	Per cent age of fertile seeds	2.26	50.46**	3.03	2.12
8	Test weight (gm)	0.01	163.31**	0.09	1.22
9	Grain yield per plant (gm)	33.68	156.69**	20.87	19.73

**Significant at 5 per cent Level of Significance

Table 3. Cluster mean values for nine quantitative traits in 52 rice landraces.

	DF50%	PH	NTPH	NETPH	PL	NGPP	PFS	TW	SYPP
Cluster. 1	84.85	120.25	13.13	11.13	22.41	221.05	93.39	23.45	22.91
Cluster. 2	85.81	120.31	13.48	11.53	22.60	213.79	93.16	31.18	22.50
Cluster. 3	87.33	110.67	12.83	10.73	23.23	210.30	93.76	15.88	24.17
Cluster. 4	97.00	121.80	11.20	9.20	21.40	221.20	90.22	26.44	29.00
Cluster. 5	100.00	142.00	18.20	16.20	23.20	257.60	94.44	25.44	28.40
Cluster. 6	100.50	122.70	12.20	10.20	22.60	216.50	94.30	37.54	23.10

DF50%= Days to 50 per cent flowering, PH = Plant height, NTPH = Number of tillers per hill, NETPH= Number of effective tillers per hill, PL= Panicle length, NGPP= Number of grains per panicle, PFP = Panicle fertility percentage, TW = Test Weight and GYPP= Grain yield per plant.

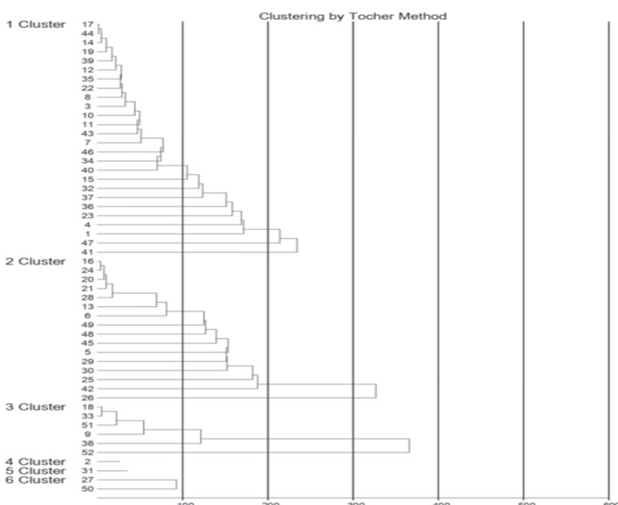

Fig. 1. Dendrogram showing clustering pattern of 52 rice landraces in *kharif*, 2021 using Tocher's method.

between clusters III and VI (73.77) followed by cluster I and VI (50.13), cluster V and VI (40.5) and cluster II and III (52.27).

In Mahalanobis' distance D^2 Statistic, the percentage contribution to genetic diversity is represented by the eigen values associated with the principal components used in the analysis (Do Rego et al., 2003). Understanding the percentage contributions helps researchers prioritize the most influential components and focus on the key sources of genetic diversity in their analysis. Test weight played the most significant role in the total genetic divergence (83.71%), being ranked first 1110 times (Table 5). Days to flowering (12.37%) followed, ranked first 164 times. similar results given by Shanmugam et al., (2023) and Sruthi et al., (2023).

Genetic diversity analysis using SSR markers

The genetic diversity (GD) varied from 0.0000 to 0.7301 (Table 6.), with an average of 0.4909. The polymorphic information content (PIC) ranged from 0.00 to 0.68, averaging 0.42. Among the markers, RM7000, RM3341, RM6842, RM1095, and RM8004 exhibited heightened discriminatory power in distinguishing genotypes due to their high PIC values of 0.68, 0.68, 0.67, 0.67, and 0.67, respectively. The primer RM3343 exhibited a lower PIC value of 0.00, indicating reduced discriminatory power for this specific primer under investigation. A PIC value above 0.5 is considered highly informative (Botstein, 1985). The identified

Table 4. Intra (diagonal) and Inter-cluster average distances for different quantitative characters in rice landraces.

	cluster 1	cluster 2	cluster 3	cluster 4	cluster 5	cluster 6
cluster 1	11.09	28.17	27.94	17.58	18.57	50.13
cluster 2		13.15	52.27	21.36	25.46	27.75
cluster 3			14.36	37.52	35.66	73.77
cluster 4				0	5.94	37.41
cluster 5					0	40.5
cluster 6						0

Table 5. Per cent contribution of different quantitative characters to genetic diversity.

Source	Times ranked 1st	Contribution %
Days to 50% flowering	164	12.37%
Plant height	7	0.53%
Number of tillers per hill	18	1.36%
Number of effective tillers per hill	1	0.08%
Panicle length	2	0.15%
panicle fertility per centage	0	0.00%
Number of grains per panicle	4	0.30%
Test weight	1110	83.71%
Grain yield per plant	20	1.51%

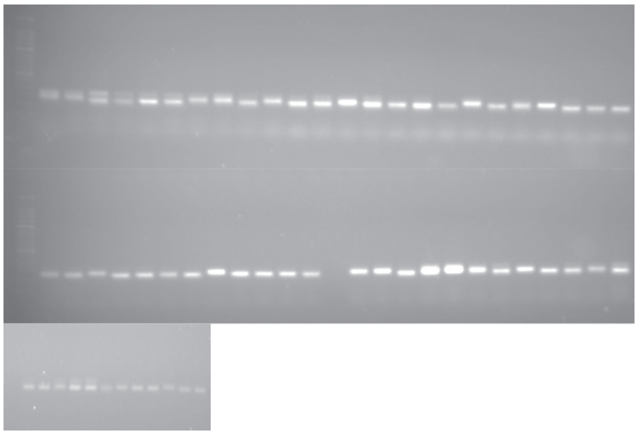

Fig. 2. Banding pattern obtained with SSR marker RM3341. L = 100 bp Ladder, 1 to 52 are the genotypes used in the present study listed in Table 1.

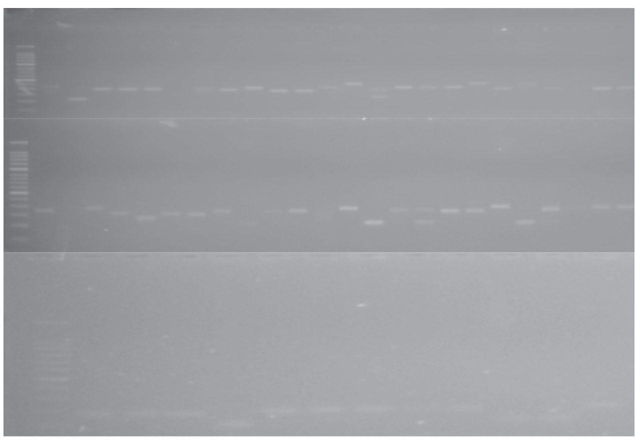

Fig. 3. Banding pattern obtained with SSR marker RM7000. L = 100 bp Ladder, 1 to 52 are the genotypes used in the present study listed in Table 1.

number of alleles ranged from 1.00 to 5.00, with an impressive average of 3.05 alleles per marker. RM8004 and RM3183 markers displayed the highest number of alleles, with each having five. Furthermore, 38 markers demonstrated four alleles each, and 63 markers exhibited three alleles each, totaling 419 alleles. The allelic variation among the rice genotypes is presented in Fig. 2 and Fig. 3. The variation in the number of alleles per locus can be attributed to the diverse rice genotypes included in the study. Additionally, the research found that 65 markers showed no heterozygosity, while the remaining markers displayed heterozygosity values ranging from 0.01 to 0.43, with an average of 0.06. The major allele frequency at the SSR loci ranged from 0.32 to 1.00, with an average of 0.61 among the 52 rice landraces, all sharing a common allele at each locus. In summary, this study provides a clear picture of the abundant genetic diversity found within rice landraces, showcasing the capabilities of modern scientific methods in uncovering the mysteries of our natural landrace diversity. Similar results reported by Vanlalsanga and Singh (2019) and Roy et al. (2023).

CONCLUSION

In summary, the investigation into North East Indian rice landraces demonstrated notable genetic diversity concerning yield and yield-related traits. D^2 Statistic analysis delineated distinct genotypic clusters showcasing unique traits associated with grain yield and related characteristics, indicating the presence of valuable genetic resources for enhancing rice improvement programs. Based on Mahalanobis D^2 it is suggested to use genotypes of cluster III and IV for hybridization which will provide a broad spectrum of variability in segregation to develop desirable genotypes for grain yield improvement in rice genotypes. The SSR markers unveiled elevated polymorphic information content and allelic diversity within this germplasm, indicating the existence of a diverse genetic foundation in this collection.

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Understanding the rice domestication concepts by experimenting with traditional rice varieties

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ABSTRACT

India conserves remarkable insights of domestication of wild rice into cultivated rice. Throughout the world the rice domestication and evolution of rice is a complex research. Many reports are evolving every day to prove the rice domestication. Our present study also aims towards the rice domestication by studying the characteristic features of Traditional Rice Varieties (TRVs) of Tamil Nadu which includes unexploited the mother gene pool. The selected varieties includes Kala Namac (Oryza sativa), Karuppu Kavuni (Oryza rufipogon), Karung Kuruvai (Oryza rufipogon), Nocora (Oryza sativa), Garudan Samba (Oryza sativa), compared with cultivated ones like Authur Samba (Oryza sativa), Salem Senna (Oryza sativa), 13 Bt (Oryza sativa) resulted how the Culm, Ligules, Panicles, Panicles Length, No of Tillers, No of Spikelet per panicle, Plant Height, seed weight, Length &Breadth ratio, Seed shattering habit that influenced the domestication of wild rice into cultivated rice. The wild rice Oryza rufipogon stand as a mother to domesticated rice Oryza sativa. This proven concept have been re-examined by with our Traditional Rive Varities. present study.

Key words: *Oryza sativa, wild rice, cultivated rice*

INTRODUCTION

Rice belonging to the family Poaceae, It is one of the most important cereal crop, ranking second to the wheat among the most cultivated cereals in the world and serves as the primary source of staple food for more than half of the global population (Emani et al., 2008; Jiang 2013). Approximately, 90% of the world's rice is grown in the Asian country and constitutes a staple food for 2.7 billion people worldwide (Salim et al., 2003).

Even though India occupy the second position for rice productivity. The demand needed ratio for rice is increasing everyday. By the year 2050, about 785 million tonnes of paddy which is 70 percent more than the current production will be needed to meet the growing demand. Therefore being the staple food of the population in India, improving rice productivity has become a crucial importance (Subbaiah et al., 2011).

To meet out the global food need high yielding and resistant varieties were developed by many rice researchers. But this practice leads to serious "generic erosion"- the loss of traditional varieties from our ecosystems and hinders efforts to improve native crops further (Porceddu et al., 1988, Singh 1999). The progressive loss of Traditional Paddy land Races implied that there were around 6,00,000 traditional seeds that were present in the past now it has dropped down to1000s. The same is case in Tamil Nadu, their numbers decreased from 2000 to 160 as they have become extinct. There were many reports on TRVs nutritional characterises and other molecular characters were studied and reported by (Roy, et al., 2021; George, 2021; Devi et al., 2020; Jegadeeswaran et al., 2017;

Venkatesan et al., 2023) but there were only few reports available on rice domestication (Nihar Ranjan Chakraborty, 2013). If so the reports were arise from China province and from USA (Molina et al., 2011; Gross et al., 2014; Fornasiero et al., 2022; Vaughan et al., 2008; Sweeney & McCouch, 2007) . Our present work on rice domestication form Tamil Nadu is a rare stand of research approach.

To evident the rice domestication there were many approaches were reported among that morphological evaluation is a fundamental step to estimate the variability and relationship among cultivars although several other tools are also used extensively (Smith et al., 1991). This phenotypic vegetative agro-morphological traits were helpful for basic characterization of varieties and also they can be used as a broad-spectrum approach to find out the morphological diversity among morphologically distinguishable rice approaches (Wijayawardhana et al., 2015).

The main stream approach of TRVs us gaining more values nowadays Traditional Rice Varieties TRV's has been considerably increased because of its low sugar content, good for diabetics, reduces weight, high amount of glutamic acid and vitamins. Report also support that the TRVs improves eyesight, fertility vocal clarity and mitigation rashes (Caius, 1999). Besides the health benefits the TRVs also has anthocyanins, anti-oxidative, anti-inflammatory and anti-carcinogenic effects (Shipp et al., 2010). In many Asian countries the coloured rice is taken as functional food (Kim et al., 2008).

In our present study based on the phenotypic or morphological characteristic features of eight different traditional rice varieties (TRVs) which includes Kala Namac (*Oryza sativa*), Karuppu Kavuni (*Oryza rufipogon*), Karung Kuruvai (*Oryza rufipogon*), Nocora (*Oryza sativa*), Garudan Samba (*Oryza sativa*), compared with cultivated ones like Authur Samba (*Oryza sativa*), Salem Senna (*Oryza sativa*), 13 Bt (*Oryza sativa*). The morphological variance showed highly significant differences between the eight different rice varieties and revealed the structure of the different phenotypes from 13 morphogenetic characters quantitative discrimination traits and characters. The studies of rice domestication explores many fields of research like the approach of neo-domestication of wild species of rice domesticated in to cultivated species (Alice Fornasiero et al., 2022). Tremendous advancements in genomics, our understanding of the genetic underpinnings of domestication has advanced significantly. Specifically, a novel path for studying the molecular genetic mechanisms and population processes of domestication has been made possible by the cloning of genes governing important domestication features in major crops (Doebley et al., 2006). Scientists are able to create rice types with longer-lasting tolerance to abiotic stressors, illnesses, and insects because to their understanding of rice domestication.

The domestication studies helps to identify the beneficial alleles in cultivated crops comparing with its wild relatives.

So the present investigation on rice domestication with our Traditional rice varieties represent important genetic reservoirs with valuable qualities.

Objective of present study

1. Collection of Traditional Rice Varieties (TRVs)
2. Germplasm maintenance of TRVs
3. Cultivation of Traditional Rice Varieties
4. Observation of morphogenetic characters of TRVs
5. Assessment of significant characters alignment with rice domestication studies.

MATERIALS AND METHODS

Collection of Traditional Rice Varieties (TRVs)

Traditional rice varieties were collected from traditional rice farmers, conservators, breeders and from private NGO. The prime locations are Madurai, Thanjavore, Sivagangai and Ulundurpet. Passport data of name of the variety its local name, season of cultivation agronomical characters, yield per acre, medicinal uses and name of the farmer were documented.

Germplasm maintenance of TRVs

The germplasms of TRVs are maintained in three forms gunny bags (Primary storage), Plastic containers (transport purpose) and at -20º C Deep freezer (Long time storage). The rice land races were stored in a appropriate containers with code TN-KN-1820 (TN - Tamil Nadu, KN - Kalanamc), TN-AS-1820 (TN - Tamil Nadu; AS - Authur Samba), TN-KK-1820 (TN - Tamil Nadu; KK - Karuppu Kavuni), TN-13Bt-1820 (TN - Tamil Nadu; 13Bt), TN-KS-1820 (TN- Tamil Nadu; KS- Karudan Samba), TN-KAK-1920 (TN- Tamil Nadu KAK- Karung Kuruvai), TN-SAM-1920 (TN- Tamil Nadu; SAM- Salem Samba, TN-NA-1920 (TN- Tamil Nadu; NA- Nacora) passport data were maintained. The percentage of germination was computed and reported (ISTA, 2015).

$$\text{Seed germination } (\%) = \frac{\text{Number of normal seedlings}}{\text{Total number of seeds sown}} * 100$$

Cultivation of Traditional Rice Varieties

The selected eight varieties of TRVs (Authur Samba, Kala Namac, Karuppu Kavuni, Karung Kuruvai, Nacora, Salem Senna, 13Bt (control), Garudan Samba) were grown on pots. Under randomozied design with 3 replicates. Watered regularly and maintained without addition of any urea and other growth substances.

Observation of morphogenetic characters and assessment of significant rice domestication characters alignment

The TRVs morphological characters like Culm, Ligule formation, No of Tillers, No of Spikelet per panicle Plant Height, 100 seed weight, Seed Length &Breadth ratio and Seed shattering habit. Were observed and recorded daily (Table 2).

RESULTS AND DISCUSSION

Collection, Cultivation and Germplasm maintenance of Traditional Rice Varieties (TRVs)

The TRVs were collected from farmers, private NGOs and rice breeding farmers (Fig. 1). The information about were collected from the farmers about their nutritional values and medicinal values were documentated. (Table 1). Among many varieties were collected selected eight varieties showing a prominet morphological variations in seeds like Brown husk, awn, ribbed with black stripes anad white rice, awn less, medium shaped grain- Kala Namac (*Oryza sativa*); black or purple rice covered with black paddy husk, medium shaped grain named as Karuppu Kavuni (*Oryza rufipogon*); reddish black rice with black paddy husk, awn less, medium shaped grain Karung Kuruvai (*Oryza rufipogon*); Short and bold grain, yellow husk, white rice, awn less- Garudan Samba (*Oryza sativa*) with cultivated lines yellow grain, slender shaped grain, white

Fig. 1. TRVs Collection.

Table 1. Selected Varieties of Traditional Rice Varieties (TRVs).

S.no	Rice variety	Accession no	Origin	Biological status
1	Authur Samba	TN-AS-1820	Tamil Nadu	Traditional cultivar/Landrace
2	Kala Namac	TN-KN-1820	Tamil Nadu	Traditional cultivar/Landrace
3	Karuppu Kavuni	TN-KK-1820	Tamil Nadu	Traditional cultivar/Landrace
4	Karung Kuruvai	TN-KAK-1920	Tamil Nadu	Traditional cultivar/Landrace
5	Nacora	TN-NA-1920	Tamil Nadu	Traditional cultivar/Landrace
6	Salem Senna	TN-SAM-1920	Tamil Nadu	Traditional cultivar/Landrace
7	13Bt	TN-13PT-1820	Tamil Nadu	Traditional cultivar/Landrace
8	Garudan Samba	TN-KS-1820	Tamil Nadu	Traditional cultivar/Landrace

Table 2. Morphological analysis of harvested TRVs.

S.no.	Variety name	Kernel breadth (cm)	Kernel length (cm)	Length & breadth ratio	1000 seed weight (g)	Plant height (cm)	No of tillers	Panicle length (cm)	Spikelet per panicle
1	Authur Samba	0.65	0.2	13:20	14.41	145	11	34	13
2	Kala Namac	0.85	0.25	17:25	15.60	146	5	32.7	14
3	KaruppuKavuni	0.95	0.35	19:35	24.85	141.5	4	33	12
4	KarungKuruvai	0.75	0.3	5:30	23.94	119	4	37	14
5	Garudan Samba	0.7	0.3	7:30	19.15	120	3	40	16
6	Nacora	0.75	0.35	15:35	20.88	109.4	5	23	8
7	Salem Senna	0.8	0.3	8.30	15.89	113.2	5	39.5	14
8	13 Bt	0.8	0.25	16.25	12.13	35.7	3	15	6

rice, awn less- Authur Samba (*Oryza sativa*); Short and bold grain, black brown in colour, white rice, awned grain - Nacora (*Oryza sativa*); White rice, yellow lemma, short slender rice, awn less - Salem Senna (*Oryza sativa*); awn less grain, short slender rice, white colour rice - 13 Bt (*Oryza sativa*) were used for further studies.

Based on the literature and research reports the available germplasm of TRVs were grouped into wild variety Karung Kuruvai (*Oryza rufipogon*), Karuppu Kavuni (*Oryza rufipogon*). Domesticated variety Kala Namac (*Oryza sativa*), Nocora (*Oryza sativa*), Garudan Samba (*Oryza sativa*). Cultivated varieties are Authur Samba (*Oryza sativa*) Salem Senna (*Oryza sativa*) and improved variety 13 Bt (*Oryza sativa*). The genus and species were confirmed by DNA barcoding studies.

Observation of morphogenetic characters and assessment of significant rice domestication characters alignment.

Assessment of Germination percentage and radicle plume measurement

In order to achieve the good germination the seeds of TRVs were sown in Petri plates with moist water content. This experiment resulted remarkable growth without any addition of any hormones or growth initiators. (Fig. 2). In this results the Authur samba and 13 Bt showed highest length of shoot and root initiation 82%. Kala Namac and Nacora showed moderated and equal amount of growth 24%. Karung Kuraivai and Garudan Samba showed Sparse amount of growth rate 12-18%. The traditional rice varieties were stored for many long years which would have initiated the dormancy that eventfully delayed germination. The HYV (High yielding varieties) like authur samba salem sanna were highly exposed to fertilizers and other growth nutrition which provoked the initiation level at high percentage.

Fig. 2. Coleoptile.

TRVs Culm initiation in pot culture

The culm is the major part of rice plant which determines the no of panicles formation and seed setting. We observed the thick dense culm in Kala Namac, Karung Kuruvai, Nacora, Salem Senna and Garudan samba. The culm types are thin in Authur Samba, Karuppu Kavuni and 13Bt compared to other varieties. The size, colour and thickness of the culm is also greater in previous said varieties than the later ones (Fig. 3).

Its inferred that the culm type of wild varieties Karung Kuruvai, Karuppu Kavuni is thick, dense measurable in size and dark in colour. This characters slowly decreased in domesticated varieties of Authur samba and in improved varieties 13Bt.

TRVs ligule observation

The ligule formation on the abaxial side of leaf branch usually 3cm long white or pale yellow in colour. The ligule of Authur Samba (*Oryza sativa*), Kala Namac

Fig. 3. Culm type in TRVs.

(*Oryza sativa*), Karuppu Kavuni (Oryza rufipogon), Salem Senna (*Oryza sativa*), 13 Bt (*Oryza sativa*) have smooth margin with sharp tip. But in Karung Kuruvai (*Oryza rufipogon*) and Garudan Samba (*Oryza sativa*) has serrated margin and broad in shape. This characteristic appearance distinguish the latter two varieties from the former.

The shape, length and margins of ligule is a key character of two different genera. In our observation the ligule of Karuppu Kavuni (*Oryza rufipogon*) and Karung Kuruvai (*Oryza rufipogon*) are different in shape, size and margin. Similarly the ligule of Kala Namac (*Oryza sativa*) and Garudan Samba (*Oryza sativa*) also differ in the ligule shape,size that two same genera and species have different type of ligules. This might be because of the evolution of TRVs in to domesticated varieties these ligule also underwent the changes. The ligule of TRV Kala Namac (*Oryza sativa*) shares similarity with Authur Samba (*Oryza sativa*), Salem Senna (*Oryza sativa*) and 13 Bt (*Oryza sativa*) this could be because of the evolutionary changes occurred in TRV Kala Namac (*Oryza sativa*) during the domestication to cultivation. The same above said phenomenon also applicable to Karuppu Kavuni (*Oryza rufipogon*) in addition the Oryza rufipogon which also shares similarity in ligule indicates that the not only the TRVs of *Oryza sativa* underwent changes even the Oryza rufipogon also underwent the changes in domestication irrespective of species difference (Fig. 4).

TRVs Panicles

Fig. 4. Ligule type in TRVs.

The panicles of Authur Samba (*Oryza sativa*) and 13 Bt (*Oryza sativa*) are white in colour and the rest of the panicles are has coloured grains of different shape and size (Fig. 5).

TRVs seeds harvest

The seeds are harvested from all varieties and stored in proper container (Fig. 6).

TRVs Panicles Length

The panicels length of Authur Samba (*Oryza sativa*), Kala Namac (*Oryza sativa*) Karuppu Kavuni (*Oryza rufipogon*) showed almost similar length of panicles (34; 32.7; 33 cm). The panicles height of Karung

Fig. 5. Panicles of TRVs.

Fig. 6. Seeds of TRVs.

Kuruvai (*Oryza rufipogon*), Garudan Samba (*Oryza sativa*) and Salem Senna (*Oryza sativa*) (37; 40; 39.5 cm). Followed by the Nacora (*Oryza sativa*) and 13Bt (*Oryza sativa*) scored 23;15 cm panicle height.

The inference from the results is the age old wild TRVs like Karuppu Kavuni (*Oryza rufipogon*) (33cm), Karung Kuruvai (*Oryza rufipogon*) (40cm) showed high panicle height compared to other TRVs. The Garudan Samba (*Oryza sativa*) showed highest range of panicle length up to 40cm which were nearer to Salem Senna (*Oryza sativa*) of 39.5cm. It clearly indicates that the wild varieties Karuppu Kavuni (*Oryza rufipogon*) (33 cm), Karung Kuruvai (*Oryza rufipogon*) little bit decreased up to 0.5 cm in domesticated variety in Garudan Samba (*Oryza sativa*) and Salem Senna (*Oryza sativa*) where as in improved 13Bt its 23 cm in size.

TRVs panicles length mean average analysis

1-Wild Variety-Karuppu Kavuni (*Oryza rufipogon*); 2-Wild Variety-Karung Kuruvai (*Oryza rufipogon*); 3- Domesticated Variety-Kala Namac (*Oryza sativa*); 4-Domesticated Variety-Garudan Samba (*Oryza sativa*); 5-Domesticated Variety-Nacora (*Oryza sativa*); 6-Cultivated Variety-Salem Senna (*Oryza sativa*); 7-Cultivated Variety-Authur Samba (*Oryza sativa*); 8-Improved Variety-13 Bt (*Oryza sativa*).

TRVs panicles length regression analysis

The panicle length of V1-Wild Variety-Karuppu Kavuni (*Oryza rufipogon*); V2-Wild Variety-Karung Kuruvai (*Oryza rufipogon*) is positively correlated results in upholding the wildness in this character similar results observed in V1-Wild Variety-Karuppu Kavuni (*Oryza rufipogon*) is also confidentially correlates with V3-domesticated Variety-Kala Namac (*Oryza sativa*). In the case of V6-Cultivated Variety-Salem Senna (*Oryza sativa)* is negatively correlates with the panicle length of V1-Wild Variety-Karuppu Kavuni (*Oryza rufipogon*). Similar results observed in V2-Wild Variety-Karung Kuruvai (*Oryza rufipogon*); V3-Domesticated Variety-Kala Namac (*Oryza sativa*). This negative correlation results in the domestication effects of wild to cultivated variety.

The flow positive correlation of V1-Wild Variety-Karuppu Kavuni (*Oryza rufipogon*) (1) to V4-Domesticated Variety-Garudan Samba (*Oryza sativa*) (0.65) and to V7-Cultivated Variety-Authur Samba (Oryza sativa) (0.33) indicates the slow evolution of rice domestication.

TRVs no of tillers

No of tillers in Karuppu Kavuni (*Oryza rufipogon*) (4) and Karung Kuruvai (*Oryza rufipogon*) (4). It indicates that these two wild varieties shows similar no of tillers representing the resemblance in their nature. Kala Namac (*Oryza sativa*), Nacora (*Oryza sativa*), Authur Samba (*Oryza sativa*) and Salem Senna (*Oryza sativa*) all of them showed 5 numbers of tillers. Similar pattern is observed in Garudan Samba (*Oryza sativa*) (3) and 13Bt (*Oryza sativa*) (3) Inference from these results are the Oryza sativa is showing not much domestication. The no of tillers is little bit decreased in domesticated and cultivated varieties because of the photosensitivity responses it slowly decreased in domesticated and cultivated varieties.

The no of tillers in V1-Wild Variety-Karuppu Kavuni (*Oryza rufipogon*) is negatively correlates with V8-Improved Variety-13 Bt (*Oryza sativa*). The reduced no of tillers implies that the due to the domestication effect. In V2-Wild Variety-Karung Kuruvai (*Oryza rufipogon*) the complete negative correlation observed in V7-Cultivated Variety-Authur Samba (*Oryza sativa*). It infers the domestication effect of the reduced tillers in cultivated variety compared to wild variety. Similar domestication results were observed in V3 against V7, V4 against V7 and

V5 against V7.

No of spikelet per panicle in TRVs

Authur Samba (*Oryza sativa*), Kala Namac (*Oryza sativa*), Karuppu Kavuni (*Oryza rufipogon*), Karung Kuruvai (*Oryza rufipogon*) and Salem Senna (*Oryza sativa*) has almost similar no. of spikelet per panicle 13;14;12;14;14 respectively.

Both the *Oryza sativa* and *Oryza rufipogon* are inter domesticated each other there were not much differentiation in no. of spikelet per panicle.

The Garudan Samba (*Oryza sativa*) stands with 16 spikelet per panicle shows the robustness in seed setting and still stands unique from the rest of the varieties. Where as the Nacora (*Oryza sativa*) and 13Bt (*Oryza sativa*) recorded 8 and 6 spikelet per panicle.

The spikelet bases of wild rice Karuppu Kavuni (*Oryza rufipogon*) and Karung Kuruvai (*Oryza rufipogon*) has shallow, round abscission scar and distinct vascular pore. The domesticated spikelet bases of Salem Senna (*Oryza sativa*) and 13Bt (*Oryza sativa*) shows deeper scar and irregular shaped base (Fig.7)

Spikelet per panicle showed a illustrative negative correlation with V1 against V4, V6 and V7. This may be due to the photosensitivity response loss of domesticated and cultivated varieties. Remarkable negative correlation is observed in V2 against V4, V6 and V7. This inference recorded due to reduce no of panicles in domesticated compared to wild and its get much reduced in cultivated and in improved varieties.

Fig. 7. Individual seeds with scission scar

Table 3. Shattering of seeds.

S. no	Accession no	Name of the varity	Seed Shattering/ Non-Shattering
1	TN-AS-1820	Authur Samba	Non-Shattering
2	TN-KN-1820	Kala Namac	Non-Shattering
3	TN-KK-1820	Karuppu Kavuni	Shattering
4	TN-KAK-1920	Karung Kuruvai	Shattering
5	TN-KS-1820	Garudan Samba	Shattering
6	TN-NA-1920	Nacora	Non-Shattering
7	TN-SAM-1920	Salem Senna	Non-Shattering
8	TN-13PT-1820	13Bt	Non-shattering

Plant height

The height of the plant generally determines the variety or race of the plant. Authur Samba (*Oryza sativa*), Kala Namac (*Oryza sativa*) Karuppu Kavuni (*Oryza rufipogon*) showed almost similar length of panicles (145;146;141.5 cm). Karung Kuruvai (*Oryza rufipogon*)-119 cm, Garudan Samba (*Oryza sativa*)-120 cm recorded resemblance in height. Nacora (*Oryza* sativa)- 109.4 cm, Salem Senna (*Oryza sativa*) -113.2 cm and 13Bt (*Oryza sativa*) scored 35.7 cm.

The height of the plant is the prime criteria for wildness. Its well observed in Karuppu Kavuni (*Oryza rufipogon*), Karung Kuruvai (*Oryza rufipogon*) and it later decreased in domesticated an cultivated varieties.

The regression analysis of plant height against wild varieties recorded negative correlation in V1 against V5. The domesticated variety decreased in plant height because of the loss of tallness due to domestication.

100 seed weight (g)

The weight of the seeds is the major trait for influential the quality of the seed. TRVs of Authur Samba (*Oryza sativa*)-14.1 g, Kala Namac (*Oryza sativa*)-15.6 g and Salem Senna (*Oryza sativa*)-15.89 g weighted almost equally. Karung Kuruvai (*Oryza rufipogon*)-23.94 g, Karuppu Kavuni (*Oryza rufipogon*)-24.85 g scaled almost equally. Garudan Samba (*Oryza sativa*)-19.15 g Nacora (*Oryza sativa*)-20.88 g and where as 13 Bt (*Oryza sativa*) stood only 12.33 g. (Fig. 8)

The key conclusions of this part of work is the TRVs has high grams of weight which implies its quality of seeds and its nutrition importance but the later

Fig. 8. Storage and maintenance of TRVs seeds

cultivated varieties like Authur Samba (*Oryza sativa*) and 13 Bt (*Oryza sativa*) weight less.

The seed weight of V1 negatively correlates with V6 of cultivated variety the seed weight loss in cultivated variety is due to many factors involved like agro climatic zones, growth additives etc., This effects also reflected in domesticated variety.

Length & breadth ratio

The size of seed measured by its Length and Breadth. The results of size measurement analysis recorded Karuppu Kavuni (*Oryza rufipogon*)-19:35, Kala Namac (*Oryza sativa*)-17:25 and 13 Bt (*Oryza sativa*)-16:25 cm large size seeds. Garudan Samba (*Oryza sativa*)-7:30 cm, Karung Kuruvai (*Oryza rufipogon*) - 5:30 cm measured wide breath than length. This size phenomenon enable seeds to hold firmly to the soil surface and resist strong wind and water current. Where as the Authur Samba (*Oryza sativa*)- 13:20 cm and 13 Bt (*Oryza sativa*)-16:25 cm were noted to be almost equal to length and breadth this modification helped these varieties to accustom to prevailing agro ecological conditions.

The panicle length decrease in Kala Namac (*Oryza sativa*) is observed compared to wild variety Karuppu Kavuni (*Oryza rufipogon*) which stands as evidence of domestication effect. The panicle length of cultivated variety Authur Samba (*Oryza sativa*) is almost similar with wild variety Kala Namac (*Oryza sativa*). This implies that the cultivated variety Authur Samba (*Oryza sativa*) maintains their panicle length even after domestication. The panicle length of the wild variety Karung Kuruvai (*Oryza rufipogon*) is comparatively high then compared with cultivated variety Salem Senna (*Oryza sativa*). It demonstrates the gradual domestication effects in the above two varieties.

A marginal decrease in panicle length is observed in domesticated variety Garudan Samba (*Oryza sativa*) compared to cultivated variety Salem Senna (*Oryza sativa*). In improved variety 13 Bt (*Oryza sativa*) a illustrative decrease in panicle length is observed compared to all varieties. The major conclusion is that the panicle length which is said to be a major domestication character which is observed in wild, domesticated, cultivated and in improved varieties were decreased during domestication.

The number of tillers is observed as in wild variety of Karuppu Kavuni (*Oryza rufipogon*), domesticated variety Garudan Samba (*Oryza sativa*), and in improved variety 13 Bt (*Oryza sativa*). This results concludes that no of tillers habit is maintained from wild to domesticated and to cultivated variety.

The number of tillers of domesticated variety Garudan Samba (*Oryza sativa*) is increased compared with wild variety Karung Kuruvai (*Oryza rufipogon*). Similar pattern is observed in domesticated variety Nacora (*Oryza sativa*) compared to wild variety Karung Kuruvai (*Oryza rufipogon*). This results clearly indicates that the decreasing effect of number of tillers from wild to domesticated variety. This may be due to the photo sensitivity responses of this varieties. The number of spikelet are increased in domesticated varieties compare to wild varieties. This is because of the usage of growth stimulants and other growth additives added to domesticated varieties.

The seed weight is increased in domesticated variety Garudan Samba (*Oryza sativa*) to wild varieties this similar pattern is observed in cultivated variety Salem Senna (*Oryza sativa*). This is because the domesticated and cultivated varieties the seed dissemination through air and other sources are not occurred because of the increased seed weight compare to wild varieties.

The length breath of ratio of seed varieties of domesticated, cultivated and improved varieties showed similar ratio. This results concludes that there is no much domestication occurred in seed length and breath ratio. If any size differences occurred its because of the growth morphogenesis occurred during the plant development in different agro climatic zones.

Seed shattering

Seed shattering phenomenon is observed in our studies because these trait it very important in domestication of wild rice to cultivated rice. The wild rice posses the ability of seed shattering and the cultivated varieties loosed the trait.

In our studies implies the TRVs of Karuppu Kavuni, Karung Kuruvai and Garudan Samba having shattering effort compared to cultivated varieties shows Non-shattering effort. Due to the domestication the Kala Namac and Nacora showed Non-shattering effort (Table 3.and Table 4).

Oryza sativa contains two major subspecies: short grained, sticky japonica variety and long grained non sticky indica rice. Our studies recorded Authur Samba (*Oryza sativa*) Japonica, Kala Namac (*Oryza sativa*) Japonica, Salem Senna (*Oryza sativa*) Indica, 13 Bt (*Oryza sativa*) Japonica, Garudan Samba (*Oryza sativa*) *Indica* or *Japonica*, Nacora (*Oryza sativa*) *Indica* or *Japonica*, Karuppu Kavuni (*Oryza rufipogon*), Karung Kuruvai (*Oryza rufipogon*). (Fig. 9)

India conserves vast diversity of two rice varieties *Oryza rufipogon* and *Oryza nivara* native to India since the Pleistocene documented by 9000 BC from the Ganges River valley (Fuller, 2010, 2011). This vast diversity of rice cultivation in India is depleting because of many parameters of both biotic and abiotic. The major attempt of this study is to conserve the genetic source of Tamil Nadu India and by improving the germplasm native to our mother land.

Though there were many research reports available regarding the nutritional values and molecular characterization studies the historic ancestral importance of TRVs of Tamil Nadu were less in documentation so our research aim on these aspect.

The top priority of germplasm conservation aspect is collection of TRVs of Tamil Nadu, India. Among the collected varieties eight selected varieties were used for further study because though the Traditional Rice varieties were found to be very good grower in previous records the ability of TRVs growth rate in present climatic zone is challenging. Due to many parameters like water availability, soil condition, moisture, pH, Nutrition uptake and energy levels the cultivation of TRVs in present day conditions is a difficult task. So we selected the varieties of same geographical locations near to Madurai and plain area varieties. The varieties collected from tribes of mountains were avoided because of the zonal variations may hinder the germination in plain areas (Harald Uhlig, 1978).

The major first aim of our studies to observe the morphological characters of grains compared with cultivated ones. Because the grain morphology provide us a proximal role in studying the rice domestication. As previous reports stated that the Compared to cultivated rice, wild rice grains are noticeably smaller (Doebley et al., 2006). In the early stages of domestication, farmers favoured choosing larger seeds in order to boost grain output and produce more food

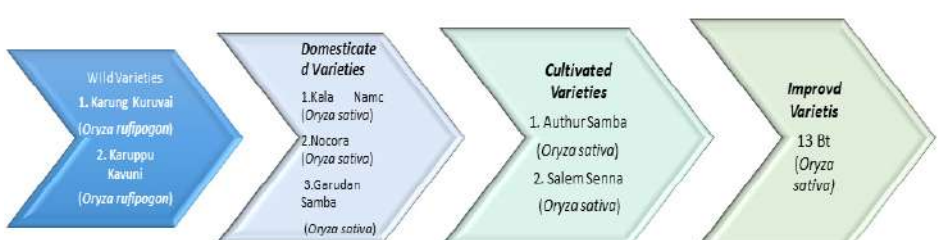

Fig. 9. Possible domestication scenario of wild *Oryza rufipogon* in to cultivated *Oryza sativa.*

Table 4. TRVs plantlet characters analysis.

S. no.	Accession	Variety name	Coleoptile colour (Color/ Colorless)	Intengity of colour in leaves Light/dark	Leaf anthoc-yanin color-ation Absent/ Present	Leaf ligule Absent/ Present	Culm type Erect/ semierect	Flag leaf attitude Horizontal Erect/ Semi erect	Panicle awn Absent/ Present	Stem anthocyanin colouration Absent/ Present
1	TN-AS-1820	Authur Samba	Colorless	Dark	Absent	Present	Erect	Erect	Absent	Absent
2	TN-KN-1820	Kala Namac	Colorless	Dark	Absent	Present	Erect	Erect	Absent	Absent
3	TN-KK-1820	Karuppu Kavuni	Colorless	Dark	Present	Present	Erect	Erect	Absent	Present
4	TN-KAK-1920	Karung Kuruvai	Colorless	Dark	Absent	Present	Erect	Erect	Absent	Absent
5	TN-KS-1820	Garudan samba	Colorless	Dark	Absent	Present	Erect	Erect	Absent	Absent
6	TN-NA-1920	Nacora	Colorless	Dark	Absent	Present	Erect	Erect	Absent	Absent
7	TN-SAM-1920	Salem Samba	Colorless	Dark	Absent	Present	Erect	Erect	Present	Absent
8	13-PT-1820	13Bt	Colorless	Dark	Absent	Present	Erect	Erect	Absent	Present

(Sundaresan, 2005). As such, selection has traditionally been applied to seed size to understand their domestication.

Our second step of TRVs promotion in to test the growth initiation in Petri plate trails the prominent outcomes provoked us for further stages. In order to conserve the native qualities and nutritive values of TRVs the addition of growth hormones were completely excluded. Because many Traditional varieties were modified or even mutated by the addition of growth hormones. (Hans Kende, 2001).

Our results on the growth promotion of Kala Namac and Nacora showed a moderate growth equally comparable with Authur samba and 13 Bt which were recognized as 70% cultivated rice. This similarity growth pattern in Kala Namac and Nacora with Authur samba and 13 Bt may be due to the similarity in their genome or similarity in the growth morphogenesis or similarity in Physiological degree or it may be nearing the verge of evolution in to cultivated rice. Where as the Karung Kuraivai and Garudan Samba showed similarity in germination pattern. Even though these two varieties were collected from two different geographical locations its shares similarity in their genome or similarity in the growth morphogenesis or similarity in Physiological degree. We came to the nearing conclusion that the Karung Kuraivai and Garudan Samba still retains its genetic identity or it does not undergo any mutational changes during all its life time. Or these varieties are morphologically still wild or yet to be domesticated. This potent genetic alleles can be used for further research studies like transgenic crop protection. These interesting findings would open up many research avenues in future.

Several traits need to be studied to understand the domestication of wild rice to cultivated rice like Seed dormancy, Panicle architecture, Pericarp Colour, tiller number, Seeds Number and size, mating type and shattering (Megan Sweeney and Susan Mccouch, 2007). The truncated ligules, straight rachilla, short spikelets, and linearly organised tubercles on the surface of the lemma and palea allow species in this complex to be clearly distinguished from one another (Nihar Ranjan Chakraborty, 2013). So our present aim is also to study the above said phenomenon.

The varieties of Kala Namac, Karung Kuruvai, Nacora, Salem Senna and Garudan samba were said to be very long ones especially the Kala Namac, Karung Kuruvai and Salem Senna and Garudan samba has long tillers. The Garudan samba by the name itself represents Garudan means eagle bird wing like type of panicles were formed. In order to hold the large panicles the culm types were recorded as thick in nature. The Thickness of the culm helps in holding the crop during the strong wind and other physiological stress. The larger the tiller larger the fodder helps in feeding animals like cow. This robustness of culm increases the milk production in cow which were reported to be greater in immune inducer A2 milk. Previous reports stands that 10,000 yeas ago cows produced only A2 milk which contains A2 beat casein protein. But later in 8000 years ago a natural single-gene mutation resulted in the production of A1 beta casein (Gonca Pasin, 2017). This underlying reports clears the view of TRVs straw eating cows will yield A2 mil with beta-casein.

The height of panicles indicates that the Karuppu Kavuni (*Oryza rufipogon*), Karung Kuruvai (*Oryza rufipogon*) still maintains their height morphology even after so many long years. It denotes that these germplasm is pure lines not subjected or not underwent any mutational or morphological changes. And interestingly these panicle length is highly remarkable (33 to 40 cm) compared to other cultivable varieties because normally the average length of a cultivable and other TRVs rice panicles reported from Sri Lanka is 20 to 25cm (Ranawake et al., 2013). So our Tamil Nadu TRVs posses uniqueness in their panicle length.

Though the Garudan Samba (*Oryza sativa*) said to be age old TRVs and the Salem Senna (*Oryza sativa*) is considered to be a cultivated one the morphological similarities in panicle length leads to the conclusion that Garudan Samba (*Oryza sativa*) would have a domestication link with Salem Senna (*Oryza sativa*). Since the domestication of wild rice in to cultivable rice is a complex debate (Megan Sweeney and Susan Mccouch, 2007) our present observation may be a key point to research.

The major finding of the numbers of tiller formation studies indicates that the *Oryza rufipogon* reported to be wild variety showing 4 tillers and the *Oryza sativa* reported to be domesticated from Oryza rufipogon (Megan Sweeney and Susan Mccouch, 2007) has 5 tillers in Kala Namac (*Oryza sativa*), Nacora (*Oryza sativa*) and Salem Senna (*Oryza sativa*) and 3 tillers in Garudan Samba (*Oryza sativa*) and 13Bt (*Oryza sativa*). This can be concluded as state with previous worker Megan Sweeney and Susan Mccouch, 2007 the *Oryza sativa* contains two domesticated species one is *Oryza sativa* and *Oryza glaberrima* and five wild species *Oryza rufipogon* and *Oryza nivra* (considered to be an ecotype of *Oryza rufipogon*) *Oryza barthii*, *Oryza meridionalis* and *Oryza glumaepatula* is since the *Oryza sativa* it self is a domesticated species from *Oryza rufipogon* this statement stands with the formation of 5 tillers is the modification during the domestication process.

Though there were many factors influencing the domestication of wild varieties in to cultivated rice variety still some traits were not at all changed to 100%. There were still some jump between one or two numbers in spikelet formation. Mostly the TRVs were reported to be more in height as evidence in Authur Samba (*Oryza sativa*), Kala Namac (*Oryza sativa*) Karuppu Kavuni (Oryza rufipogon). and cultivable rice are dwarf in nature like Salem Senna (*Oryza sativa*) and 13Bt (*Oryza sativa)*. This results supports that the due to the less availability of water and nutrients the plants undergo modification in their height during their evolution (Rathore et al., 2015).

The increased weight of the grains was considered to be a key quality trait besides the more weight grains were difficult in cooking and eating. Due to this major reason many farmers and rice breeders transformed TRVs in to commercial varieties which are less in weight and easy cooking.

An comparative size analysis between size and weight of the seeds inferenced the following observations. Garudan Samba (*Oryza sativa*), Karung Kuruvai (*Oryza rufipogon*) seeds lower in size but greater in weight. This rare quality is absent in cultivated varieties. This data of analysing the length and breadth ratio is a key trait to understand the domestication of rice (Jiang and Liu, 2006). Later reports by Fuller in 2007 concluded that the grain size and shape cannot be considered for rice domestication because the grain size may vary according to plant responses to environmental factors. But we still assume that this aspect can be disproved. Based on the hypothesis that the size of the finger millet, Foxtail millet, Wheat, maize and many grains have not changed in their size even after many more years of cultivation in different agro-environmental conditions. If still variation occurs it may be in few millimetres that's too negligible.

The spikelet changes noted in our results substantiate with the finding of Zhao and Gu in 2011. Seed shattering is a key trait in domesticated crops which differentiates it's from its wild lines (Fuller in 2007). The process of domestication in respective of seeds shattering trait takes very long time to reach non-shattering stage and this remains consistent and inherited. This phenomenon was observed in our experiment studies the which substantiates with earlier reports by Briana Grossa and Zhijun Zhao in 2013.

During the evolution of TRVs in to cultivated rice there were many changes occurred in the TRVs genome (He et al., 2011; Molina et al., 2011; Huang et al., 2012; Yang et al., 2012; Civán et al., 2015,2016,2017; Huang and Han et al., 2015). Our study on the germplasm conservation and characteristic study of TRVs of Tamil Nadu, India may open many research views on trait studies, evolution aspects and rice domestication prospects.

As our object of studying grain size, colour and shape which has underlying several significant QTLs related to grain size have undergone molecular characterisation, and their regulatory functions in dictating grain size or weight have been investigated (Fan et al., 2006; Mao et al., 2010; Shomura, 2008; Weng, 2008; Duan, 2017; Liu, 2017; Wang et al., 2008, 2012, 2015, 2018; Duan et al., 2017; Si, 2016; Yu, et al., 2017; Zhou, et al., 2015). Our studies on grain shape, size, length and breadth awn and awn less also contribute more insights in rice domestication its evident from the above references and our research results.

Overall it has been observed that the traditional rice varieties have a moderate amount of dormancy in seedling stage as we overserved in our results . Similar outcomes of seed dormancy in traditional rice varieties were observed in Kuruvai kalangium, Kothamalli samba, Nootripathu, Karunkuruvai, Senthooram selection, Thengaipoo samba, Arasamba, Panamara samba selection, Sembarai, and Sarapillai samba selection (Raja et al., 2021; Hanumanthappa et al., 2015)

Additionally, during a varietal development program, these types can be employed as donors to transfer characteristics and stop pre-harvest sprouting or in situ seed germination in the field (Sivakumar et al., 2022). The benefits of an outcrossing progenitor with greater genetic diversity are highlighted by proponents of the theory that *O. rufipogon* is the ancestor of cultivated rice (Oka, 1988). More work should go into accurately identifying and differentiating these two ecologically distinct taxa in order to better characterise rice's wild ancestor (Zhu et al., 2007). Due to a shift in environment, *O. nivara* evolved from an ancestor that resembled *O. rufipogon* (Grillo et al., 2009).

India is rich in Traditional Knowledge and heritage which inherited to many generations. Rapid development on science and technology replaced many traditional traits form us. People were started to find new technologies and innovations from non existing ones. But seldom effects were done on the reviving the traditional traits into modern era.

Our present aim towards the revitalizing our mother land with traditional traits a novel approach. Especially the traditional rice varieties of Tamil Nadu rich in nutrition and good heritable genetic traits. Our pilot study on germplasm conservation of TRVs from all over Tamil Nadu is a great effort towards strengthening our rice germplasm. The characteristic analysis of TRVs compared with cultivated rice will emphasize the importance on TRVs. The yield analysis of TRVs recorded robust results.

CONCLUSION

The evolution and domestication of wild rice into cultivable rice still a complex problem. To answer this rice researchers all over the world working on rice domestication. Our present study helps in addressing the key factors for rice domestication and to study its evolution. The major conclusion of this work is TRVs of Tamil Nadu was first addressed in this paper and the cultivation of TRVs in pot trails are the pioneer study which can be adopted to farmers land in future.

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Amelioration of Zn with appropriate doses, methods and time of application under low land rice

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ABSTRACT

A field experiment was conducted at university's experimental farm, Gayeshpur, West Bengal during kharif (2018) to evaluate the right dose, method and time of application of Zn in low land rice (IET-4786). Experiment was undertaken with twelve treatments containing different doses of Zn application through soil and foliar at different growth stages of rice. The treatments are, viz T_1 =No Zn, T_2= Soil application of Zn @2.5 kg ha^{-1} through $ZnSO_4.7H_2O$ at the time of transplanting, T_3= Soil application of Zn @5.0 kg ha^{-1} through $ZnSO_4.7H_2O$ at the time of transplanting, T_4= Soil application of Zn @10.0 kg ha^{-1} through $ZnSO_4.7H_2O$ at the time of transplanting, T_5= Two foliar sprays of Zn as $ZnSO_4.7H_2O$ @0.5% (One spray at maximum tillering stage and one at pre-flowering stage), T_6 = Two foliar sprays of Zn as Zn-EDTA @0.5% (One spray at maximum tillering stage and one at pre-flowering stage), T_7= T_2 + Zn spray as $ZnSO_4.7H_2O$ @0.5% at the maximum tillering stage, T_8 = T_3 + Zn spray as $ZnSO_4.7H_2O$ @0.5% at the maximum tillering stage, T_9 = T_4+ Zn spray as $ZnSO_4.7H_2O$ @0.5% at the maximum tillering stage, T_{10} = T_2 + Zn spray as Zn-EDTA @0.5% at the maximum tillering stage, T_{11}= T_3+ Zn spray as Zn-EDTA @0.5% at the maximum tillering stage and T_{12}= T_4+ Zn spray as Zn-EDTA @0.5% at the maximum tillering stage. The statistical design of the field experiment was laid out in Randomized Complete Block Design (RCBD). The results of the study revealed that soil application of Zn @ 5 kg ha^{-1} before transplanting of rice along with foliar application of $ZnSO_4.7H_2O$ @ 0.5% at max. tillering stage was found highly suitable for sustaining the rice production and it might be recommended to the farmers not only for sustainable production but also for getting the quality produce. Results also showed that a very small amount of applied Zn (<1%) is utilized by rice and its utilization is high when it is applied at lower level. Zn translocation factor from root to shoot and shoot to grain, agronomic efficiency (AE) and apparent recovery efficiency (ARE) were also higher at lower level of Zn applications compared to the higher levels.

Key words: *Foliar application, Zn translocation, Zn utilization, agronomic efficiency (AE), apparent recovery efficiency (ARE) and sustainable production*

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food crop worldwide for nearly half of the world population, particularly for those living in the developing countries like India (Barua and Saikia, 2018). West Bengal is one of the most important rice growing states in India and it plays a vital role in the socio-economic and cultural life of the people of the state. Production and quality of rice now a day's hampered by some micronutrient deficiencies in West Bengal. Among the micronutrients, zinc (Zn) deficiency is widespread throughout the world particularly in lowland rice areas causing decreased crop yields and poor quality (low Zn) grains (Hazra et al., 2015). In West Bengal about 33% soils are deficient in zinc, where as in India it is about 39.9% of cultivated area (Shukla et al., 2016) specially in the state of Bihar, Gujarat and Madhya

Pradesh (Kumar et al, 2019). Dietary deficiencies of Zn and Fe are a serious global public health problem affecting over two billion people mostly pregnant women or children under five years old and causing a loss of thousands of lives every year (Myers et al., 2014 and Stanton et al., 2022). This is mainly because cereals that constitute about two-thirds of the energy intake of humans particularly in developing countries are low in Zn and Fe. Fortification of cereal grains with Zn offers a cost-effective solution of the problem of Zn deficiency (Saha et al., 2017). Increasing grain zinc (Zn) concentration of cereals for minimizing Zn malnutrition in two billion people represents an important global humanitarian challenge. Unfortunately, the potential of agriculture system to supply nutritious food to overcome "hidden hunger" (*e.g*., micronutrient malnutrition) for human health has received less attention than the common malnutrition and related issues (such as calorie intake, food demand, crop yield and environmental sustainability) In last two decades, developing micronutrient-enriched staple food crops to alleviate human micronutrient deficiencies (bio-fortification) has increasingly been considered in addition to traditional strategies such as supplementation, food fortification and dietary diversification. Bio fortified staple foods by either genetic (*e.g*., plant breeding) or agronomic strategies can increase micronutrient intake for the resource-poor people who consume them daily, and thus complement existing strategies. Agronomic practices, such as water management and zinc fertilizer, are established strategies for improving rice yield, which are also considered complementary strategies for Zn bio-fortification in rice. Both strategies are employed to improve soil Zn availability that could meet plant demand for Zn (Bhatt et al., 2020). Water management strategies, such as pre flooding and alternate wetting and drying, expose soils to both aerobic and anaerobic conditions that change soil pH, and Zn adsorption capacity of soil resulting in either more or less available soil Zn to plants. Pre-flooding reduces oxides of Fe to amorphous Fe oxide which has high scavenging action of Zn thereby reduced the mobility of Zn in soils (Mondal et al., 1992) (Bunquin et al., 2017).

In India, Zn fertilization is an accepted practice and applied in different rates in different crops/cropping systems (Kumar et al., 2023). Zinc fertilization at the initial growth stages of low land rice may become irrelevant owing to immobilization of applied Zn resulting in the unavailability of Zn to plants. Alternate wetting and drying, on the other hand, reverses the flooding effect where amorphous iron oxides again reverse back to its original position which in turn reduces its scaving action of Zn in soil with a greater mobility of Zn in soil (Mandal et al., 2000,). This suggests that zinc fertilizer should be combined with drainage to ensure aerobic conditions when the fertilizer is applied to soils. This further implies that, Zinc Fertilizer should be applied in times when plant's demand is high for Zn for physiological or reproductive development which in turn may help to achieve sustainable production as well as grain Zn accumulation can be expected (Tuiwong et al., 2022). Besides, Zn requirement at various growth stages of rice is also deficient. So, for sustaining the crop growth Zn should be applied at right stages with appropriate doses of Zn. But Zn-use efficiency of soil-applied Zn by plants hardly exceeds 2% of the applied amount. Improving the uptake of Zn in rice from the soil and enhancing their movement to edible parts of plant will provide benefits for plants, animal and human nutrition (Hamzah Saleem et al., 2022). However, this may still depend on genotypic response to agronomic management. But information in this regard is meagure. So, to alleviate Zn-deficiency of rice or its hidden hunger, zinc fertilization with right dose, right method and right time of application is very much essential to sustain crop production as well as to get the value-added quality product. With this view this research programme was undertaken to evaluate the appropriate stage, method and dose for Zn fertilization of low land rice in Zn deficient field.

MATERIALS AND METHODS

A field experiment was carried out in *kharif* season 2018 for growing low land rice variety at the central research farm Gayeshpur, Bidhan Chandra Krishi Viswavidyalaya, West Bengal. Soil of field experiment is new alluvial in native with order of Entisol and class (Dystrochrepts-Udifiuvents). Represented soil samples were taken in bulk from 0-15 cm depth before the growing season. The samples were air-dried, ground and passed through 2-mm sieve. Composite samples were taken and analyzed for physical and chemical characteristics namely, EC, pH, organic carbon and DTPA extractable micronutrients.

Field experiment was laid out in Randomized Complete Block Design (RCBD) with twelve (12) treatments taking plot size of 8x6 m^2 which is represented in Table 1.

All plots received 80 kg ha^{-1} Nitrogen (N) as urea, 40 kg ha^{-1} of Phosphorus (P) as SSP and 72 kg ha^{-1} Potassium (K) as KCL. Full dose of P and K was applied just before transplanting. Nitrogen fertilizers were applied in two split applications- the first split (40 kg ha^{-1}) was applied at the time of finial land preparation and second split (40 kg ha^{-1}) was given at the booting stage. Zn was applied as $ZnSO_4.7H_2O$ at the stage of transplanting and Zn EDTA was added only through foliar sprays. The foliar spray was done at two different stages namely, maximum tillering (40-45 days after transplanting) stage and panicle initiation (60-65 days after transplanting) stage. Transplanting of rice seedlings was done after 10 days of flooding followed by puddling. Two rice seedlings in a single bench were transplanted with plant-to-plant distance of 10 cm and row to row distance about 20 cm at 21 days after sowing. All other cultural practices were kept same and uniform for all treatments. Plant height and number of tillers were recorded during the growth stage. At crop harvesting, rice plants were manually harvested from 1 m^2 area in the centre of each plot. These plants were used to determine grain yield and its components after separating into straw and grains. Dry weight of straw was determined after oven-drying at 70°C to constant weight**.**

Table 1. Treatments of the field experiment for low land rice in *kharif* season.

Notation	Treatment
T_1	No Zn
T_2	Soil application of Zn @2.5 kg ha^{-1} through $ZnSO_4.7H_2O$ at the time of transplanting
T_3	Soil application of Zn @5.0 kg ha^{-1} through $ZnSO_4.7H_2O$ at the time of transplanting
T_4	Soil application of Zn @10.0 kg ha^{-1} through $ZnSO_4.7H_2O$ at the time of transplanting
T_5	Two foliar sprays of Zn as $ZnSO_4.7H_2O$ @0.5% (One spray at maximum tillering stage and one at pre-flowering stage
T_6	Two foliar sprays of Zn as Zn-EDTA @0.5% (One spray at maximum tillering stage and one at pre-flowering stage)
T_7	T_2 + Zn spray as $ZnSO_4.7H_2O$ @0.5% at the maximum tillering stage
T_8	T_3 + Zn spray as $ZnSO_4.7H_2O$ @0.5% at the maximum tillering stage
T_9	T_4 + Zn spray as $ZnSO_4.7H_2O$ @0.5% at the maximum tillering stage
T_{10}	T_2 + Zn spray as Zn-EDTA @0.5% at the maximum tillering stage
T_{11}	T_3 + Zn spray as Zn-EDTA @0.5% at the maximum tillering stage
T_{12}	T_4 + Zn spray as Zn-EDTA @0.5% at the maximum tillering stage

Soil samples were collected randomly from five different places from each plot and then made a composite sample. After harvesting grain yield and straw yield were measured and harvest index and BCR were also calculated.

pH of the experimental soil was determined by glass electrode method in 1: 2.5: soil: water suspension using a Systronics pH meter (Jackson 1973). EC was estimated in 1: 2.5: soil: water suspension with the help of Wheatstone Conductivity Bridge as described by Jackson (1973). Organic carbon (OC) was determined by Walkley and Black (1934) potassium-di-chromate wet digestion method DTPA extractable soil zinc (Zn), Copper (Cu) Iron (Fe) and Manganese (Mn) were extracted with DTPA extractant (Lindsay and Norvell 1978). After two hours of horizontal shaking solution was filtrated using Whatman No. 42 filter paper. The filtrate was used for estimating zinc, copper, iron and manganese by Atomic Absorption Spectrometer (Perkin Elmer PinAAcle 90^0F).

Ground and dried plant materials, 0.5 g, were weighed into 30 mL porcelain crucibles. The crucibles were placed in a muffle furnace at room temperature. The furnace temperature was set at 550°C, and the temperature was gradually raised. The plant samples were ashed for 4 hours and after that they were left overnight for cooling. Cool crucibles were taken out and the ash was moistened with a few drops of water, followed by 10 ml of 6 N hydrochloric acid (HCl). After cooling the flask, 20 ml of double distilled water was added. The volume was made up to 50ml with double distilled water and filtered the solution using Whatman No. 42 filter paper. The filtrate was used for estimating zinc by AAS (Perkin Elmer PinAAcle 90^0F).

Other parameters like AE, ARE, Utilization of applied Zn, Translocation factor and B:C ratio were estimated.

AE *i.e.*, Agronomic efficiency is calculated by following equation,

$$AE = \frac{(Y - Y0)}{F}$$

where Y = yield of harvested portion of crop with nutrient applied; Y0 = yield with not nutrient applied; F = amount of nutrient applied.

ARE % *i.e.*, Apparent recovery Efficiency Percentage is calculated by following equation,

$$ARE\% = \frac{(U - U0)}{F} * 100$$

Where U = total nutrient uptake in aboveground crop biomass with nutrient applied; U0 = nutrient uptake in aboveground crop biomass with no nutrient applied, F = amount of nutrient applied.

Utilization of applied Zn is calculated by following equation,

$$\text{Utilization \% of applied Zn} = \frac{(\text{Zinc uptake in Zn treated plot - Zinc uptake in control plot})}{\text{Amount of applied Zn}} * 100$$

TF *i.e.*, Translocation factor is calculated by following equation,

$$TF_{\text{Root to Shoot}} = \frac{(\text{Zn Conc. in Shoot})}{\text{Zn Conc. in Root}}$$

$$TF_{\text{Shoot to grain}} = \frac{(\text{Zn Conc. in Grain})}{(\text{Zn Conc. in Shoot})}$$

B:C ratio *i.e.*, Benefit: Cost ratio is calculated by following equation,

$$\text{B:C ratio} = \frac{\text{Monitory return from the cultivated plot}}{\text{Cost of Cultivation for the plot}}$$

Initial soil properties of the experimental field

Some important chemical properties of the initial soils collected from the experimental field were estimated and result showed that pH of the soil is neutral in reaction (6.93) and EC is below 4 dSm-1 (0.136) which means it is not salt affected. Organic carbon status of the experimental soil is medium (0.54%) and there is no deficiency of available Zn (DTPA Zn: 2.15 ppm) as well as other available cationic micronutrient contents viz; Cu (DTPA Cu: 6.124 ppm), Fe (DTPA Fe: 105.7 ppm) and Mn (DTPA Mn: 24.80 ppm).

RESULTS AND DISCUSSION

Zn uptake at various growth stages of rice

The experiment was conducted with a view to evaluate the most appropriate stage or peak demanding stage for Zn in rice growth and development. For this purpose, soil application of Zn as $ZnSo_4$ was done before transplanting the rice seedling. This soil application of Zn was also combined with foliar spray of Zn at the high nutrient demanding stage *i.e.*, at the maximum tillering stage. Besides this, Zinc was also applied through sole foliar sprays at the two most important growth stages of rice viz. maximum tillering stage (40-45 DAT) and panicle initiation (60-65 DAT) stage. The yields of root, shoot, grain, their Zn content as well as uptake were estimated at various growth stages of rice which are depicted below.

The yields of the root, shoot, and their Zn contents and uptakes, at maximum tillering stage under various treatments are presented in Table 3.

Experimental results (Table 2) showed that among the twelve treatments, T_{12} (Zn @ 10 kg ha^{-1} $ZnSO_4$ + 0.5% foliar spray of Zn-EDTA at MTS) recovered the highest Zn content in both root (63.9 ppm) and shoot (38.05 ppm) followed by T_{11} and T_9 in case of root, however, for the shoot this was just reverse *i.e.*, T_9 followed by T_{11}.

Table 2. Effect of Zn application on the root and shoot yields of rice and their Zn contents as well as uptakes at maximum tillering stage.

Treatments (Maximum tillering)	Root yield (q ha^{-1})	Zn in root (mg kg^{-1})	Zn Uptake by root (g ha^{-1})	Shoot yield (q ha^{-1})	Zn in shoot (mg kg^{-1})	Zn Uptake by shoot (g ha^{-1})	Zn Uptake by plant (g ha^{-1})
T_1 = No Zn	4.93^g	32.45^i	16.00^h	19.00^g	21.40^g	40.65^h	56.65^i
T_2 = Zn @2.5 kg ha^{-1} $ZnSO_4$.	5.62^f	37.60^h	21.13^f	20.46^f	27.55^f	56.35^g	77.49^h
T_3 = Zn @5 kg ha^{-1} $ZnSO_4$.	7.68^c	52.30^e	40.16^e	27.96^c	28.05^f	78.39^d	118.55^e
T_4 = Zn @10 kg ha^{-1} $ZnSO_4$.	8.14^b	56.76^d	46.20^c	29.63^b	32.05^d	94.96^b	141.17^c
T_5 = 2 foliar spray of $ZnSO_4$.	4.45^h	42.80^f	19.03^g	19.58^{fg}	27.20^f	53.25^g	72.28^h
T_6 = 2 foliar spray of ZnEDTA	4.55^h	40.29^g	18.32^g	20.02^{fg}	26.95^f	53.94^g	72.26^h
T_7 = T_2 + foliar spray of $ZnSO_4$	6.01^e	44.00^f	26.44^e	21.88^e	29.30^e	64.09^f	90.53^g
T_8 = T_3 + foliar spray of $ZnSO_4$	7.06^d	58.50^c	41.30^e	25.70^d	34.26^c	88.01^d	129.31^d
T_9 = T_4 + foliar spray of $ZnSO_4$	8.58^a	59.65^c	51.18^b	31.23^a	36.72^b	114.64^a	165.82^b
T_{10} = T_2 + foliar spray of ZnEDTA	6.05^e	41.30g	24.99^e	22.02^e	32.30^d	71.21^e	96.20^f
T_{11} = T_3 + foliar spray of ZnEDTA	7.26^d	62.20^b	45.16^d	26.43^d	34.77^c	91.89^{bc}	137.05^c
T_{12} = T_4 + foliar spray of ZnEDTA	8.47^a	63.90^a	54.13^a	30.83^{ab}	38.05^a	117.31^a	171.43^a
LSD/CD	0.55	2.70	3.08	1.89	1.72	7.89	9.18
Sem	0.15	0.74	0.85	0.52	0.47	2.17	2.52
CV %	2.80	1.85	3.08	2.60	1.88	3.45	2.79

Zn uptake by root and shoot was highest in the treatment T_{12} with values of 54.12 g ha^{-1} and 171.4 g ha^{-1} respectively followed by treatments T_9 and T_{11}. Shoot and root dry matter yields were also higher in T9 treatment (Zn @10 kg ha^{-1} + 0.5% foliar spray of $ZnSO_4$ at MTS) followed by T_{12} than the other treatments. The results e revealed that soil application of Zn @ 10 kg ha^{-1} along with foliar spray of $ZnSO_4$ @ 0.5% at the maximum tillering stage (MTS) enhanced both the Zn contents in root and shoot, as well as their dry matter yields in comparison to sole application either through soil or foliar sprays. Maximum tillering stage (MTS) is the very active growth stage of rice where nutrient enrichment is generally high and from the results it was found that soil application of Zn @10 kg ha^{-1} at the time of transplanting along with foliar spraying of Zn at MTS would be effective to meet this requirement.

Reports have highlighted (Karak et al., 2005) regarding application of $ZnSO_4$ and Zn-EDTA fertilizer for alleviation of Zn deficiency in rice field but information regarding timing of application growth stages of rice as well as mode of application was not highlighted. This research study takes these factors into account and the results revealed that both soil application as well as foliar application of Zn at the maximum tillering stage and booting stage are highly effectivenot only to increase the yield but enrichment of grain Zn also. Since Zn requirement of rice at the maximum tillering stage and flowering stage is supposed to be very high, this is also supported by Tuiwong et al. (2022).

The panicle initiation stage (PIS) is the most critical growth stage (60-65 DAS) of rice where nutrient elements particularly Zn plays a critical role for the growth and development of rice. The influence of the treatments on the dry matter yields and Zn contents in the PIS has been presented in Table 4.

From the results (Table 3) it was found that both root and shoot dry matter yield of rice at this stage are comparatively higher than the MTS, but regarding Zn content the reverse results were observed where Zn content in both root and shoot are lower than at the MTS, which might be related to the dilution effect resulting from the higher dry matter yield in this stage compared to MTS. In this case T_{12} treatment *i.e.*, Zn @10 kg ha^{-1} $ZnSO_4$ + foliar spray of Zn-EDTA. superior to the others superior to the others in terms of dry matter yields, Zn content and Zn uptake of both root and shoot followed by T_9 *i.e.*, Zn @10 kg ha^{-1} $ZnSO_4$ + foliar spray of $ZnSO_4$ treatment similar to the MTS growth stage. The results also revealed that T_{12} treatment is superior to T_9 treatment in respect of dry matter yield, Zn uptake by root and shoot but their magnitudes are quite similar. From the results it can be concluded that soil application of Zn @10 kg ha^{-1} along with foliar spray of Zn @ 0.5% would be very effective

Table 3. Effect of Zn on the root and shoot yields of rice, their Zn contents as well as uptakes at the Panicle initiation stage.

Treatments	Root yield (q ha^{-1})	Zn in root (mg kg^{-1})	Uptake of root (g ha^{-1})	Shoot yield (q ha^{-1})	Zn in shoot (mg kg^{-1})	Uptake of shoot (g ha^{-1})	Uptake of plant (g ha^{-1})
T_1 =No Zn	7.05^{g}	27.69^{l}	19.52^{h}	32.90^{g}	17.83^{h}	58.67^{h}	78.19^{j}
T_2 = Zn @2.5 kg ha^{-1} $ZnSO_4$.	8.37^{f}	32.84^{k}	27.48^{g}	39.06^{f}	22.96^{fg}	89.68^{g}	117.12^{i}
T_3 = Zn @5 kg ha^{-1} $ZnSO_4$.	$9.24d^{e}$	47.54^{f}	43.93^{e}	43.12^{de}	23.38^{f}	100.79^{e}	144.72^{g}
T_4 = Zn @10 kg ha^{-1} $ZnSO_4$.	9.96^{abc}	52.00^{e}	51.79^{c}	46.48^{abc}	26.71^{d}	124.17^{d}	175.97^{d}
T_5 = 2 foliar spray of $ZnSO_4$.	8.88^{e}	38.04^{h}	33.76^{f}	41.44^{ef}	22.67^{fg}	93.91^{efg}	127.68^{h}
T_6 = 2 foliar spray of ZnEDTA	8.40^{f}	35.53^{j}	29.84^{g}	39.20^{f}	22.46^{g}	88.02^{g}	117.87^{i}
T_7 = T_2 + foliar spray of $ZnSO_4$	8.43^{f}	39.24^{g}	33.08^{f}	39.34^{f}	24.42^{f}	96.07^{ef}	129.15^{h}
T_8 = T_3 + foliar spray of $ZnSO_4$	8.85^{e}	53.74^{d}	47.55^{d}	41.30^{ef}	28.55^{c}	117.90^{d}	165.45^{e}
T_9 = T_4 + foliar spray of $ZnSO_4$	10.14^{ab}	54.89^{c}	55.66^{b}	47.32^{ab}	30.60^{b}	144.80^{b}	200.46^{b}
T_{10} = T_2 + foliar spray of ZnEDTA	9.57^{cd}	36.54^{i}	34.97^{f}	44.66^{cd}	26.92^{d}	120.11^{d}	155.08^{f}
T_{11} = T_3 + foliar spray of ZnEDTA	9.78^{bc}	57.44^{b}	56.19^{b}	45.64^{bc}	28.98^{c}	132.24^{c}	188.43^{c}
T_{12} = T_4 + foliar spray of ZnEDTA	10.35^{a}	59.14^{a}	61.21^{a}	48.30^{a}	31.71^{a}	153.16^{a}	214.36^{a}
CD	0.64	1.22	3.47	2.13	1.30	8.41	9.17
Sem	0.18	0.34	0.95	0.59	0.36	2.31	2.60
CV %	2.38	0.92	2.83	1.69	1.71	2.54	2.11

than sole application of Zn either through soil or foliar to enhance the crop growth as well as Zn uptake similar to the results found in MTS growth stage. Foliar spray of Zn either through $ZnSO_4$ or Zn-EDTA along with soil application was done with an assumption that Zn requirement of rice will be in high demand at the panicle initiation stage since it has a function of protein synthesis through the activities of various enzymes (Perumal et al., 2019).

The yields of the root, shoot, grain and their Zn contents and uptakes, at harvesting stage under different treatments are presented in Table 4.

The results showed that similar to the earlier two growth stages of rice, the treatment T_{12} recorded the highest dry matter yields of root, shoot and grain followed by T_{11} for root and shoot. However, for grain yield T_{12} and T_8 *i.e.*, Zn @ 5 kg ha^{-1} along with foliar spray of $ZnSO_4$ @0.5% at MTS were at par. Results also revealed that treatments showed differential responses regarding Zn contents in root, shoot and grain. For root Zn content T_{12} *i.e.*, 10 kg ha^{-1} with Zn-EDTA spray at MTS was the best but for shoot and

Table 4. Effect of Zn on the dry matter yields of root, shoot and grain and their Zn contents as well as uptake at the Harvesting stage.

Treatments	Root yield (q ha^{-1})	Zn in root (mg kg^{-1})	Zn Uptake by root (g ha^{-1})	Shoot yield (q ha^{-1})	Zn in shoot (mg kg^{-1})	Zn Uptake by shoot (g ha^{-1})	Grain yield (q ha^{-1})	Zn in grain (mg kg^{-1})	Zn uptake by grain (g ha^{-1})	Total Zn uptake by plant (g ha^{-1})
T_1 = No Zn	9.29^{f}	33.50^{j}	31.01^{i}	58.33^{e}	23.51^{g}	137.12^{d}	50.92^{h}	22.70^{d}	115.62^{e}	283.75^{g}
T_2 = Zn @2.5 kg ha^{-1} $ZnSO_4$.	9.67^{de}	41.63^{gh}	40.23^{g}	60.89^{cd}	25.15^{ef}	153.14^{c}	54.17^{h}	24.70^{abcd}	133.85^{d}	327.22^{f}
T_3 = Zn @5 kg ha^{-1} $ZnSO_4$.	10.05^{bc}	42.44^{fg}	42.65^{f}	63.32^{b}	26.45^{de}	167.48^{b}	55.76^{g}	25.07^{abc}	139.83^{bcd}	349.97^{de}
T_4 = Zn @10 kg ha^{-1} $ZnSO_4$.	10.11^{b}	42.86^{f}	43.33^{ef}	63.68^{b}	28.45^{a}	181.14^{a}	57.39^{ef}	26.55^{ab}	152.36^{ab}	376.83^{c}
T_5 = 2 foliar spray of $ZnSO_4$.	9.76^{cde}	41.15^{h}	40.14^{g}	61.46^{bcd}	25.35^{ef}	155.79^{c}	58.03^{cd}	24.62^{bcd}	142.88^{bcd}	338.82^{ef}
T_6 = 2 foliar spray of ZnEDTA	9.59^{e}	39.25^{i}	37.64^{h}	60.42^{d}	25.90^{ef}	156.47^{c}	56.94^{f}	24.12^{cd}	137.33^{cd}	331.45^{f}
T_7 = T_2 + foliar spray of $ZnSO_4$	9.66^{de}	46.52^{e}	44.94^{e}	60.86^{cd}	27.60^{bc}	167.97^{b}	58.08^{cd}	25.57^{abc}	148.50^{abc}	361.40^{d}
T_8 = T_3 + foliar spray of ZnSO4	9.97^{bcd}	51.34^{c}	51.15^{c}	62.78^{bc}	27.26^{bc}	171.13^{b}	59.57^{a}	26.55^{ab}	158.15^{a}	380.43^{c}
T_9 = T_4 + foliar spray of ZnSO4	10.47^{a}	53.26^{b}	55.77^{b}	65.97^{a}	27.85^{ab}	183.71^{a}	58.48^{bc}	27.03a	158.06^{a}	397.54^{ab}
T_{10} = T_2 + foliar spray of ZnEDTA	10.04^{bc}	48.39^{d}	48.59^{d}	63.25^{b}	26.35^{de}	166.65^{b}	57.00^{f}	24.60^{bcd}	140.22^{bcd}	355.47^{d}
T_{11} = T_3 + foliar spray of ZnEDTA	10.49^{a}	52.35^{b}	54.94^{b}	66.11^{a}	25.90^{ef}	171.26^{b}	59.19^{ab}	26.89^{ab}	159.16^{a}	385.37^{bc}
T_{12} = T_4 + foliar spray of ZnEDTA	10.63^{a}	54.75^{a}	58.20^{a}	66.96^{a}	27.05^{cd}	181.12^{a}	60.13^{a}	26.97^{ab}	162.17^{a}	401.49^{a}
CD Value (P=0.05)	0.45	1.79	2.89	2.16	1.00	10.58	1.61	3.43	20.57	20.97
SEm	0.12	0.49	0.79	0.59	0.27	2.91	0.44	0.94	5.65	5.77
CV (%)	1.53	1.32	2.13	1.16	1.27	2.15	0.95	4.54	4.75	1.98

grain Zn contents treatment, *i.e.*, T_9 (Zn @ 10 kg ha^{-1} with spray @ 0.5% as $ZnSO_4$ at MTS) recorded the highest value.

Results, therefore, revealed that soil application of Zn @ 10 kg ha^{-1} at the time of transplanting along with Zn spray at 0.5% either through Zn-EDTA or $ZnSO_4$ was effective to increase the dry matter yields of root and shoot though soil was not deficient in Zn (2.15 ppm DTPA Zn) but in case of grain yield it was more or less at par with the addition of Zn @ 5 kg ha-1along with the spraying @ 0.5% $ZnSO_4$ at MTS. Zn contents at various plant parts also showed more or less the similar trend of results. The influences of various treatments on grain yield and Zn contents of the various plant parts are also depicted through graphical representation as Fig. 1 and Fig. 2 respectively, which are given below:

From graphical representation, it is clearly observed that the percentage yield increase of rice grain over control upon the treatments T_{12} *i.e.*, Zn @ 10 kg ha^{-1} ZnSO4 + 0.5% foliar spray of Zn-EDTA at MTS (18.09%) and T8 *i.e.*, Zn @ 5 kg ha^{-1} $ZnSO_4$ + 0.5% foliar spray of $ZnSO_4$ (16.99%) are more or less similar corroborating our earlier views. Biswas et al. (2018) showed yield increase in rice after zinc application was 2 to 18 % over control which is more or less similar to the present study. Khan et al. (2007) showed, basal application of zinc @ 5kg and 10 kg ha^{-1} increased rice productivity by 38 % and 50 % over control which is quite higher than the present study.

Results also showed that basal application of Zn either @ 5 kg ha^{-1} or 10 kg ha^{-1} caused an increase in grain yield to the tune of 9.51 to 12.71 % over control. However, when this basal application @ 5 kg ha^{-1} or 10 kg ha^{-1} was combined with foliar spray of Zn either through $ZnSO_4.7H_2O$ @ 0.5% or Zn-EDTA @ 0.5% caused an increased grain yield to the tune of 16.24 to 16.99 and 14.85 to 18.09% over control respectively.

Graphical representation of Zn contents in various plant parts *viz*., root, shoot and grain (Fig. 2), showed that Zn contents in rice shoot and grain were more or less similar in T_{12}, T_{11}, T_9 and T_8 treatments However, in the case of the root, it was highest in T_{12} (63.43%) followed by T9 (58.99%) though soil was sufficient in available Zn as per the DTPA soil test (Lindsey & Norwell, 1978) which might be related to the hidden hunger for Zn of rice. Since rice is Zn loving crop and Zn plays a very critical role for its growth and development. Cereals specially Rice is more prone to zinc deficiency in compared to legumes and other crops which leads sustainable yield loss as well as a loss in nutritional quality (Rehman et al., 2012).

In this study, various Zn fertilizers were used with different times of application to study the effect of fertilizer on growth and yield of rice. It is assumed that Zn-EDTA is superior to ZnSO4 and several reports support this (Karak et al., 2005; Dhaliwal et al., 2022). However, the results of this study revealed that the effectiveness of both is more or less similar but B:C

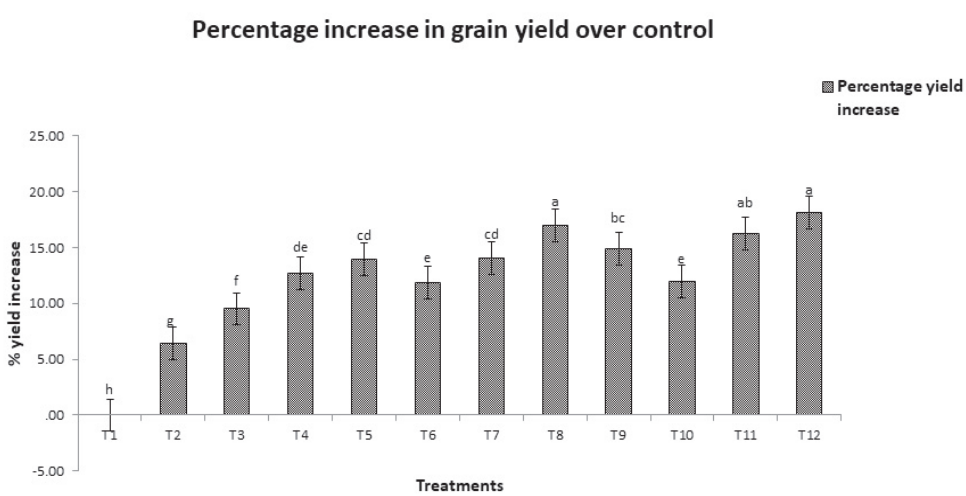


Fig. 1. Percentage increase in grain yield of rice over control upon Zn fertilizer applications.

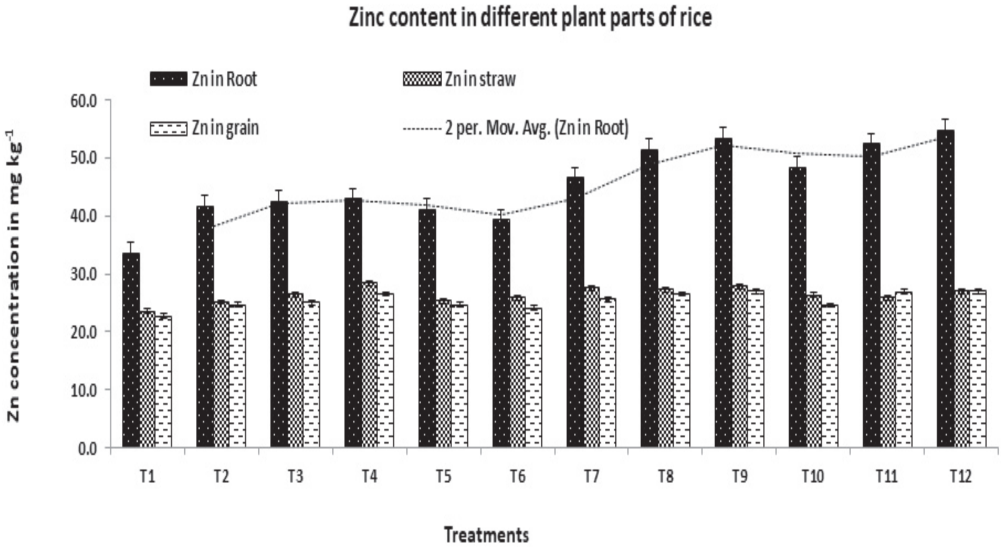


Fig. 2. Zinc concentration in rice root, shoot and grain.

ratio (benefit cost ratio) of Zn-EDTA (1.38) is very low as compared to $ZnSO_4$ (1.51) which making it less cost-effective to the farmers.

Zn fertilizer is generally applied through soil application as well as foliar application. Soil application is typically done at the time of planting. As a result, its translocation to the plant is restricted by various factors and use efficiency is not more than 2% (Khampuang et al., 2020;). In contrast, foliar application is done according to the plant's demand at flowering stage (Boonchuay et al., 2013; Tuiwong et al., 2022;) which have been very effective to sustain the yield and quality of the crop, Zn enrichment of the grain was found to be higher with foliar application than with soil application

Table 5. Percentage utilization of applied Zinc by rice.

Treatments	Applied Zn in kg ha^{-1}	Zn uptake by root (kg ha^{-1})	% utilization by roots	Zn uptake by straw (kg ha^{-1})	% utilization by straw	Zn uptake by grain (kg ha^{-1})	% utilization by grain	Total utilization % by rice plant
T_1 = No Zn	0.0	0.031^{i}	0.000^{g}	0.137^{d}	0.000^{d}	0.116^{e}	0.000^{c}	0.000^{d}
T_2 = Zn @2.5 kg ha^{-1} $ZnSO_4$.	2.5	0.04^{g}	0.369^{a}	0.153^{c}	0.646^{a}	0.134^{d}	0.729^{a}	1.732^{a}
T_3 = Zn @5 kg ha^{-1} $ZnSO_4$.	5.0	0.043^{f}	0.233^{c}	0.167^{b}	0.610^{a}	0.140^{bcd}	0.484^{ab}	1.327^{b}
T_4 = Zn @10 kg ha^{-1} $ZnSO_4$.	10.0	0.043^{ef}	0.123^{f}	0.181^{a}	0.441^{bc}	0.152^{ab}	0.367^{b}	0.932^{c}
T_5 = 2 foliar spray of $ZnSO_4$.	6.0	0.04^{g}	0.152^{e}	0.156^{c}	0.313^{c}	0.143^{bcd}	0.454^{b}	0.920^{c}
T_6 = 2 foliar spray of ZnEDTA	6.0	0.038^{h}	0.110^{f}	0.156^{c}	0.325^{c}	0.137^{bcd}	0.362^{b}	0.797^{c}
T_7 = T_2 + foliar spray of $ZnSO_4$	5.5	0.045^{e}	0.253^{c}	0.168^{b}	0.563^{a}	0.148^{abc}	0.598^{ab}	1.414^{b}
T_8 = T_3 + foliar spray of $ZnSO_4$	8.0	0.051^{c}	0.252^{c}	0.171^{b}	0.427^{bc}	0.158^{a}	0.532^{ab}	1.210^{b}
T_9 = T_4 + foliar spray of $ZnSO_4$	13.0	0.056^{b}	0.190^{d}	0.184^{a}	0.359^{c}	0.158^{a}	0.326^{b}	0.876^{c}
T_{10} = T_2 + foliar spray of ZnEDTA	5.5	0.049^{d}	0.320^{b}	0.167^{b}	0.539^{ab}	0.140^{bcd}	0.448^{b}	1.306^{b}
T_{11} = T_3 + foliar spray of ZnEDTA	8.0	0.055^{b}	0.299^{b}	0.171^{b}	0.428^{bc}	0.159^{a}	0.544^{ab}	1.272^{b}
T_{12} = T_4 + foliar spray of ZnEDTA	13.0	0.058^{a}	0.209^{d}	0.181^{a}	0.339^{d}	0.162^{a}	0.358^{b}	0.907^{c}
CD Value (P=0.05)	NA	0.003	0.039	0.011	0.192	0.021	0.405	0.362
SEm	NA	0.001	0.011	0.003	0.053	0.006	0.111	0.100
CV (%)	NA	2.130	6.340	2.150	15.570	4.750	21.480	11.600

*NA- Not available

(Hazra et al., 2015).

Agronomic efficiency and apparent recovery efficiency

Agronomic efficiency (AE) is calculated in units of yield increase per unit of nutrient applied. It closely reflects the direct production impact of an applied fertilizer and relates directly to economic return.

Agronomic efficiency of different doses of Zn fertilizer application was measured to determine how much productivity improvement was achieved through Zn fertilization. Results showed that T_7 *i.e.*, Zn @ 2.5 kg ha^{-1} with foliar spray of $ZnSO_4$ @ 0.5% at MTS (130.18 kg kg^{-1}) has given the highest value followed by T_2 *i.e.*, Zn @ 2.5 kg ha^{-1} as basal application (130 kg kg^{-1}). Zulfiqar et al. (2020) found that values of agronomic efficiency for soil application of zinc ranges between 75 to 108 kg ha^{-1} which is more or less similar to the results of the present study. Apparent recovery efficiency was also measured to know how much amount of applied Zn taken by the plant and results showed that its highest magnitude was recovered by the treatment T_2 (1.37 %) *i.e.*, Zn addition @ 2.5 kg ha^{-1} before transplanting of rice seedlings followed by T_7 (1.16%) *i.e.*, soil application of Zn before transplanting @2.5 kg ha^{-1} through Zinc Sulphate + foliar application of Zinc Sulphate @ 0.5%, one at maximum tillering stage and T_3 (1.09%) basal application of Zinc sulphate @ 5 kg ha^{-1}. Farooq et al. (2018) also reported an apparent recovery efficiency as high as 1.33 % apparent recovery efficiency using soil and foliar application of zinc which is more or less similar to the present study.

Through graphical representation (Fig. 3) it was also found that treatments like T_2, followed by T_7 and T_3, were superior to the other treatments regarding apparent recovery efficiency of Zn. This might be related to the Zn status of soil which is not deficient in available Zn. Therefore, higher recovery percentage was recorded at lower doses of Zn than the higher doses.

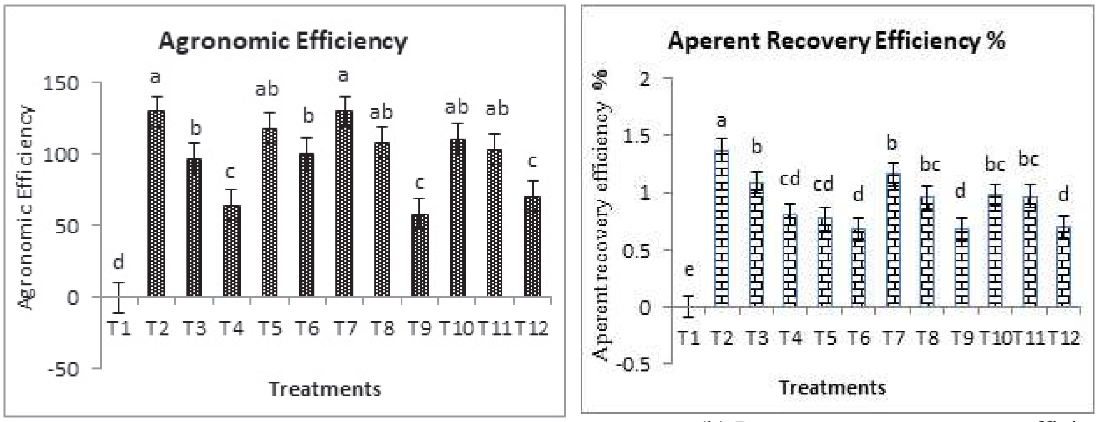
Percentage utilization of applied Zinc by rice

When Zn is applied to the soil through various fertilizers is mostly retained by the soil constituents. Only a very small portion of it is utilized by the plant which also varies under the influence of various forms, doses and methods of application. Therefore, its estimation under the influence of various treatments will help immensely in determining the right form, dose and method of application for Zn enrichment of rice.

Results (Table 5) showed that similar to AE and ARE, percentage utilization of applied Zn was also higher at lower level of Zn application than the higher levels. In particular, T_2 treatment i.e., soil application of Zn @ 2.5 kg ha^{-1} recovered its highest magnitude in root, shoot and grain. Regarding Zn utilization of rice generally the economic parts *viz*., shoot and grain are considered. In case of shoot, treatment T_2 was followed

Table 6. Amount of Zinc required to produce unit weight of rice grain (g t^{-1}).

Treatments	Grain yield (q ha^{-1})	Zn in grain (mg kg^{-1})	Zn uptake by grain (kg ha^{-1})	Zn require to yield per unit wt of grain (g t^{-1})
T_1 = No Zn	50.92^{h}	22.70^{d}	0.12	22.70^{d}
T_2 = Zn @2.5 kg ha^{-1} $ZnSO_4$.	54.17^{h}	24.70^{abcd}	0.13	24.70^{abcd}
T_3 = Zn @5 kg ha^{-1} $ZnSO_4$.	55.76^{g}	25.07^{abc}	0.14	25.07^{abc}
T_4 = Zn @10 kg ha^{-1} $ZnSO_4$.	57.39^{ef}	26.55^{ab}	0.15	26.55^{ab}
T_5 = 2 foliar spray of $ZnSO_4$.	58.03^{cd}	24.62^{bcd}	0.14	24.62^{bcd}
T_6 = 2 foliar spray of ZnEDTA	56.94^{f}	24.12^{cd}	0.14	24.12^{cd}
T_7 = T_2 + foliar spray of $ZnSO_4$	58.08^{cd}	25.57^{abc}	0.15	25.57^{abc}
T_8 = T_3 + foliar spray of $ZnSO_4$	59.57^{a}	26.55^{ab}	0.16	26.5^{ab}
T_9 = T_4 + foliar spray of $ZnSO_4$	58.48^{bc}	27.03^{a}	0.16	27.03^{a}
T_{10} = T_2 + foliar spray of ZnEDTA	57.00^{f}	24.60^{bcd}	0.14	24.60^{bcd}
T_{11} = T_3 + foliar spray of ZnEDTA	59.19^{ab}	26.89^{ab}	0.16	26.89^{ab}
T_{12} = T_4 + foliar spray of ZnEDTA	60.13^{a}	26.97^{ab}	0.16	26.97^{ab}
CD Value (P=0.05)	1.61	3.43	0.021	3.43
SEm	0.44	0.94	0.006	0.94
CV (%)	0.95	4.54	4.750	4.54



(a) Agronomic efficiency of zinc in rice. (b) Percent apparent recovery efficiency.

Fig. 3. Graphical representation of Agronomic Efficiency and Apparent Use Efficiency of added Zinc

by T_3 *i.e.*, soil Zn application @ 5 kg ha^{-1} but for grain, treatment T_2 was followed by T_7 *i.e.*, soil Zn application @ 2.5 kg ha^{-1} with foliar spray of $ZnSO_4$ @ 0.5% at MTS. Total utilization of applied Zn also recorded its highest magnitude upon T_2 (1.732%) followed by T_7 (1.41%). Therefore, the results revealed that a very little amount of applied Zn (less than 2%) is utilized by rice and lower levels of soil Zn application @ 2.5 kg ha^{-1} along with foliar spray of $ZnSO_4$ @ 0.5% at MTS is very effective to increase its magnitude. This finding is corroborated by the findings of Mondal et al. (2000), where they reported percent utilization of applied Zn in rice varied from 0.61% to 2.29%.

Zn translocation

Translocation factor is also a very important physiological parameter that indicates the movement of nutrient elements within the plant system. Rice roots absorb nutrient Zn from soil, and it is translocated to the above ground plant parts, *viz*., from root to shoot and shoot to grain which in turn helps to increase the productivity as well as Zn content. Zn translocation factor in various plant parts was estimated under various treatments and the results (Table 6, Fig. 4) showed that translocation of Zn from root to shoot recorded its highest magnitude (0.7) in T_1 (native soil Zn), followed by T_4 (soil application of Zn @ 10 kg ha^{-1} before transplanting). However translocation of Zn from shoot to grain showed the reverse results where it recorded the highest magnitude (1.0) in T_{11} *i.e*., soil Zn @ 5 kg ha^{-1} with foliar spray of Zn-EDTA at MTS followed by T_2 (soil application of Zn @ 2.5 kg ha^{-1}) and T5 (two foliar sprays of Zn through ZnSO? @ 0.5% at MTS and PI).

Results revealed that the translocation of Zn from root to shoot was effective at lower doses of soil Zn application whereas in case of shoot to grain it was more effective when Zn was applied before transplanting at 5 kg ha^{-1} with foliar spray of Zn-EDTA @ 0.5% at MTS or two foliar sprays of Zn as "$ZnSO_4 \cdot 7H_2O$ @ 0.5% one at MTS and another at PI. This further strengthens the earlier views that lower levels of Zn are effective to increase the AE, ARE, Zn utilization and the Zn translocation factor which might be due to the medium status of soil Zn. Results also showed that translocation of Zn from shoot to grain was higher than root to shoot which might be due to higher affinity of grain Zn accumulation in rice. Wu et al. (2010) also reported that the accumulation of Zn from flag leaf to grain is much higher when Zn is applied at the booting stage of rice.

The amount of Zn sequestrated per unit weight of rice grain under various treatments was also estimated, and the results (Table 7) showed that it was highest (27 g t^{-1}) in treatment T_9 *i.e*., soil Zn @ 10 kg ha^{-1} with foliar spray of $ZnSO_4$ @ 0.5% at MTS followed by T_{12} (26.97 g ha^{-1}) soil Zn @ 10 kg ha^{-1} with foliar spray of $ZnSO_4$ @ 0.5% at MTS

Results revealed that soil application of Zn @ 10 kg ha^{-1} along with foliar spray of Zn, either through Zn-EDTA or $ZnSO_4$, is very effective for Zn enrichment of rice grain which resulting in an increase in its amount to the extent of 18.81 to 19.07% over

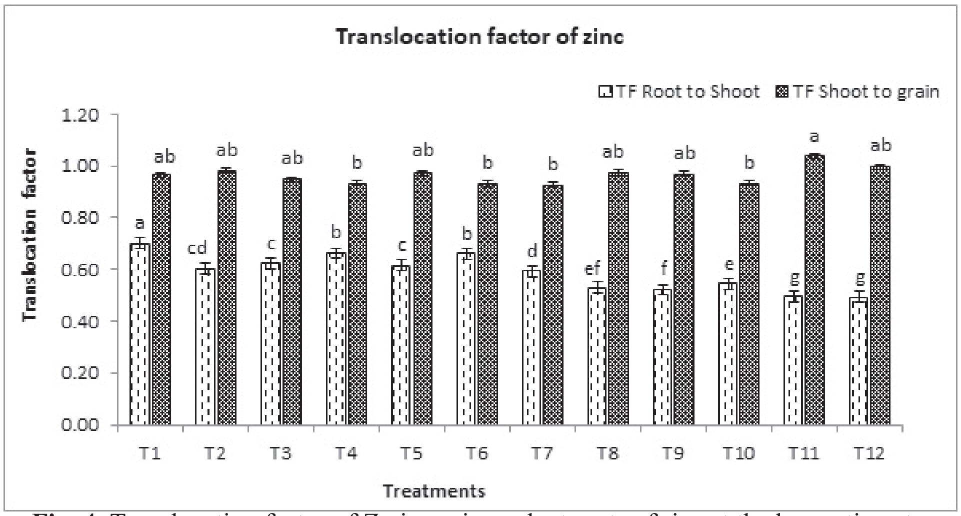


Fig. 4. Translocation factor of Zn in various plant parts of rice at the harvesting stage.

control respectively. Yadav et al. (2018) also reported a similar type of findings where it was found that Zn Fertilization caused an increase in the grain Zn content from 21.7 to 28.7 mg kg^{-1}.

Benefit-cost ratio

The benefit-cost ratio (BCR) was estimated under all the treatments, and the results (Table 7 and Fig. 5) showed that its magnitude was highest (1.54) in the treatment T_8 *i.e.*, soil application of Zn @ 5 kg ha^{-1} along with foliar application of ZnSO4.7 H2O @ 0.5% at MTS followed by T_7 (soil application of Zn @ 2.5 kg ha^{-1} along with foliar application of $ZnSO_4.7H_2O$ @ 0.5% at MTS) and T_5 (two foliar sprays of $ZnSO_4$ @ 0.5%, one at MTS and another at PI).

The results therefore revealed that although the grain yield of rice was highest (60.13 q ha^{-1}) in T12 treatment soil Zn (10 kg ha^{-1} with Zn-EDTA spraying @ 0.5% at MTS) compared to T_8 (59.57 q ha^{-1}) *i.e.*, soil application of Zn @ 5 kg ha^{-1} along with foliar application of $ZnSO_4.7H_2O$ @ 0.5% at MTS, it should not be recommended to farmers because of its lower BCR (Benefit Cost Ratio) (1.47) than T_8 (1.53). So,

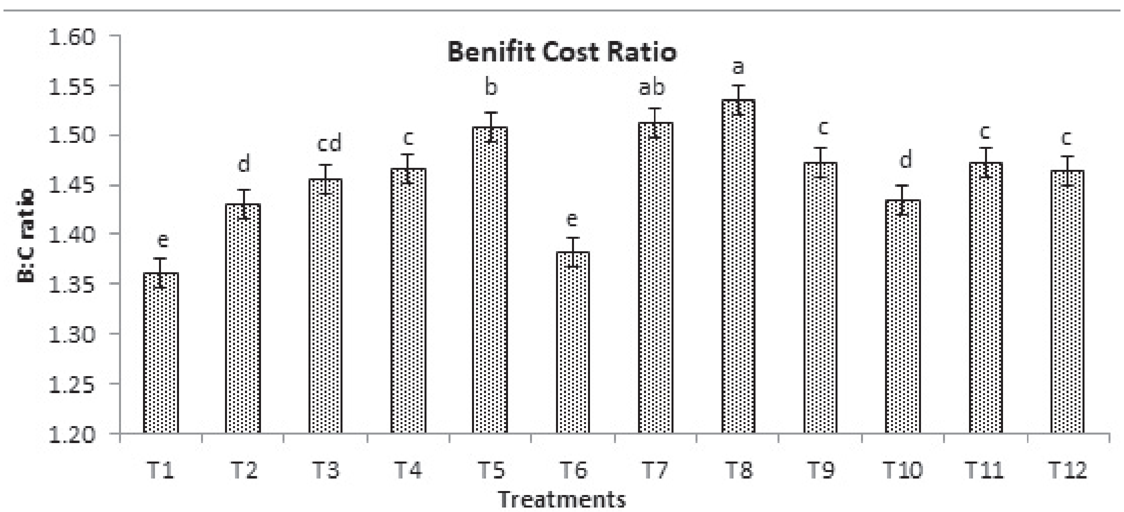


Fig. 5. Graphical representation of benefit cost ratio (B:C).

from the practical point of view, the treatment T8 i.e., soil application of Zn @ 5 kg ha^{-1} before transplanting along with the foliar application of $ZnSO_4.7H_2O$ @ 0.5% at MTS recorded the highest BCR in comparison to other treatments will be very effective for sustaining crop production. Fig. 5 shows benefit-cost ratio (B:C) of different treatments of the experiment.

CONCLUSION

From the experimental results, it may be concluded that though Zn requirement in rice is very low (with maximum Zn uptake 0.401 kg ha^{-1}), it plays a vital role for the growth and development of rice and resulting in a yield response to the tune of 18.09 % over no Zn application even in soil which was not deficient in available Zn. It was also found that for sustaining the rice production soil application of Zn (@ 5 kg ha^{-1}) before transplanting of rice seedlings along with foliar application of $ZnSO_4.7H_2O$ @ 0.5% at the maximum tillering stage of the growth of rice would be very effective over other treatments owing to optimum grain yield as well as highest benefit cost ratio which might be due to higher Zn uptake, higher translocation of Zn from root to shoot and shoot to grain. Results also revealed that Zn translocation from shoot to grain was always greater than from root to shoot upon all the treatments emphasizing the role of foliar application of Zn for better grain Zn enrichment.

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Impact of the different doses of urea application on the damaging potentiality and number of live cases of Paddy caseworm, *Nymphula depunctalis* on popular rice cultivar at Cauvery command area

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ABSTRACT

Nitrogen is one of the most important factors in development of herbivore populations. The application of nitrogen fertilizer in crops can normally increase herbivore feeding preference, food consumption, survival, growth, reproduction, and population density. This experiment was comprised of 10 treatments and laid out in a RCBD with 3 replications. The treatments included a standard check i.e., 100:50:50 kg/ha of N.P.K as per package of practice (POP-UASB-2021) and other treatments 10, 20, 30, 40, 50, 60, 70, 80, 90 % of excess nitrogen application above recommendation (standard check), It was observed that high doses of nitrogen i.e., RDF+90 % N of treatment recorded comparatively highest incidence (20.58 % leaf damages), nitrogen content (2.21% of N), whereas, the lowest leaf damage (7.36 %) and nitrogen content (0.97 %) observed in RDF treatment respectively. Live cases on some local cultivars were recorded and it is revealed that, the highest number of live cases (6.60 live cases) of paddy caseworm was recorded in Jaya cultivar, whereas the lowest number of live cases on BR-2655 cultivar (0.37) among the different cultivars. The present study provides an insight into how excess nitrogen fertilizer shape paddy- caseworm interactions and the optimal regime of nitrogen fertilizer is proposed to improve the fertilizer-nitrogen use efficiency and reduce outbreak of herbivores insects.

Key words: *Nitrogen, urea,leaf damage, paddy caseworm, N. depunctalis*

INTRODUCTION

After 'Green Revolution' in the world on 1966s, the important characterized by introduction of successful breeding and widespread adoption of high yield varieties (HYV), application pesticides and nitrogen fertilizers, has achieved double in production of some of the important crops such as Wheat, Rice and Maize crops etc., however, the continuous and excessive application of pesticides and nitrogen-based fertilizers have resulted in the negative impact due to emergence of biotic factors in field crops.

Among these mentioned filed crops, Rice (*Oryza sativa* L) is most important and essential among cereal crops, and it serves as staple food crop for half the world's population, especially in Asian continents. It belonging to family Poaceae (Graminae) and is self-pollinated crop (Davla et al., 2013), and India is second largest producer of rice in world after China. However, rice is attacked by 800 species of insect out of which 20 (i.e., rice leaf folder, stem borer, plant hopper, grass hopper, gall midge and paddy caseworm etc.) cause economic damage and are considered as major pests (Jena et al., 2018).

Nitrogen plays a crucial role in the development of herbivore populations. The application of nitrogen fertilizers typically enhances herbivore feeding preferences, food consumption, survival, growth, reproduction, and population density. However, there are a few instances where nitrogen fertilizers have been shown to reduce herbivore performance. In most rice-growing regions across Asia, significant increases in the populations of major rice insect pests, such as planthoppers (*Nilaparvata lugens* and *Sogatella furcifera*), leaf folders (*Cnaphalocrocis medinalis*), and stem borers (*Scirpophaga incertulas*, *Chilo suppressalis*, *Scirpophaga innotata*, *C. polychrysus*, and *Sesamia inferens*) have been closely linked to the long-term excessive use of nitrogen fertilizers (Zhong-xian et al., 2007)

The significant difference in nitrogen content between animal and plant tissues is likely the primary reason most herbivores have the ability to seek out host plants with higher nitrogen levels (Southwood, 1973). Although the heavy application of nitrogen fertilizer rarely affects insects directly, it can influence the morphological, biochemical, and physiological traits of host plants. This, in turn, improves the nutritional conditions for herbivores by playing a crucial role in their population dynamics, influencing factors like host selection, ecological fitness, survival, growth, fecundity, reproductive capacity, and notably reducing the host plant's resistance to herbivores (Barbour et al., 1991).

The chemical or inorganic fertilizer is one the most important nutrition factors of increasing the productivity of rice, but due to high doses of application by Indian famers this leads vigorous growth and more attractive to insect pests feeding and causes drastic damages and finally yields loss, (Chakraborty et al., 2012). The incidence of leaf folder and stem borer increased with increase in nitrogen level application (Randhawa and Aulka, 2014).

However, in Karnataka, particularly Cauvery command area, for past 5 years, the farmers delaying for transplanting of rice crop due to unavailability canal water for sources irrigation, excess of application nitrogen base fertilizer and using indiscriminate and same molecules of insecticides for continuously, and introduction of susceptible cultivars, like Jaya and IR-64 etc., that trigger and boost the outbreak of some secondary insect pests of rice among them, the paddy caseworm, Nymphula depunctalis, it earlier consider as minor pest, but now it becoming emerging as major pest and causes sever infestation (Pandit et al., 2024). The paddy caseworm's larvae are known for their distinctive behaviours and feeding habits, especially the larvae scrape the green matter (chlorophyll content) at early vegetative stage of rice, which can lead to reduced crop productivity if left unchecked. Understanding the life cycle, behavior, and management strategies of the paddy caseworm is essential for sustainable rice production and minimizing economic losses for farmers (Pandit et al., 2024).

Judicious and proportion-based application of inorganic fertilizer i.e., nitrogen based fertilizes in relation to rice growth stage can suppress the pest incidence without any conciliation of the final yield generation. In this contemplation a study was undertaken in the field of widely adopted paddy Jaya cultivar at Agriculture College, Mandya, Karnataka, India where no study even preliminary in nature relating to the assessment of the impact of inorganic nitrogen fertilizer on paddy caseworm incidence and number of live cases were done earlier.

MATERIAL AND METHODS

Different levels of nitrogen application

The present investigations were taken to know the feeding behavior and infestation level of paddy caseworm, Nymphula depunctalis at different levels of nitrogen application in the form of urea above the normal recommendation (RDF), this field experiment was carried out at 'A' block, College of Agriculture, V. C. Farm, Mandya, during late *kharif* season of 2021-2022. Geographically, Mandy is situated at 690 meters above mean sea level, having latitude 12° 31' 25.43" North and longitude 76° 53' 40.186" East and average annual rainfall of about 751 mm, with the mean maximum temperature and minimum temperature vary between 28-35°C and 24-26°C, respectively, and it under southern dry region of Karnataka.

A field experiment was carried out in Randomized Completely Block Design (RCBD) with ten treatments and three replications with different doses of nitrogen applied, including an untreated control as standard check (Normal RDF application treatment). The popular and highly susceptible variety Jaya was sown and 25 days old seedlings were transplanted in the main field with a spacing of 20×15 cm, between rows and plants, respectively. For each replication, a plot size of 4×3 m was maintained (Plate 1). The crop was raised as per the package of practice, except for the plant protection measures (Anon., 2017). The details of treatments include a standard check i.e., 100:50:50 kg/ha of N.P.K as per Package of practice (POP-UASB-2021) and other treatments 10, 20, 30, 40, 50, 60, 70, 80, 90 % of excess nitrogen application above recommendation (standard check), the application was made 15 days after transplanting (DAT).

The observation on the incidence was recorded on ten hills randomly selected in each treatment by counting the number of damaged, cut leaves and also recorded the number of live cases on different growth phases on ten selected cultivars (MTU-1001, Gangavati Sona, Rajamudi, Thanu, IR-64, MTU-1010, Jaya, KRH-4 and BR-2655) Jyothi of rice per hill a week after application in 10 days interval *i.e.*, 30, 40, 50, 60, 70, 80, 90 and 100 days after transplanting. This incidence was expressed in terms of percentage. Further, nitrogen (N) content was estimated by using standard procedures and protocols which is prescribed below.

Estimation of nitrogen (N) content in plant sample

The nitrogen content in the plant sample was determined by the micro-Kjeldhal method as suggested by Piper (1966). The leaf sample was collected 40 days after transplanting, from the treatments of a different level of nitrogen application in the form of urea and during higher larval activity and damage. The leaf sample was collected separately in polyethylene covers from ten randomly selected hills from each treatment (Fig. 1). A sufficient sample size of leaf samples were collected and brought to the laboratory. The samples were cleaned using sterilized water. Subsequently, the cleaned samples were placed in a hot oven set at a temperature of 45°C for one week to ensure thorough drying. To prevent any degradation, the dried samples were stored in a dry place until they could be analyzed for nitrogen content.

Estimation of nitrogen

0.5 g of finely powdered oven dried Na_2CO_3 samples were taken in the digestion tubes. To this 1-2 g of digestion mixture and 10-15 ml concentrated H_2SO_4 and the samples were added in Kjheldahl digestion assembly till a light bluish green residue is obtained. Then the content was cooled and some distilled water was added. Receiving flask was placed at the receiving end of distillation unit. The digestion was loaded on tube along with digested sample to the distillation apparatus one at a time. By keeping all reserve tanks loaded with appropriate reagents such as 4 per cent boric acid with mixed indicator and 40 per cent NaOH the content was distilled for 6 minutes and the released ammonia was collected in boric acid solution by programming the instrument. Once the distillation is completed, the receiving flask was removed and titrated against standard H_2SO_4 till the colour changes from green to pink. Titre value (TV) was noted and nitrogen content was calculated using equation

$$\text{Nitrogen (\%)} = \frac{\text{(TV x normality of } H_2SO_4 \text{ x 0.014}}{\text{Weight sample x 100}}$$

Statistical analysis: The obtained mean data on per cent leaf damage and nitrogen content of each treatment was processed after arc-sine transformation and subjected to ANOVA (Gomez and Gomez, 1984 and Hosmand, 1988) and means was separated by Tukey's HSD (Tukey, 1965) for interpretation after statistical data conversion were performed using SPSS software (SPSS 24 Version) and for figures, used Graph pad prime 10 (10.0.1 Version).

RESULTS AND DISCUSSION

Incidence of paddy caseworm at different level of nitrogen application

The treatment includes a recommended dose of fertilizers (RDF) *i.e.*, 100:50:50 kg/ha of NPK along with 10, 20, 30, 40, 50, 60, 70, 80, 90% of excess nitrogen application at 15-20 DAT. Therefore, the present investigation was undertaken to find out the effect of different doses of application of nitrogen-based fertilizer i.e., Urea on the level of infestation (incidence) of paddy caseworm, during, *kharif,* 2021.

The results of present findings revealed that, application of different doses of urea fertilizer above the recommend dose of fertilizer, that up-regulate (boost) the level of paddy caseworm and more vegetative growth in paddy plants and infestation was found significantly high at RDF+90% nitrogen application at 30, 40 and 50 day after transplanting (DAT), Table 1; Fig. 1.

However, at 30 days after transplanting, among

Table 1. Incidence of paddy caseworm at different levels of nitrogen application and estimation of N content, *kharif* 2021.

Sl. no.	Treatments	Mean per cent leaf damage				Overall mean	Nitrogen content (%)
		30 DAT	40 DAT	50 DAT	60 DAT		
T_1	RDF + 10 % N	9.93 (18.36)^{ab}	15.09 (22.86)^{a}	5.16 (13.13)^{a}	1.92(7.97)^{ab}	8.03±3.33	1.04
T_2	RDF + 20 % N	11.56 (19.88)^{b}	16.15 (23.69)^{ab}	6.45 (14.71)^{ab}	2.09 (8.31)^{abc}	9.06±4.10	1.15
T_3	RDF + 30 % N	15.62 (23.28)^{c}	18.86 (25.74)^{bc}	8.43 (16.87)^{c}	2.28 (8.67)^{abcd}	11.30±4.52	1.29
T_4	RDF + 40 % N	17.12 (24.44)^{c}	20.15 (26.67)^{c}	11.27 (19.61)^{c}	2.36 (8.8)^{abcd}	12.73±4.48	1.48
T_5	RDF + 50 % N	19.69 (26.35)^{d}	23.51 (29.00)^{d}	11.19 (19.53)^{c}	2.62 (9.31)^{bcd}	14.25±4.13	1.74
T_6	RDF + 60 % N	21.68 (27.75)^{de}	24.66 (29.78)^{de}	15.27 (23.00)^{d}	2.94 (9.87)^{ced}	16.14±5.50	1.87
T_7	RDF + 70 % N	23.15 (28.76)^{ef}	26.23 (30.80)^{de}	17.75 (24.92)^{de}	3.15 (10.22)^{ef}	17.57±4.48	1.96
T_8	RDF + 80 % N	24.44 (29.63)^{fg}	27.53 (31.65)^{ef}	18.83 (25.69)^{de}	3.63 (10.98)^{ef}	18.61±4.63	2.08
T_9	RDF + 90 % N	26.74 (31.14)^{g}	31.01 (33.83)^{f}	20.47 (26.89)^{e}	4.08 (11.66)^{f}	20.58±3.12	2.21
T_{10}	RDF	8.49 (16.93)^{a}	14.16 (22.09)^{a}	5.00 (12.92)^{a}	1.78 (7.67)^{a}	7.36±2.79	0.97
SE m ±		0.38	0.53	0.61	0.16		0.049
CD @ p = 0.05		1.13	1.57	1.83	0.47		0.146

Recommended dose of fertilizer (RDF)= 100 N: 50 P_2O_5: 50 K_2O kg ha^{-1}; N = Nitrogen; DAT = Day after transplanting; Values in the column followed by common letters are non-significant at p = 0.05 as per Tukey's HSD (Tukey, 1965). Figures in the parenthesis indicate arc-sine transformed values; T - Treatment; *sample drawn at 40 DAT.

Fig. 1. General view experimental plot of different levels of nitrogen applications, *kharif* 2021.

the different doses of urea applications, the highest per cent leaf damage was noticed in RDF + 90% N (T_9) with 26.74 per cent of leaf damage incidence by paddy caseworm, while a lowest percentage of leaf damage was recorded in Treatement$_{10}$-RDF (8.49%) where normal RDF was applied. This was followed by treatment$_1$-RDF +10% N (T_1) with 9.93 per cent leaf damage, and which was par with (T_2) RDF+20% N (11.56%). Other treatments recorded significantly higher per cent leaf damage as compared to T10-RDF, viz., at treatment 3 (T_3)-RDF+30% N (15.6%), treatment-RDF+40% N (T_4) (17.12%) which was on par with each other, whereas, T_5-RDF+50 % N (19.68%), T_6- RDF+60% N (21.68), T_7- RDF+70% N (23.15%) and T_8- RDF+80% N (24.44%), respectively (Table 1).

Overall, of the results evident that highest per cent leaf damage (20.58%) was found in the treatment with RDF+90% N (treatment-9) application in all the observations dates in compared with treatment-RDF (T_{10}-CNT) (7.36%) where, treatment-1 (RDF+10% N) (8.03 %) and treatment-2 (RDF+20% N) (9.06%) were on par with treatment-RDF(T10-CNT) in all the observations after application of fertilizers, and there was no much statistically difference between them. It was clearly indicated that the leaf damage by paddy caseworm was increased with increase in nitrogen fertilizer level, (Table 1).

This was mainly due to adequate supply of nitrogen, with more than recommended dose (RDF) resulted in sufficient availability from soil for plant uptake and same might use in optimum biosynthesis of carbohydrates leads to taller plant at initial stages of growth and this nitrogen fertilizers, associated with the stimulating effects on various physiological processes including softness of tissues and increasing cell sap. The peak infestation level was noticed at 40 DAT at all the doses of urea which declined later on. These are the-some reasons might be increasing in damage percentage by paddy caseworm.

The results of the present study are in line with the reports of Das et al. (2001) who recorded infestation of paddy caseworm, N. depunctalis significantly in different levels of urea (130, 150, and 190 kg of N / ha). However, the maximum infestation was observed from plot treated with 190 kg urea ha^{-1}, and the minimum from 130 kg ha^{-1}. The lowest yield was also obtained from the plot treated with high level of urea. Similarly, the results are close with agreement with Panda and Sontakke (1993) who also reported that, where higher doses N fertilizer (150 kg N/ha) were recorded highest per cent leaf damage of, and dead heard and whit ear of rice stem borer.

The present results are in conformity with Paramasiva et al. (2020) who observed the infestation of insect pests in rice field at different levels N application (40, 80, 120, 160, and 200 kg ha^{-1}). The result exploited that the pest infestation rates were higher (31.43% and 43.86% for stem borer, 9.23% and 13.59% for leaf folder, and 2.75% and 3.00% for gall midge) in treatments with higher nitrogen rates of 160 and 200 kg/ha, respectively, compared to lower nitrogen rates of 40 and 80 kg ha^{-1}. Similarly, Kraker et al. (2000) also observed average density of rice leaf folder larvae at the highest nitrogen level (150 kg N ha^{-1}).

The present results are corroborated with the finding of Kulagod et al. (2011b) who recorded highest per cent of leaf damage of leaf folder in treatment of 200 kg N/ ha plots. Whereas, lowest per cent leaf damage was recorded for the control and 100 kg N and K/ha, and plant damage by leaf folder was positively correlated with the levels of N and P, but negatively correlated with the K rate, respectively. Harish and Singh (2004) reveled that, among the different levels nitrogen fertilizer (0, 50 and 150 kg of N/ha) application, for the occurrence of leaf folder (*Cnaphalocrocis medinalis*), rice hispa (*Dicladispa armigera*), and grasshopper (*Hieroglyphus banian*), the highest incidences of leaf folder, rice hispa, and grasshopper were observed in the plot treated with 150 kg/ha dose of nitrogen fertilizer, resulting in leaf damage percentages of 12.05, 5.83, and 28.31 per cent, respectively, as comparing the 0 and 50 kg of N/ha.

According to Chakraborty et al. (2012) the current investigation is closely in agreement with their previous reports. They found highest incidence of adult yellow stem borer population (1.93±0.12 individuals/5 hills) and egg masses (2.82±0.52 egg mass/quadrate), as well as the percentage of "dead heart" (16.12%) and "white head" (12.56%) when applying 160 kg N/ ha in the form of urea granules. Conversely, the lowest incidence of YSB population (0.56±0.12 individuals/5 hills) and egg masses (1.12±0.35 egg mass/quadrate) occurred when the field was solely fertilized with 3.5 tons of vermicompost. However, it was noted that this high dose of N fertilizer treatment resulted in lower yields due to a relatively higher incidence of YSB pest and excessive vegetative and luxurious foliage growth of the plants with a short span of time. Similarly reports of Prasad et al. (2004) have noted that the extent of rice crop damage increases at significant level following the application of 200 kg N / ha. They further noted that the extent of damage was 5.4% and 4.8% at 120 kg N / ha and no N/ha application, respectively. But in their experiment, they have studied only the incidence of DH and WH under the combined application of organic and inorganic sources of fertilizer. Assessment of the incidence of YSB egg masses was not duly done.

Estimation of nitrogen level

The results revealed that increasing application of N fertilizers to the rice plants more than the RDF leads to increase in N concentration in rice plants. The nitrogen content among treatments with different dose of N fertilizers application varies from 0.97 to 2.21 per cent. The lower amount of nitrogen content was recorded in treatment-10 (T_{10}) of the normal recommend doses fertilize (RDF) *i.e.*, 100 N: 50 P_2O_5: 50 K_2O kg ha^{-1} with 0.97 per cent and this was followed by the treatments like treatmnet-1 (RDF+10% N), treatment-2 (RDF+20% N) and treatmnet-3 (RDF+30% N) which were recorded 10.4, 1.15 and 1.29 per cent of nitrogen content, respectively and which non-significant difference with treatment-RDF (T_{10}). Whereas

Table 2. The number of live cases of *N. depunctalis* recorded on different age of popular cultivars, during *kharif*, 2021.

Sl. no.	Popular cultivars	Mean number of live cases of *N. depunctalis*					Overall mean
		10 DAT	20 DAT	30 DAT	40 DAT	50 DAT	
1	MTU-1001	2.10	2.30	3.25	4.20	1.60	2.24
2	Gangavati Sona	0.00	1.20	3.15	4.20	3.60	2.03
3	Rajamudi	0.00	1.10	3.00	3.60	2.80	1.75
4	Thanu	0.00	1.00	3.60	3.80	1.60	1.67
5	IR-64	0.00	2.00	4.80	5.60	2.90	2.55
6	MTU-1010	0.00	1.20	5.20	4.60	1.50	2.08
7	Jaya	3.30	3.90	6.40	6.60	3.00	3.87
8	KRH-4	0.00	2.50	5.20	4.20	1.60	2.25
9	BR-2655	0.00	1.00	1.20	0.00	0.00	0.37
10	Jyothi	1.50	1.60	2.20	3.70	1.10	1.68

Mean of 10 hills per cultivars; DAT - Day after transplanting.

treatment-4 (RDF+40% N), treatment-5 (RDF+50% N), treatment-6 (RDF+60% N), and treatment-7 (RDF+70% N) with nitrogen content of 1.48, 1.74, 1.87and 1.96 per cent, respectively and were on par with each other. Likewise, the highest nitrogen content was recorded in treatment-9 (RDF+90% N) with 2.21 per cent. This was followed by treatment-8 (RDF+80% N) by recording 2.08 per cent of nitrogen content, respectively (Table 1).

The overall results of nitrogen content indicated that, when the nitrogen-based fertilizers, application increases over normal recommend dose of fertilizer (RDF) that increases the nitrogen content in crop plants as well soil, this helps the plants to grow more vigorously and more succulents and proved to more susceptible to more insect pests. which will lead to drastically reduction in production and productivity of crops. Hence, the host nutrients play an important role in population abundance of insect pests of rice and there by yield performance of the crop. Over-dose of N fertilizer resulted in higher incidence of insect pests. Higher doses of nitrogen fertilizer supported higher incidence of pest population. The highest leaf damage was recorded from the dose of nitrogen fertilizers might be associated with the stimulating effect of urea (nitrogen) on various physiological processes such as softness of tissues, increasing the sap which make the plant more vulnerable to insect attack. However, to avoid the excess application of nitrogenous fertilizers in order to reduce insect pests and use judicial nitrogen fertilizer.

The Table 2 presents the number of live cases of paddy caseworm (*N. depunctalis*) recorded in different rice cultivars at various stages, ranging from 10 to 100 days after transplanting (DAT) during the *kharif* 2021 season. The data indicates the mean number of live cases per cultivar across different time intervals. Result revealed that highest number of live cases in Jaya cultivar had the overall mean (3.87) live cases of paddy caseworm, with a peak infestation of 6.60 at 40 DAT. Whereas, Lowest is the cultivar BR-2655 exhibited the lowest overall mean (0.37), with minimal live cases recorded throughout the observation period. The overall, infestation of *N. depunctalis* peaked between 30 to 50 DAT in most cultivars, with a noticeable decline after 60 DAT across all varieties. Jaya was the most susceptible cultivar, while BR-2655 showed strong resistance to paddy caseworm. This information can be useful for selecting more resistant rice cultivars for future cultivation (Table 2; Fig. 2).

CONCLUSION

From above results, we can conclude that excessive nitrogen (N) fertilization in rice cultivation increases susceptibility to paddy caseworm (Nymphula depunctalis) infestations, particularly around 40 days after transplanting (DAT), which corresponds with the peak incidence of larval cases. This heightened vulnerability not only leads to increased pest-related damage but also signifies a misallocation of resources, as the surplus nitrogen does not translate into proportional yield benefits. Therefore, it is crucial for the farming community to adhere to recommended nitrogen application rates or balance nitrogen fertilizer to optimize crop health and minimize pest infestations. Additionally, selecting resistant rice cultivars can further enhance the effectiveness of integrated pest management strategies against paddy caseworm.

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Interaction studies on *Rhizoctonia solani*, sheath blight pathogen and brown plant hopper (*Nilaparvata lugens*) in rice

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ABSTRACT

The association of sheath blight (Rhizoctonia solani), a widespread fungal disease in rice and the brown planthopper (Nilaparvata lugens), a major rice pest, presents a significant challenge in rice production. Brown planthopper feeding may increase plant susceptibility to sheath blight by weakening plant defenses and facilitating fungal invasion through the wounds they create. The synergistic effects of brown planthopper and sheath blight infestation result in significant yield losses, complicating management strategies. Three sets of interactions were conducted, among the three sets, the highest disease severity was recorded in the set 1 (Simultaneous inoculation of BPH and Rhizoctonia solani) (73.33%) followed by set 2 (Release of BPH and subsequent inoculation of Rhizoctonia solani after 7 days) (60%) and least disease severity was observed in set 3 (BPH release after 7 days of inoculation with Rhizoctonia solani) (57.04%). This study aims to investigate the complex interaction between brown planthopper and sheath blight in rice, focusing on how these interactions influence disease progression and pest dynamics, and to provide insights into improved management strategies for rice farmers facing both threats.

Key words: *Interaction, rice, sheath blight, brown plant hopper*

INTRODUCTION

Sheath blight, caused by the fungal pathogen *Rhizoctonia solani*, is a prevalent disease in rice that leads to significant yield losses. The disease is characterized by blight symptoms on the leaf sheath, resulting in reduced photosynthetic efficiency and lower grain production. Environmental conditions, such as humidity and temperature, play a significant role in the disease's progress, making it particularly problematic in rice cultivation areas that favor fungal growth (Cheng et al., 2013). The brown planthopper is a notorious pest in rice (*Oryza sativa*) cultivation, known for causing significant damage through its feeding activities. BPH feeds on the phloem sap of rice plants, leading to reduced plant vigor, stunted growth, and even complete crop failure under severe infestations. BPH infestation can be devastating, especially in tropical regions, where it poses a substantial risk to rice yield (Ghobadifar et al., 2016). Additionally, BPH acts as a vector for several viral diseases, importantly impacting the overall health of rice plants and enhancing the severity of sheath blight infections. The feeding habits of BPH influence sheath blight development in several ways. Firstly, the mechanical damage inflicted by BPH feeding can open pathways for fungal spores, facilitating infection. Secondly, the stress on plants due to BPH infestation may weaken their defences against pathogens, making them more susceptible to sheath blight (Ramli et al., 2018). As such, the synergy between BPH feeding and sheath blight presents a dual threat to rice production that requires comprehensive study and management approaches.

MATERIALS AND METHODS

The experiment has been conducted during *kharif* 2023 in greenhouse, College of Agriculture, V. C. Farm, Mandya to know the effect of different population densities of brown plant hopper on sheath blight of rice where the interaction between sheath blight and brown plant hopper was studied.

Mass rearing of brown plant hopper (*Nilaparvata lugens*)

Initially, the field population of BPH was collected from unsprayed rice fields of COA, Mandya and surrounding villages. The culture was maintained for 3-4 generations on susceptible rice varieties TN1 and Jaya. The susceptible plants with BPH culture in glass cages was identified and removed natural enemies such as mirid bugs on regular basis along with wilted and dried plants. The adults were confined to 30-35 days old plants of Jaya raised in a plastic tray filled with fertilizer enriched puddled soil and placed in oviposition cages (45×45×60 cm) with GI frames, glass top and the wire mesh along the sidewalls with glass door for further studies.

Maintenance of inoculum of sheath blight

The sorghum seeds were boiled and placed in autoclavable covers each containing 250g and autoclaved. After autoclaving, 3-5 mycelial discs of *Rhizoctonia solani* were inoculated into covers containing sorghum seeds to promote luxuriant pathogen growth. After autoclaving -The full grown pathogen on sorghum seeds was inoculated to field (Punya et al., 2021).

Interaction studies

Simultaneous inoculation of brown plant hopper and *Rhizoctonia solani*

The 1 cm mycelial disc containing sclerotial bodies from 7 days old *R. solani* culture was taken for inoculation. Each plant was inoculated with a mycelial disc containing a sclerotial body between tillers and cotton was placed on that to maintain moisture at 40DAS. The second stage nymphs were collected from the cage through the aspirator and released on to the plant at 40DAS which was covered by mylar tubes.Inoculation of Rhizoctonia solani seven days after BPH release

The 1 cm mycelial discs containing sclerotial bodies from 7 days old *R. solani* culture was taken for inoculation. Every plant was inoculated with one mycelial disc consisting sclerotial body between tillers and cotton was placed on that to maintain moisture at 47DAS. The second stage nymphs were collected from the cage through the aspirator and released on to the plant at 40DAS which was covered by mylar tubes.

Inoculation of *Rhizoctonia solani* seven days before BPH release

The 1 cm mycelial discs containing sclerotial bodies from 7 days old *R. solani* culture were taken for inoculation. Each plant was inoculated with a mycelial disc placed between the tillers, with cotton over it to maintain moisture at 40DAS. The second stage nymphs were collected from the cage using an aspirator and released on to the plant at 47 DAS which was then covered with mylar tubes.

RESULTS AND DISCUSSION

The experiment was conducted in greenhouse to know the effect of different population densities of brown plant hopper (BPH) on sheath blight of rice where the interaction between sheath blight and BPH was studied. The experiment was conducted by three sets (Set-1: Simultaneous inoculation of BPH and *R. solani*, followed by Set-2: Release of BPH and after 7 days inoculation of *Rhizoctonia solani*, Set-3: Inoculation of *Rhizoctonia solani* and after 7 days release of BPH) with seven treatments (T_1- 0, T_2 - 5, T_3 - 10, T_4- 20, T_5- 30, T_6- 40, T_7- 50) consisting of different populations of BPH (Table 1). BPH infestation was recorded by following Standard Evaluation system(SES) for rice(IRRI, 2013) as per Table 2 and disease severity of sheath blight was recorded by following SES for rice

Table 1. Formulation of treatments based on different population densities of BPH.

Treatment	Population
T_1	0
T_2	5
T_3	10
T_4	20
T_5	30
T_6	40
T_7	50

The experiment consisted of seven treatments with three replications each.

Table 2. Standard Evalution System for BPH (IRRI, 2013).

Scale	Symptoms
0	No damage
1	Very slight damage
3	First and second leaves partially yellowing
5	Pronounced yellowing and wilting
7	Mostly wilting, the plant still alive
9	Plant completely wilted or died

Table 3. Disease scoring scale as per IRRI (2013) for sheath blight of rice.

Sc-ale	Symptoms
0	No infection
1	Vertical spread of the lesions up to 20% of plant height
3	Vertical spread of the lesions up to 21- 30% of plant height
5	Vertical spread of the lesion up to 31 - 45% of plant height
7	Vertical spread of the lesion up to 46 - 65% of plant height
9	Vertical spread of the lesions up to 66-100% of plant height

(IRRI, 2013) as in Table 3.

Set-1: Simultaneous inoculation of BPH and *Rhizoctonia solani*

All treatments exhibited sheath blight severity except the untreated control. On the basis of mean insect infestation percentage and mean disease severity, treatment T_6 (40 BPH) recorded the highest value BPH infestation and disease severity (88.15% and 73.33 %) followed by treatment T_5 (30 BPH) (71.85 % and 70.37 %) and treatment T_4 with 20 population of BPH (62.96 % and 64.44 %) and least severity shown by (T_7) of 50 population of BPH treatment (100% and 0.00%) followed by treatment T_2 of 5 population of BPH (35.56 % and 37.78 %). Treatment (T_1) untreated control had no insect infestation or disease severity.But treatment with highest mean insect infestation percentage of 100 % showed zero per cent disease severity due to complete plant drying caused by insect (Table 5, Fig. 1).

Set-2: Inoculation of *R. solani* seven days after BPH release

On the basis of mean insect infestation percentage and mean disease severity, treatment (T_6) consisting of 40 population of BPH (83.70% and 60.00 %) followed by treatment (T_5) of 30 population of BPH (65.93 % and 55.56 %) and treatment (T_4) of 20 population of BPH (55.56 % and 52.50 %) and least severity shown by (T_7) of 50 population of BPH treatment (98.52% and

Table 5. Effect on sheath blight severity due to simultaneous inoculation of BPH and *R. solani* under greenhouse condition.

Treatment No.	BPH infestation (%)						Sheath blight severity (%)						Plant height (cm)	Plant tillers (No.)
	43 DAS	46 DAS	49 DAS	52 DAS	55DAS	Mean	50 DAS	60 DAS	70 DAS	80 DAS	90DAS	Mean		
T_1	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00	96.00	12.33
T_2	7.41(15.80)	11.11(19.48)	25.93(30.62)	55.56(48.21)	77.78(61.91)	35.56	11.11(19.48)	25.93(30.62)	33.33(35.28)	48.15(43.96)	70.37(57.05)	37.78	92.33	11.33
T_3	18.52(25.50)	25.93(30.62)	48.15(39.68)	70.37(57.05)	92.59(74.24)	51.11	25.93(30.62)	40.74(39.68)	48.15(43.96)	62.96(52.54)	77.78(61.91)	51.11	90.67	10.67
T_4	25.93(30.62)	40.74(39.68)	62.96(43.96)	85.19(67.40)	100.00(90.05)	62.96	33.33(35.28)	55.56(48.21)	62.96(52.24)	77.78(61.91)	92.59(74.24)	64.44	88.00	9.33
T_5	40.74(39.68)	48.15(43.96)	77.78(61.91)	92.59(74.24)	100.00(90.05)	71.85	33.33(35.28)	62.96(52.54)	70.37(57.05)	85.19(67.40)	100.00(90.05)	70.37	85.67	8.67
T_6	70.37(57.05)	77.78(61.91)	92.59(74.24)	100.00(90.05)	100.00(90.05)	88.15	33.33(35.28)	70.37(57.05)	77.78(61.91)	85.19(67.40)	100.00(90.05)	73.33	83.67	8.00
T_7	100.00(90.05)	100.00(90.05)	100.00(90.05)	100.00(90.05)	100.00(90.05)	100.00	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00	81.00	7.33
SEm ±	2.36	2.21	1.86	2.35	1.50	2.06	2.28	2.66	1.80	2.56	2.68	2.40	2.54	2.32
C.D @5%	8.56	8.21	9.32	7.05	6.32	7.89	8.62	8.40	7.92	7.12	6.56	7.72	9.11	9.18

Note: Figures in the parenthesis are arcsin transformed values

T_1: Control (0), T_2: 5 population of BPH, T_3: 10 population, T_4: 20 population, T_5: 30 population, T_6: 40 population, T_7: 50 population.

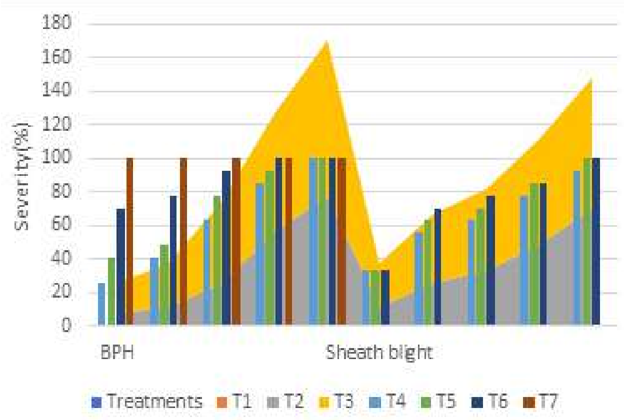

Fig. 1. Effect on sheath blight severity due to simultaneous inoculation of BPH and *R. solani* under greenhouse condition.

0.00%) followed by treatment (T_2) of 5 population of BPH (30.37 % and 30.92 %) whereas treatment (T_1) untreated control, but treatment (T_7) of 50 population of BPH with highest mean insect infestation percentage of 98.52 % showed zero per cent disease severity due to complete plant dried out because of insect infestation (Table 6, Fig 2).

Set-3: Inoculation of *R solani* seven days before release of BPH

On the basis of mean insect infestation percentage and mean disease severity, treatment (T_4) consisting of 20 population of BPH (54.07% and 57.04 %) followed by treatment (T_5) of 30 population of BPH (65.93 % and

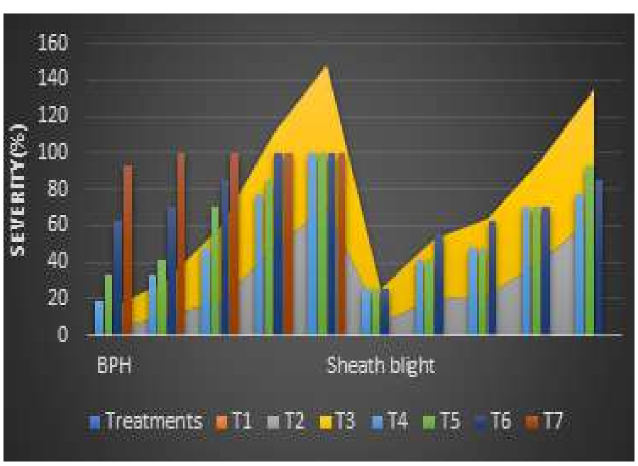

Fig. 2. Effect on sheath blight severity due to inoculation of *R. solani* 7 days after BPH release under greenhouse condition.

Table 6. Effect on sheath blight severity due to inoculation of R. solani 7 days after BPH release under greenhouse condition

Treatment No.	BPH infestation (%)						Sheath blight severity (%)						Plant height (cm)	Plant tillers (No.)
	43 DAS	46 DAS	49 DAS	52 DAS	55DAS	Mean	50 DAS	60 DAS	70 DAS	80 DAS	90DAS	Mean		
T_1	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00	95.67	12.00
T_2	3.70(11.10)	11.11(19.48)	18.52(25.50)	48.15(43.96)	70.37(57.05)	30.37	7.41(15.80)	19.30(26.07)	22.63(28.42)	40.74(39.68)	64.52(53.47)	30.92	91.67	11.00
T_3	11.11(19.48)	18.52(25.50)	40.74(39.68)	62.96(52.54)	77.78(61.91)	42.22	18.52(25.50)	33.33(35.28)	40.74(39.68)	55.56(48.21)	70.37(57.05)	43.70	90.00	10.33
T_4	18.52(25.50)	33.33(35.28)	48.15(43.96)	77.78(61.91)	100.00(90.05)	55.56	25.93(30.62)	40.74(39.68)	48.15(43.96)	70.37(57.05)	77.78(61.91)	52.50	87.67	9.00
T_5	33.33(35.28)	40.74(39.68)	70.37(57.05)	85.19(67.40)	100.00(90.05)	65.93	25.93(30.62)	40.74(39.68)	48.15(43.96)	70.37(57.05)	92.59(74.24)	55.56	85.00	8.33
T_6	62.96(52.54)	70.37(57.05)	85.19(67.40)	100.00(90.05)	100.00(90.05)	83.70	25.93(30.62)	55.56(48.21)	62.96(52.24)	70.37(57.05)	85.19(67.40)	60.00	83.00	7.78
T_7	92.59(74.24)	100.00(90.05)	100.00(90.05)	100.00(90.05)	100.00(90.05)	98.52	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00	80.33	7.19
SEm±	3.25	3.63	2.58	2.23	2.02	2.74	3.62	2.96	2.41	2.03	1.92	2.59	3.25	2.23
C.D@5%	9.56	9.42	8.11	7.65	5.01	7.95	8.47	9.51	8.13	7.20	5.63	7.79	9.62	8.85

Note: Figures in the parenthesis are arcsin transformed values

T_1: Control (0), T_2: 5 population of BPH, T_3: 10 population, T_4: 20 population, T_5: 30 population, T_6: 40 population, T_7: 50 population.

Table 7. Effect on sheath blight severity due to inoculation of *R. solani* 7 days before release of BPH under greenhouse condition.

Treatment No.	BPH infestation (%)						Sheath blight severity (%)						Plant height (cm)	Plant tillers (No.)
	43 DAS	46 DAS	49 DAS	52 DAS	55DAS	Mean	50 DAS	60 DAS	70 DAS	80 DAS	90DAS	Mean		
T_1	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00(0.00)	0.00	94.67	11.67
T_2	3.70(11.10)	11.11(19.48)	18.52(25.50)	48.15(43.96)	70.37(57.05)	30.37	7.41(15.80)	18.52(25.50)	25.93(30.62)	40.74(39.68)	55.56(48.21)	29.63	91.00	10.88
T_3	7.41(15.80)	18.52(25.50)	33.33(35.28)	62.96(52.54)	70.37(57.05)	38.52	18.52(25.50)	25.93(30.62)	33.33(35.28)	48.15(43.96)	62.96(52.24)	37.78	89.67	10.23
T_4	18.52(25.50)	25.93(30.62)	48.15(43.96)	77.78(61.91)	100.00(90.05)	54.07	25.93(30.62)	48.15(43.96)	55.56(48.21)	70.37(57.05)	85.19(67.40)	57.04	85.00	8.97
T_5	33.33(35.28)	40.74(39.68)	70.37(57.05)	85.19(67.40)	100.00(90.05)	65.93	25.93(30.62)	40.74(39.68)	48.15(43.96)	62.96(52.24)	85.19(67.40)	52.59	87.00	9.67
T_6	62.96(52.54)	62.96(52.54)	85.19(67.40)	100.00(90.05)	100.00(90.05)	82.22	18.52(25.50)	40.74(39.68)	55.56(48.21)	62.96(52.24)	62.96(52.24)	48.15	88.33	10.67
T_7	92.59(74.24)	100.00(90.05)	100.00(90.05)	100.00(90.05)	100.00(90.05)	98.52	11.11(19.48)	11.11(19.48)	11.11(19.48)	11.11(19.48)	11.11(19.48)	11.11	80.00	7.15
SEm ±	2.52	2.03	1.98	2.33	1.65	2.10	2.65	2.06	1.52	2.69	2.37	2.26	2.89	2.34
C.D @5%	8.63	7.99	6.65	7.44	6.20	7.38	8.52	7.86	6.98	6.36	6.19	7.18	8.76	9.29

Note: Figures in the parenthesis are arcsin transformed values.

T_1: Control (0), T_2: 5 population of BPH, T_3: 10 population, T_4: 20 population, T_5: 30 population, T_6: 40 population, T_7: 50 population.

52.59 %) and treatment (T_6) of 40 population of BPH (82.22 % and 48.15 %) and least severity shown by treatment (T_7) of 50 population of BPH treatment (98.52% and 11.11%) and (T_2) of 5 population of BPH (30.37 % and 29.63 %) followed by treatment (T_3) of 5 population of BPH (38.52 % and 37.78 %) whereas treatment (T_1) untreated control, but treatment (T_7) of 50 population of BPH with highest mean insect infestation percentage of 98.52 % showed 11.11 % disease severity due to early inoculation of *Rhizoctonia solani* after seven days release of insects causes complete infestation where the disease spread ceases (Table 7, Fig. 3).

Among the three interaction types, the highest disease severity was recorded in simultaneous inoculation of BPH and *Rhizoctonia solani*, followed by BPH release and Rhizoctonia solani inoculation after 7 days. The least disease severity was observed in (Rhizoctonia solani inoculation after 7 days of BPH release) (Table 5, 6 and 7).On the basis of plant height tiller count, in set 1, the highest value were recorded in treatment (T_1) untreated control (96.00 cm and 12.33 tillers) followed by treatment (T_2) of 5 population of BPH (92.33 cm and 11.33 tillers) and treatment (T_3) of 10 population of BPH (90.67cm and 10.67 tillers). the lowest values were observed in treatment (T_7) of 50 population of BPH (81.00cm and 7.33), followed by treatment (T_6) of 40 population of BPH (83.67cm and 8.00), treatment (T_5) of 30 population of BPH (85.67cm and 8.67) and treatment (T_4) of 20 population of BPH (88.00cm and 9.33) (Table 5, fig 1).

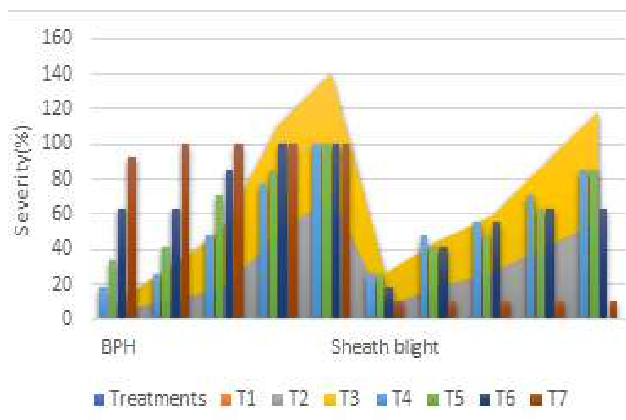

Fig. 3. Effect on sheath blight severity due to inoculation of *R. solani* 7 days before release of BPH under greenhouse condition.

In set 2 the treatment (T_1) recorded higher value (95.67 cm and 12.00), followed by treatment (T_2) of 5 population of BPH (91.67cm and 11.00) and treatment (T_3) of 10 population of BPH (90.00 cm and 10.33). the lower values were recorded in treatment (T_7) of 50 population of BPH (80.33 cm and 7.19) followed by treatment (T_6) of 40 population of BPH (83.00 cm and 7.78), treatment (T_5) of 30 population of BPH (85.00 cm and 8.33) and treatment (T_4) of 20 population of BPH (87.67cm and 9.00) (Table 6, Fig 2).

In set 3 the treatment (T_1) untreated control (94.67 cm and 11.67) shown a highest value, followed by treatment (T_2) of 5 population of BPH (91.00 cm and 10.88) and treatment (T_3) of 10 population of BPH (89.67 cm and 10.23) and least shown by treatment (T_7) of 50 population of BPH (80.00cm and 7.15) followed by treatment (T_4) of 20 population of BPH (85.00 cm and 8.97), treatment (T_5) of 30 population of BPH (87.00 cm and 9.67) and treatment (T_6) of 40 population of BPH (88.33 cm and 10.67) (Table 7, Fig. 3). Among the three sets, the highest plant height and plant tillers was recorded in the set 1 (Simultaneous inoculation of BPH and *R. solani*) followed by set 2 (Release of BPH and after 7 days inoculation of R. solani) and least was observed in set 3 (Inoculation of R. solani and after 7 days release of BPH) (Table 5, 6 and 7).

The present study was in accordence with Lee et al. (1984) who carried out the study using two susceptible varieties of BPH (IR22 and TN11). Both varieties, were inoculated with the sheath blight pathogen (*R. solani*) at maximum tillering stage with a hill of 10 tillers at 7 days after infestation with 100 fourth instar BPH/plot. Disease severity was significantly higher in the treatment where BPH and the sheath blight pathogen were present in combination than when the sheath blight pathogen was inoculated alone.

Similarly, Latin and Reed (1985) evaluated incidence and severity of Fusarium wilt on muskmelon seedlings grown in a soil-less substrate infested with microconidia of *Fusarium oxysporum* f. sp. *melonis* (FOM) and eggs of the striped cucumber beetle (*Acalymma vittatum*) (STCB). Disease incidence was observed at a lower inoculum level where treatment included STCB infestation. The increased incidence and severity of Fusarium wilt due to root-feeding by STCB larvae provide the rationale for controlling root-feeding stages of insects on muskmelon.

Fermaud and Lemenn (1992) conducted a greenhouse trial showed that conidia were introduced inside larval galleries by larvae contamination with fugus. The germination of conidia and resulting mycelial colonization followed on the inner surfaces of galleries. As for damage to ripe berries, third-generation larvae carrying viable conidia caused a 2.4% increase in disease severity at harvest as compared with larvae carrying dead conidia.

Correlation of the effect of Brown plant hopper on sheath blight disease severity inoculated simultaneously

All of the treatments showed sheath blight severity except untreated control. On the basis of mean insect infestation percentage, disease severity varied among the treatments.Treatment (T_2) of 5 population of BPH recorded highest value of 37.78 %, followed by treatment (T_5) of 30 population of BPH (70.37 %), (T_3) of 5 population of BPH (51.11 %), treatment (T_4) of 20 population of BPH (64.44 %), treatment (T_5) of 30 population of BPH (70.37 %) (T_3) and treatment (T6) consisting of 40 population of BPH (73.33 %). As the population density of BPH increased, sheath blight severity also showed corresponding increaseThe results revealed that there was a significant positive correlation between BPH and sheath blight severity in the rice. The regression coefficient (R^2) was 0.3163 indicating that 1 unit change in BPH infestation increase 31.63 per cent of the sheath blight severity. The difference in sheath blight disease severity with respect to BPH was significant, according to a significant test of correlation coefficient at the probability level of $p = 0.01$ (Table 4, Fig. 4).

Note: p value represents significance of correlation coefficient

N=30, ** Correlation is significant at 0.01 level (2- tailed)

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

Linear regression equation $Y= bx + a$

Where y= dependent variable *i.e.*, severity a = constant (y intercept)

b = regression coefficient

x = independent variable *i.e.*, respective nutrient

Correlation of the effect of Brown plant hopper on sheath blight disease severity inoculated after seven days of insect release

All of the treatments showed severity of sheath blight except untreated control. On the basis of mean insect infestation percentage disease severity changes , treatment (T_2) of 5 population of BPH (30.92 %) followed by treatment (T_3) of 10 population of BPH (43.70 %), treatment (T_4) of 20 population of BPH (52.50 %), treatment (T_5) of 30 population of BPH (55.56 %) and treatment (T_6) consisting of 40 population of BPH (60.00 %) as population density increases sheath blight severity also increases along with it.

The results revealed that there was a significant positive correlation between nitrogen content and sheath blight severity in the rice genotypes with a correlation coefficient (R) of 0.344. The regression coefficient (R^2) was 0.2238, indicating that 1 unit change in BPH infestation in the plant affects the 22.38 per cent severity of sheath blight. According to a significant test of correlation coefficient at the probability level of p = 0.01, the difference in sheath blight disease severity with respect to BPH infestation was significant (Table 4, Fig. 4).

Correlation of the effect of Brown plant hopper (inoculated after 7 days) on sheath blight disease severity

All of the treatments showed severity of sheath blight except untreated control. On the basis of mean insect infestation percentage disease severity changes , treatment (T2) of 5 population of BPH (29.63 %) followed by treatment (T_3) of 10 population of BPH (37.78 %), treatment (T_6) of 40 population of BPH (48.15 %), treatment (T_5) of 30 population of BPH (52.59 %) and treatment (T_4) consisting of 20 population of BPH (57.04 %) as population density increases from

Table 4. Correlation (R) and regression coefficient (R2) between sheath blight severity and brown plant hopper.

SI. no.	Sets of interaction	Disease severity	
		R	Regression equation
1	Simultaneous inoculation of BPH and *Rhizoctonia solani*	0.339**	Y = 0.3163x + 23.927
2	Release of BPH and followed by after 7 days inoculation of *Rhizoctonia solani*	0.344**	Y = 0.2238x + 21.94
3	Inoculation of *Rhizoctonia solani* and after 7 days release of BPH	0.263*	Y= 0.2024x + 23.8

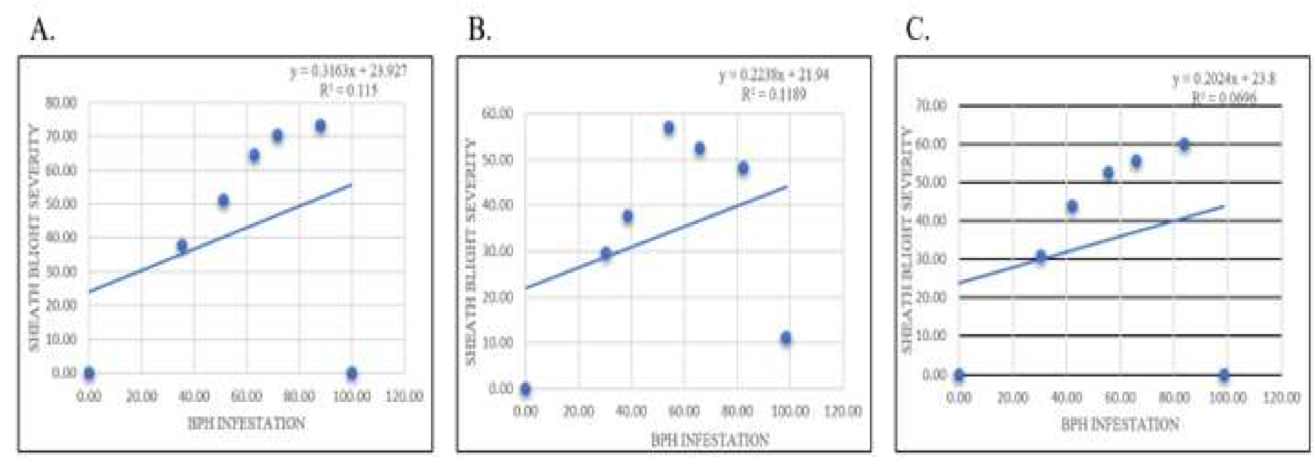

Fig. 4. Correlation (R) and regression coefficient (R^2) between sheath blight severity and brown plant hopper infestation.
A. Correlation of the effect of Brown plant hopper on sheath blight disease severity inoculated simultaneously.
B. Correlation of the effect of Brown plant hopper on sheath blight disease severity inoculated after 7 days of insect release.
C. Correlation of the effect of Brown plant hopper (inoculated after 7 days) on sheath blight disease severity.

T_2 to T_3 sheath blight severity also increases from T_4 to T_7 severity decreases (Table 4, Fig. 4).

The results showed a positive relationship between BPH infestation and sheath blight severity in the genotypes with a correlation value (R) of 0.263. The regression coefficient (R^2) was 0.2024, indicating that a 1unit change in BPH infestation in plant affects 20.24 per cent severity of sheath blight. The difference in sheath blight disease severity with respect to BPH infestation was significant, according to a significant test of correlation coefficient at the probability level of $p = 0.01$.

The present study was in accordance with Duncan et al. (2004) findings, where populations of Tylenchulus semipenetrans and Phytophthora parasitica were measured weekly during 27 months in an orchard of mature grapefruit trees on rough lemon rootstock. The study was conducted to identify potential key climatic and host factors affecting population changes in both parasites. The average monthly levels of P. parasitica protein in roots were best fit ($R^2 = 0.76$) by linear models incorporating soil moisture, temperature, and concentrations of ketone sugars in roots. Root mass density and concentration of ketone sugars explained 86% of the monthly variation in P. parasitica propagule densities in soil. Experimental verification of causality in these relationships would help explain seasonal and annual variation in the parasite burden posed by these two pathogens.

The findings of the present study align with Leath and Hower (1993), who reported a strong association between insect herbivory and fungal pathogen dynamics in plants. Their research highlighted the role of the clover root curculio (CRC) in facilitating fungal infections, particularly by Fusarium oxysporum f. sp. medicaginis, which was the predominant pathogen isolated. The interaction between CRC feeding and fungal colonization suggests that mechanical damage caused by insect feeding creates entry points for fungal pathogens, thereby intensifying disease severity.

CONCLUSION

In this study, the interaction between *R. solani*, the sheath blight pathogen, and the brown planthopper (*N. lugens*) was explored to understand the combined impact on rice. Our findings revealed a synergistic relationship, where N. lugens infestation increased rice susceptibility to sheath blight infection. The feeding activity of the brown planthopper caused physiological stress and weakened the host plant, creating favorable conditions for R. solani infection. Conversely, sheath blight lesions provided additional feeding sites, potentially enhancing N. lugens survival. This dual infestation led to significantly higher yield losses compared to individual infections. Our results underscore the synergistic effect of BPH infestation on sheath blight severity. Further research on the underlying mechanisms could aid in the development of effective control strategies.

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Planting environment, nutrition and storage effects on yield and quality of red-husked non-basmati aromatic rice

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ABSTRACT

Two separate experiments were conducted at University Farm and at Aromatic Rice Laboratory, Bidhan Chandra Krishi Vishwavidyalaya, West Bengal, India, to assess the effect of planting environment, nutrition, and storage methods on flowering behaviour, yield, and quality of red-husked non-Basmati aromatic rice (cv. Lal Badshabhog). The flowering and day length relations under varied planting times were: 10 July [D10J (105-109 d and 11.85-11.91 hr)], 25 July [D25J (102-103 d and 11.71-11.74 hr)], and 10 August [D10A (96-98 d and 11.55-11.60 hr). The mean light extinction coefficient (k) of the variety was 0.37 at the flowering stage, indicating its semi-erect leaves. Planting on D10A led to less duration (123.3 d), greater grain yield (2.72 t ha^{-1}), amylose content (17.5%) and medium aroma (score 1.74) compared to early plantings (D_{10J} and D_{25J}); while close planting ($S_{15\times15}$, or 44 hills m^{-2}) improved the grain yield by 4.0% over wider spacing ($S_{20\times15}$ or 33 hills m^{-2}). Sole application of chemical fertilizers ($N_{50}P_{25}$K25 kg ha^{-1}) resulted in higher grain yield and protein content (7.8%) compared to organic or integrated nutrient management. Storage of paddy for a 3-month (3-m) period in an earthen pot (CE_p) had a favourable effect on head rice recovery (HRR), protein content, and aroma compared to jute-made gunny bag (C_{Gb})) and polypropylene bag (C_{Pb}). The cool storage environment (8±2^{o}C) favoured greater HRR (56.6%) and aroma in 3-m storage, while it improved HRR and elongation ratio (1.81) than ambient condition (28±4^{o}C) during 6-m post-harvest period.

Key words: *Aromatic rice, grain quality, nutrition, planting time, storage methods, yield*

INTRODUCTION

India is a leader in aromatic rice production and export in the world. Basmati is prized for its super-fine kernel (>7.5 mm, L/B ratio >3.0), soft fluffy rice, and pleasant aroma (Juliano 1972; IARI, 1980), but small and medium-grain non-Basmati aromatic rice varieties are traditionally grown in different regions and states of the country. The state of West Bengal has a great diversity in aromatic rice landraces since long (Singh et al., 2000; Shobharani and Krishnaih, 2001), and it is estimated that aromatic rice occupies about 90,000-1,00,000 ha with a production of 2.5-3.0 lakh tonnes paddy every year (Ghosh, 2019).

Lal Badshabhog, a small-grain non-Basmati type red-husked aromatic rice, reported from Purulia district of West Bengal (Deb, 2005) has been cultivated in Red and Laterite Zone of the state. The nomenclature of Lal Badshabhog paddy probably originated from three Bengali words 'Lal' meaning red, 'Badsha' meaning King or emperor, particularly in the Mughal regime, and 'blog' meaning pulse-mixed rice offered to Hindu God or Goddess (Ghosh et al., 2019). Lal Badshabhog is mainly adapted in rainfed medium land with a duration of 130-135 days, whose major characteristic features are: plant height 140-150 cm, grain yield 2.2-2.6 t ha^{-1}, red-husked grain, short-bold type white kernel and medium aroma.

Lal Badshabhog, being an indigenous photosensitive rice crop, is highly influenced by temperature, day length, and sunshine hours toward its performance in terms of growth, yield, and quality. Sharma et al. (2011) observed that Basmati rice could not express its full yield potential due to indefinite transplanting time and poor plant population per unit area. Therefore, the sowing or planting period is an important non-monetary practice considering the weather-dependent correlations with yield and quality parameters of aromatic rice. Spacing determines the plant population in a unit area, thereby influencing the efficiency and yield of the crop. Plant population above optimum increases competition among plants for light and nutrients that weaken the plant, and ultimately, low yield is obtained (Mondal et al., 2013). The nutrition of such non-Basmati rice is traditionally provided by locally available organic manures or combined with chemical fertilizers in native districts of the state. Such untested varied nutrient schedules practiced by the farmers need refinement, taking different organic sources like farm yard manure (FYM), mustard cake, vermicompost, green manure, bio-fertilizer, etc., along with straight or compound chemical fertilizers available in the market. Moreover, combining organic and inorganic fertilizers could change the phenological events in rice by modulating the photosynthetic capacity of leaves (Shaukat et al., 2020). Keeping these in view, the study on the performance of non-basmati rice under varied planting environments, spacing, and nutrient management is important for increased yield and better grain quality, hopefully leading to timely accommodation of such premium rice in multi-crop sequence for longer sustainability and greater profitability of the system.

Rice aging is a complicated process, which involves changes in the physical and chemical properties of a rice grain, with variations due to storage methods, time, and environment. Although the overall starch, protein, and lipid contents in rice grain remain essentially unchanged during storage, structural changes occur, which affect the flavour and texture of cooked rice (Zhou et al., 2002). On the other hand, low-capacity storage structures have varied influences on grain damage, milling quality, protein content, etc., during a year-long storage period (Ilyas et al., 1983). Yoshihashi et al. (2005) investigated the effect of package and temperature on 2-acetyl 1-pyrroline (2AP) content in milled aromatic rice during storage, where 2AP was decreased faster at higher storage temperature. However, the information on storage effects on short-grain non-Basmati aromatic rice is insufficient, which is necessary for standardization of locally-feasible storage methods for non-Basmati paddy for sustenance of the grain quality including aroma during post-harvest period.

MATERIALS AND METHODS

Experimental details

Experiment 1

The field experiment was conducted at 'C' Block Farm (22°58' N latitude, 88°26' E longitude and 15.9 m altitude) of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Kalyani, Nadia, West Bengal, India. The experiment was laid out in a split split-plot design replicated thrice comprising three planting dates [10 July (D_{10J}), 25 July (D_{25J}) and 10 August (D10A)] in main plots, two spacings [20 cm × 15 cm ($S_{20\times15}$), and 15 cm × 15 cm ($S_{15\times15}$)] in subplots, and three nutrient management practices [100% RD Organic (NO, 25% RD FYM+75% RD Mustard cake), 100% RD Inorganic (NCF, $N_{50}P_{25}K_{25}$), and 50% RD Organic+50% RD Inorganic (NO + CF, 25% RD FYM + 25% RD Mustard cake + $N_{25}P_{12.5}K_{12.5}$)] in sub-sub plots during rainy (*kharif*) seasons of 2014 and 2015. Seeds of Lal Badshabhog paddy collected from the RKVY Project on 'Bengal Aromatic Rice' of BCKV were sown @ 20 kg ha^{-1} in the wet nursery. 24-25 days old seedlings @ 2-3 $hill^{-1}$ were transplanted as per planting time and spacing schedule at a shallow depth (3-4 cm) in puddled field. Manual weeding was done at 3 and 6 weeks after transplanting (WAT), and other crop management practices were adopted as per standard recommendations.

The daily meteorological data were collected from the automated weather observatory under AICRP on Agro-meteorology, BCKV, West Bengal. The month-wise maximum and minimum temperature (T_{max} and T_{min}) during the cropping period was found to vary between 15.9 and 35.4°C in 2014, and 18.8 and 34.6°C in 2015, and the rainfall received for the growing seasons was 130.7 cm and 127.3 cm, respectively. The variation in monthly average maximum relative humidity (RH) was 79.7 and 95.8% during the first year and 90.3 and 97.8% during the second year. The bright sunshine (BS) hour was lower in high rainfall months mainly due to cloud-cast days and that ranged between 4.6 (Aug.) and 7.5 h (Nov.) during 2014, and between 2.5 (Oct.) and 7.3 h (Nov.) during 2015. The weather parameters at different phenophases under varied sowing and planting dates are shown in Figure 1.

Experiment 2

The second experiment on storage was done at Aromatic Rice Laboratory, Department of Agronomy, BCKV, Mohanpur, Nadia, West Bengal, India. The effect of storage containers and temperature on grain quality of Lal Badshabhog rice during different length of storage periods was tested in a 2-factor completely randomized design (CRD) with 3 replications during 2014-15 and 2015-16. The first factor consisted of three storage containers, viz. Gunny bag (C_{Gb}, jute-made mainly used for storing paddy, pulses and seeds in rural areas), Polypropylene bag (C_{Pb}, generally used for storing sugar, and seeds in recent times) and Earthen pot (C_{Ep}, locally available, generally used for sweets, curd and small quantity seeds); while the second factor included two storage temperature, 8±2^{o}C (T_8, lower case of domestic refrigerator) and 28±4^{o}C (T_{28}, ambient condition at laboratory).

The average T_{max} and T_{min} during the storage period of first year (Jan. to June of 2015) were 32.1ºC and 19.6ºC respectively, while those were 32.7ºC and 19.3 ºC during second year storage period (Jan. to June of 2016). The month-wise recorded RH_{min} and RH_{max} revealed that it was varied between 48.1 and 96.5% during the storage period of 2015 and between 48.3 and 97.8% during 2016.

METHODOLOGY AND FIELD DATA COLLECTION

Plant characters, light interception, yield and lodging

Final plant height, yield attributes and grain yield were recorded at maturity. But leaf area index (Watson, 1958), light transmission ratio (LTR) and light extinction coefficient (Saeki, 1963) were recorded at flowering stage. The rating of lodging of plants was done at hard dough stage following Standard Evaluation System for Rice (IRRI, 1996) in a 9-point scale (1: no lodging, 3: most plants slightly lodge, 5: most plants completely lodge, 7: most plants nearly flat, and 9: all plants flat).

Thermal indices

Growing degree days (GDD) at different phenophases as well as for entire life cycle was determined as the difference between the mean daily temperature (T_{mean}) and the base temperature (T_b) of crop [GDD= {(Tmax + T_{min}) / 2} - T_b] (Nuttonson, 1955), where 10^{o}C was the base temperature for rice. The heliothermal units (HTU) was calculated [PTU= GDD × Bright sunshine hour] following Singh et al. (1990).

Determination of grain quality

Milling quality

100 g clean paddy sample of each experimental unit was processed in 3 steps: dehulling (Rice Sheller, Type THU 35B, Satake make, Japan), milling (Rice Miller, Type TM 050, Satake make, Japan) and grading (Indosaw make, India) to determine the head rice recovery [HRR= Weight of head rice (g) / Weight of rough rice (g) × 100].

Cooking and nutritional quality

The amylose content in milled rice was estimated following the method of Juliano (1971) with some modifications. Nitrogen content in oven-dried grain samples was determined by micro-Kjeldahl method, and that was multiplied by 5.95 to determine the protein content of rice.

The alkali digestion test was carried out by putting 10 whole milled rice kernels having no cracks in a Petridish containing dilute aqueous KOH at room temperature (±25^{0}C) for 23 hours (Bhattacharya and Sowbhagya, 1972), and thereafter the kernels were rated for alkali spreading value (ASV) in 7-point scale: (1: kernel not affected; 2: kernel swollen, 3: kernel swollen, collar complete and narrow, 4: kernel swollen, collar complete and wide, 5: kernel split or segregated, collar complete and wide, 6: kernel dispersed, merging with collar, and 7: kernel completely dispersed and intermingled) to estimate the gelatinization temperature (GT).

Processing quality

The length of 10 raw kernels for each treatment was recorded by a dial micro-meter, while the lengths of cooked kernels were measured by placing them on graph paper. The elongation ratio (ER) was calculated as: [ER = Length of cooked rice kernel (mm) / Length of raw rice kernel (mm)]

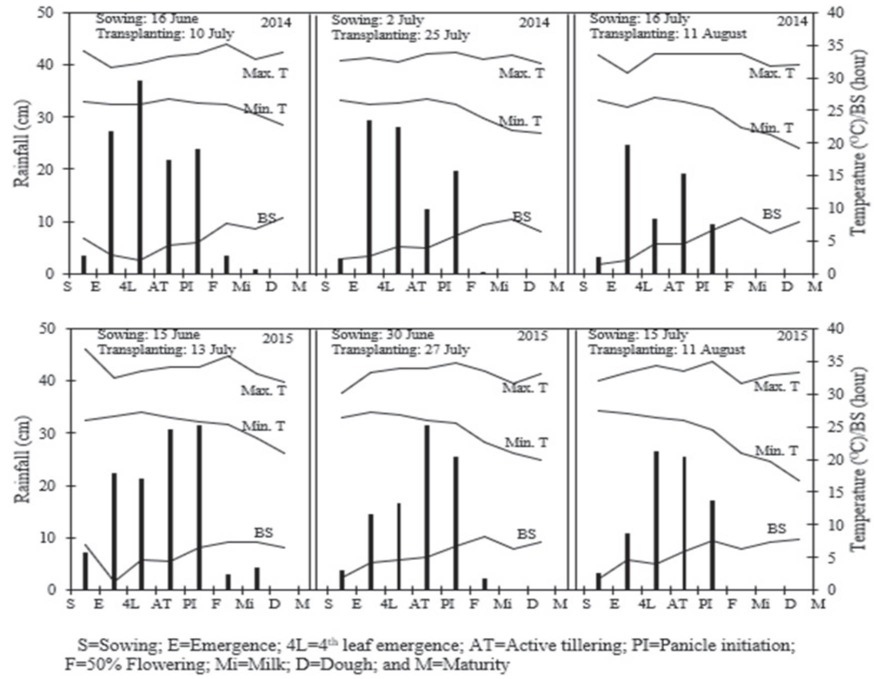


Fig. 1. Meteorological parameters at different phenophases of aromatic rice (cv. Lal Badshabhog) under different sowing and planting dates during 2014 and 2015.

The aroma of milled rice was estimated in 0.1 N KOH solution following the method of Nagaraju et al. (1991). A panel of experts scored the intensity of aroma on a 3-point scale (1: mild, 2: medium, and 3: strong).

Nutrient uptake

Total and available N, P, K in plant were determined by standard methods (Jackson 1973), and nutrient uptake was estimated by multiplying the dry matter yield of grain and straw by their respective nutrient percentages.

Statistical analysis

The data recorded in the study were analysed using Fisher's 'Analysis of Variance' technique as per the procedures described by Gomez and Gomez (1984), and the mean differences were compared at 5% level of significance. Correlations among yield and grain quality parameters with thermal indices at ripening stage were determined.

RESULTS AND DISCUSSION

Flowering, duration and yield

Flowering and phenology

The field-based observation on flowering behaviour revealed that early (10 July), mid (25 July) and late (10

Table 1. Flowering period and day length of aromatic rice (cv. Lal Badshabhog) under varied dates of sowing and planting at Kalyani, West Bengal, India.

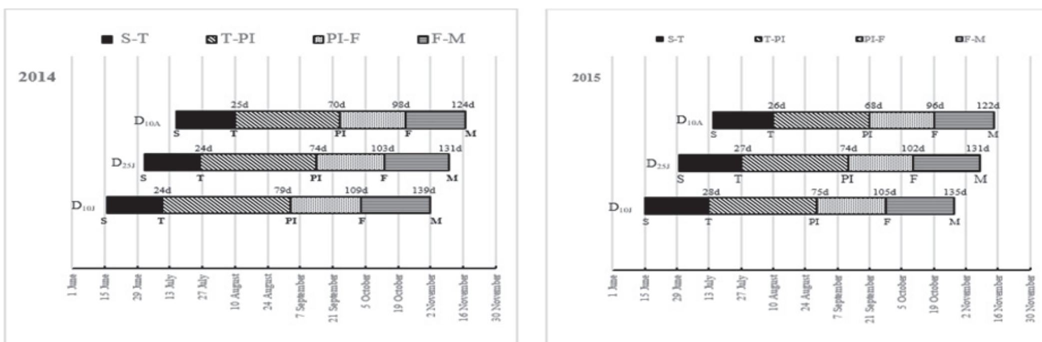
Year	Sowing date	Planting date	Flowering 50% flowering (d)	Period (date)	Day length (hour)
2014	16 June	10 July	29 September - 4 October	11.85	109
	2 July	25 July	9 - 15 October	11.71	103
	16 July	11 August	19 - 25 October	11.55	98
2015	15 June	13 July	25 - 30 September	11.91	105
	30 June	27 July	7 - 12 October	11.74	102
	15 July	11 August	16 - 22 October	11.60	96

The range of T_{max}, T_{min}, and BS during flowering period were 33.5 - 36.4°C, 20.4 - 25.5°C and 5.10-9.4 hr during 2014, and 33.3 - 35.1°C, 23.8 - 25.8°C and 6.8 - 8.8 hr during 2015.

Table 2. Meteorological parameters at ripening phases under different planting dates of aromatic rice (cv. Lal Badshabhog) during wet season.

Year	Planting time	Flowering to Milk				Milk to Dough				Dough to Maturity			
		Tmax (°C)	Tmin (°C)	BS (hr)	R_{total} (mm)	Tmax (°C)	Tmin (°C)	BS (hr)	R_{total} (mm)	Tmax (°C)	Tmin (°C)	BS (hr)	R_{total} (mm)
2014	10 July	35.8	25.3	7.4	2.9	33.1	23.2	7.3	4.5	31.8	20.9	6.5	0.1
	25 July	33.5	22.7	8.3	2.4	31.7	20.9	6.3	0.1	33.2	19.9	7.4	0
	11 August	31.7	21.0	6.3	0.1	33.0	19.8	7.5	0	33.2	16.7	7.7	0
2015	13 July	35.1	25.9	3.3	60.6	32.9	24.4	6.8	0.9	33.9	22.8	8.6	0
	27 July	32.9	23.8	0.2	0	33.4	21.9	8.3	0	32.2	21.5	6.6	0
	11 August	33.7	22.4	0.0	8.0	31.9	21.3	6.3	0	32.1	19.3	8.0	0

T_{max}: Maximum temperature; T_{min}: Minimum temperature; BS: Bright sunshine; R_{total}:Total rainfall



S: Sowing; T: Transplanting; PI: Panicle Initiation; F: Flowering; M: Maturity

Fig. 2. Phenophase duration of aromatic rice (cv. Lal Badshabhog) under different planting dates during 2014 and 2015.

August) planted Lal Badshabhog rice recorded days to 50% flowering and average day length as: D_{10J} (109 d and 11.85 h), D_{25J} (103 d and 11.71 h), and D_{10A} (98 d and 11.55 h) in 2014, and D_{10J} (105 d and 11.91 h), D_{25J} (102 d and 11.74 h), and D_{10A} (96 d and 11.60 h) in 2015 at Kalyani, West Bengal (Table 1 and Fig. 2). The meteorological parameters during flowering to milk (F-Mi), milk to dough (Mi-D) and dough to maturity (D-M) stages are presented in Table 2, which shows the ripening period of delayed planted crops advances to autumn in West Bengal, India. The days to maturity of Lal Badshabhog rice was reduced by 13.7 d due to delay in planting from D_{10J} (137.0 d) to D_{10A} (123.3 d) (Table 3). Spacing could not influence the duration of Lal Badshabhog rice, but nutrient management had significant effect on days to maturity. Plants under 100%RD organic nutrient system (NO) had longer life duration (132.9 d) compared to chemical fertilizer-based (129.4 d) or integrated nutrient management (128.6 d) in the study. Shaukat et al. (2020) reported that organo-mineral treatments promoted the early tillering and anthesis, but prolonged the grain-filling period thereby the physiological maturity of rice. Besides, Vilayvong et al. (2015) observed that differential planting density affected days to anthesis and maturity of rice.

Leaf growth, light interception and extinction coefficient

Lal Badshabhog rice plants produced long, narrow and green leaves with split-type ligule and sickle-shaped auricles at leaf base. The attitude of flag leaf was semi-erect at flowering and near maturity stage. The foliage growth of rice in terms of LAI was increased consistently up to 84 DAT (*i.e.*, post-anthesis stage) and declined thereafter due to drying and withering of lower leaves during the ripening phase. Later planted crops (10 August) recorded a maximum LAI (4.88) at the flowering stage in the experiment (Table 3). The planting geometry had significant effect on foliage growth of Lal Badshabhog rice in later phase of life cycle, *i.e.*, at flowering stage during *kharif* season; where closely spaced crop ($S_{15×15}$) recorded greater leaf area due to more number of hills in unit area. However, Singh et al. (2012) reported that wider spacing

Table 3. Effect of planting date, spacing and nutrient management on plant characters, light interception and yield of aromatic rice (cv. Lal Badshabhog).

Treatment	Duration (d)	Plant hei -ght at harvest (cm)	Leaf area index at flowering	Light transmission ratio at flowering	Light extinction coefficient at flowering(k)	Panicles per m^2	Filled grains per panicle	Grain yield (t ha^{-1})	Lodging (score)
Planting date (D)			4.88a	19.6a	0.34c				
10 July (D_{10J})	137.0a	160.2a	4.44b	19.9a	0.37b	260b	125.8c	2.41c	3.4b
25 July (D_{25J})	130.6b	153.0b	3.99c	20.8a	0.40a	272ab	133.0b	2.62b	2.8b
10 August (D_{10A})	123.3c	152.6b				281a	142.6a	2.72a	2.2a
Spacing (S)									
20 cm × 15 cm ($S_{20×15}$)	130.4a	156.1a	4.30b	20.0a	0.38a	262b	132.5a	2.53b	2.9a
15 cm × 15 cm ($S_{15×15}$)	130.3a	154.4a	4.57a	20.2a	0.35b	279a	135.1a	2.63a	2.7a
Nutrient management (N)									
100% RD organic (N_O)	132.9a	153.1a	4.27b	21.3a	0.37a	262c	130.9b	2.50b	2.2c
100% RD inorganic (N_{CF})	129.4b	156.9a	4.55a	18.7b	0.38a	278a	136.0a	2.64a	3.3a
50% RD organic + 50% RD inorganic (N_{O+CF})	128.6c	155.8a	4.49a	20.3a	0.36a	272b	134.5a	2.61a	2.8b
D	*	*	*	ns	*	*	*	*	*
S	ns	ns	*	ns	*	*	ns	*	ns
N	*	ns	*	*	ns	*	ns	*	*
D × S	ns	ns	ns	ns	*	*	ns	ns	ns
D × N	ns	ns	ns	ns	ns	*	ns	ns	ns
S × N	ns	ns	ns	ns	ns	ns	ns	ns	ns
D × S × N	ns	ns	ns	ns	ns	*	ns	ns	ns

Values followed by the same letter in the same case in each column are not significantly different ($P < 0.05$)

(30 cm × 30 cm) favoured the production of more leaves with greater size leading to higher LAI values of three aromatic rice varieties (Pusa Basmati 1; Pusa Sugandh 5 and Pusa Sugandh 3) at 30 and 60 DAT compared to closer spacing (25 cm × 25 cm) at New Delhi. Sole application of chemical fertilizers ($N_{50}P_{25}K_{25}$) usually resulted in highest LAI values at flowering stage (4.55) being mostly on par with integrated nutrient dose (50% RD inorganic + 50% RD organic) in gangetic alluvial soil of West Bengal. On the other hand, 100% organic sources of nutrients (FYM + mustard cake) had slow and less effect toward the foliage growth of Lal Badshabhog paddy in the investigation.

Mean light intensity at crop canopy and ground level, averaged over treatments and years, were 94869 and 19069 lux at the flowering stage, while mean light interception by the canopy of Lal Badshabhog paddy was 20.1% at the same stage, which might be due to panicle exertion at pre-flowering stage. The light transmission ratio (LTR) values, in general, showed an inverse relationship with their respective LAI values because of the influence of foliage toward penetration of light at the ground. The mean light extinction coefficient (k), averaged over either planting time or spacing or nutrient management, was 0.37 at the flowering stage, indicating its semi-erect leaves. Widely spaced crops (20 cm × 15 cm) recorded greater k values at the flowering stage compared to closely planted ones (15 cm × 15 cm) in the study.

Yield and correlations with thermal indices

Among rice yield deciding factors, transplanting date, plant population per unit area and nutrition are considered important. The yield components (*viz*. number of panicles per m^2, number of filled grains per panicle and test weight) and grain yield usually differed significantly among planting time, spacing and nutrient management treatments in the investigation (Table 3). Lal Badshabhog paddy planted on 10 August recorded a maximum number of panicles (281 m^{-2}) and filled grains per panicle (142.6) compared to two July plantings (D_{10J} and D_{25J}) during the wet season. Close planting ($S_{15×15}$) resulted in a greater number of panicles per m^2 (279.4) compared to a widely-spaced one

($S_{20×15}$). However, the field data indicated that there was more panicles per hill in a widely spaced plot (mean 7.9 vs. 6.4) than in a closely planted unit. Thus, it could be concluded that more number of hills in the unit area (44 hills per m^2) under closer spacing ($S_{15×15}$) could produce a greater number of tillers per se panicles per m^2 after the flowering stage, even with less tillering ability than wider spacing ($S_{20×15}$ or 33 hills per m^2). The chemical fertilizer-based nutrient management (100% RD inorganic) recorded maximum number of panicles in 1 m^2 area (278.0), filled grains per panicle (136.0), and 1000 grain weight (10.60 g) compared to organic (N_O) and combined nutrient sources (N_{O+CF}) in the study.

Lal Badshabhog rice planted on 10 August (D_{10A}) produced the highest grain yield (2.72 t ha^{-1}), which was 3.8 and 12.8% greater over D_{25J} and D_{10J} plantings, respectively (Table 3). The ANOVA of the experiment showed that total variation in sum of squares (SS) due to environment [Year (Y) + Planting time (D)] was 42.90%, of which D was responsible for the greatest amount of total variation in SS (33.50%) for grain yield, while year was a lesser factor (9.40%) (Table 4). The negative correlation of GDD and HTU with grain yield (P<0.01) during the ripening period indicated that the delayed planted crop could get a favourable environment for grain-filling and yield (Table 5). Square planting ($S_{15×15}$ or 44 hills per m^2) improved the grain yield of Lal Badshabhog paddy by 4.0% (2.63 vs. 2.53 t ha^{-1}) over wider spacing ($S_{20×15}$ or 33 hills per m^2) mainly due to greater production of panicles (279 vs. 262 m^{-2}). A similar type of finding was reported with the adoption of 25 cm × 25 cm spacing in scented rice fields compared to wider planting density (30 cm × 25 cm) in New Delhi, India (Singh et al., 2012). Among three nutrient management practices, sole application of chemical fertilizers @ $N_{50}P_{25}K_{25}$ kg ha^{-1} resulted in significantly highest grain yield (2.64 t ha^{-1}) being at par with integrated dose (50%RD organic +50%RD chemical fertilizer) during wet season in lower gangetic plains. It might be due to the fact that chemical fertilizers (urea, single super phosphate and muriate of potash) supplied plant nutrients at faster rate and improved the yield components of Lal Badshabhog rice over the organic nutrient sources (FYM and mustard cake) in New Alluvial Zone of West Bengal. Swamy and Singh (2020) recommended application of 60 kg ha^{-1} through urea in combination with Azolla @ 1.6 t ha^{-1} for optimum

Table 4. Effect of planting date, spacing and nutrient management on grain quality and nutrient uptake of aromatic rice (cv. Lal Badshabhog).

Treatment	Head rice (%)	Amylose (%)	Protein (%)	Aroma (score)	N uptake at harvest (kg ha^{-1})	P uptake at harvest (kg ha^{-1})	K uptakeat harvest (kg ha^{-1})
Planting date (D)					40.6a	15.6a	30.9a
10 July (D_{10J})	56.2b	17.0b	7.6a	1.5c	41.2a	15.7a	30.4a
25 July (D_{25J})	58.2a	17.4a	7.7a	1.6b	41.6a	16.1a	29.7a
10 August (D_{10A})	56.7b	17.5a	7.8a	1.7a			
Spacing (S)							
20 cm × 15 cm ($S_{20×15}$)	57.2a	17.4a	7.7a	1.6a	40.6a	15.8a	29.6b
15 cm × 15 cm ($S_{15×15}$)	56.9a	17.1b	7.7a	1.6a	41.7a	15.8a	31.0a
Nutrient management (N)							
100% RD organic (N_O)	57.4a	17.2a	7.6b	1.6a	40.4b	15.2b	28.4b
100% RD inorganic (N_{CF})	56.7b	17.3a	7.8a	1.6a	41.7a	16.1a	31.6a
50% RD organic + 50% RD inorganic (N_{O+CF})	57.1ab	17.4a	7.7ab	1.6a	41.4ab	16.1a	31.0a
D	*	*	ns	*	ns	ns	ns
S	ns	*	ns	ns	ns	ns	*
N	*	ns	*	ns	*	*	*
D × S	ns	*	*	*	ns	ns	ns
D × N	*	ns	ns	ns	ns	ns	ns
S × N	*	*	ns	*	ns	ns	ns
D × S × N	*	*	ns	ns	ns	ns	ns

Values followed by the same letter in the same case in each column are not significantly different (P <0.05) production of rice in Meghalaya.

Lodging

The field-based observation indicated that most (>50%) of Lal Badshabhog plants had a general tendency to lodge down slightly (score ±3.0) at hard dough stage. The lodging habit was found to decrease with delay in planting from 10 July (score 3.4) to 10 August (score 2.2), but spacing and nutrient management caused non-significant variation in the lodging tendency of Lal Badhabhog rice in the study (Table 3). On the contrary, Unan et al. (2013) reported an increase in sowing density led to increase lodging tendency of two rice varieties in Turkey (Karadeniz and Osmancik 1997).

Grain quality

Milling quality

The variation in planting time, spacing and nutrient management caused significant influence on some grain quality parameters of Lal Badshabhog rice in the first experiment (Table 4). The grains obtained from 25 July planted crop resulted in maximum HR yield (58.2%) compared to early (D_{10J}) and late planting (D_{10A}), which could partly be supported by Hossain et al. (2007) for aromatic rice varieties at Dinajpur, Bangladesh. The ANOVA showed that year (Y) and planting time (D) contributed 0.02 and 24.03%, combining 24.05% due to the environment toward the HRR of Lal Badshabhog rice (Table 5). Moreover, the negative influence of HTU on HR yield (r = -0.200*) during milk to dough stage was noted (Table 6). The application of 100%RD through organic sources (FYM + mustard cake) resulted in highest HRR (57.4%) compared to N_{CF} or N_{O+CF}, and similar effects of organic manures or integrated nutrient management for better HRR of Basmati rice varieties compared to chemical fertilizer-based practices were also reported (Prakash et al. 2002; Longanadhan and Rajeswari 2005 and Saha et al. 2007).

Storage container had significant effect on HRR of 3-m aged Lal Badshabhog paddy only during both the years and pooled values in second experiment (Table 7). The paddy grains stored in CEp or CPb usually yielded higher head rice, while CGb always recorded lowest HRR during both the years. Based on pooled data of HRR for 3-m aged paddy, three storage containers could be arranged as: Earthen pot (56.0%) > Polypropylene bag (55.7%) > Gunny bag (55.0%). The HR yield of 3-m stored Lal Badshabhog paddy was significantly influenced due to variation in storage temperature during both the years of investigation and pooled values; while that of 6-m aged paddy varied significantly in first year only. Cool storage condition

Table 5. ANOVA for grain yield, head rice recovery and aroma of aromatic rice (cv. Lal Badshabhog).

Source	df	Grain yield			Head rice recovery			Aroma		
		% TSS	F value	Pr (>F)	% TSS	F value	Pr (>F)	% TSS	F value	Pr (>F)
Year	1	9.40	12.96	0.023	0.02	0.04	0.857	3.34	4.42	0.103
Rep. within year	4	2.90	3.05	0.010	2.37	1.23	0.372	3.02	0.88	0.516
Planting date (D)	2	33.50	162.87	0	24.03	24.83	0.0004	32.91	19.23	0.001
Y × D	2	3.17	15.39	0.002	6.47	6.69	0.020	0.77	0.45	0.654
Pooled error (a)	8	0.82	-	-	3.87	-	-	6.85	-	-
Spacing (S)	1	5.17	14.07	0.003	0.85	2.67	0.128	0.53	1.63	0.227
D × S	2	0.19	0.24	0.793	0.57	0.90	0.434	3.43	5.24	0.023
Y × S	1	0.25	0.62	0.447	0.27	0.85	0.375	0.31	0.94	0.351
Y × D × S	2	0.26	0.32	0.730	4.48	7.03	0.010	2.49	3.81	0.052
Pooled error (b)	12	4.87	-	-	3.83	-	-	3.92	-	-
Nutrient management (N)	2	7.58	10.65	0.0001	2.54	4.05	0.024	1.18	1.07	0.350
D × N	4	0.81	0.57	0.687	6.57	5.24	0.001	2.40	1.09	0.372
S × N	2	0.87	1.22	0.304	3.36	5.36	0.008	5.04	4.57	0.015
Y × N	2	0.51	0.72	0.490	5.13	8.18	0.001	0.06	0.06	0.946
D × S × N	4	0.80	0.56	0.690	12.16	9.69	0	3.70	1.68	0.171
Y × D × N	4	2.62	1.84	0.137	4.95	3.94	0.008	1/85	0.84	0.506
Y × S × N	2	7.36	10.35	0.0002	0.71	1.14	0.329	0.01	0.01	0.990
Y × D × S × N	4	1.31	0.92	0.461	2.76	2.20	0.083	1.74	0.79	0.537
Pooled error (c)	48	17.07	-	-	15.05	-	-	26.44	-	-
Total	107									

Table 6. Correlations of thermal indices at post-anthesis stages with yield and quality parameters of aromatic rice (cv. Lal Badshabhog).

Yield and quality parameter	Flowering to Milk		Milk to Dough		Dough to Maturity	
	GDD	HTU	GDD	HTU	GDD	HTU
Filled grains per panicle	-0.415**	-0.186	-0.187	-0.166	-0.347**	-0.147
1000 grain weight	-0.183	-0.188	-0.120	0.146	-0.130	-0.148
Grain yield	-0.492**	-0.260**	-0.333**	-0.318**	-0.314**	-0.126
Head rice recovery	-0.136	0.084	-0.055	-0.200*	-0.127	-0.103
Amylose	-0.045	-0.121	-0.074	-0.118	-0.171	-0.011
Protein	-0.229*	-0.033	-0.118	-0.204*	0.001	0.094
Aroma	-0.470**	-0.392**	-0.429**	-0.354**	-0.367**	-0.275**

Sample size: n = 108; r value = 0.189* and 0.247** at 5% and 1% level of significance, respectively.

(8±2°C) resulted in higher HR yield irrespective of containers compared to ambient environment (28±4°C). On the contrary, El-Kady et al. (2013) reported greater head rice (%) of four special rice varieties stored at room temperature (23±2°C) than low temperature (2°C) in refrigerator in Egypt.

Amylose content

Late planting (10 August) of Lal Badshabhog rice recorded highest amylose content (17.5%) compared to 25 July (17.4%) and 10 July (17.0%) plantings at Kalyani, West Bengal (Table 4). On the contrary, Chowdhury et al. (2011) reported decreasing trend in amylose content of rice (cv. Rajendra Suwasani) with delay in planting from 5 July to 4 August in Bihar. Mean amylose content in Lal Badshabhog rice grain either of planting times or spacing or nutrient management practices was 17.3±0.9%, which indicated that the variety belonged to low (9-20%) amylose group. Widely spaced plants ($S_{20\times15}$) recorded higher amylose content compared to closely planted ones ($S_{15\times15}$) during wet season. The second experiment indicated a slight improvement in amylose content (pooled mean 17.4 vs. 17.6%) with increase in storage duration from 90 to180 d (Table 6); but Abeysundara et al. (2015) reported 0.5-1.7% reduction in amylose content of three rice varieties stored for 40 weeks in Sri Lanka. The amylose content of Lal Badshabhog rice was not significantly affected at low (T_8) and room temperature (T_{28}) for both short (3-m) and medium post-harvest duration (6-m) in both the years and pooled values. According to Butt et al. (2008), amylose content of two Basmati rice varieties (Basmati Super and Basmati 385) was decreased gradually with the increase in storage temperature from 5°C (22.81 g/100 g sample) to 45°C (22.01 g/100 g sample) at Faisalabad, Pakistan.

Protein content

Mean protein content in Lal Badshabhog rice grain, averaged over treatment combinations, was 7.7±0.1% in the study. There was gradual improvement in grain protein content with increment in chemical fertilizers from integrated (N_{O+CF}) to 100%RD inorganic (N_{CF}) over 100%RD organic (N_O) (Table 4), which might indicate greater protein synthesis in crop nourished with chemical fertilizers only (N_{CF}) compared to either integrated (N_{O+CF}) or sole organic nutrient sources (N_O). Protein content of Lal Badshabhog rice in Experiment 2 varied significantly among three containers for 3-m and 6-m storage (Table 7); where C_{Ep} recorded the highest protein content (7.4%) after 3-m storage, and C_{Pb} noted the maximum value (7.6%) after 6-m storage. Such variation in protein content among the storage containers under different storage periods could not be properly explained, which indicated the need of some other bio-chemical analyses to understand the changes in protein fractions during the ageing process. There was no significant influence of storage temperature on protein content of Lal Badshabhog rice stored for a quarter or half a year period during 2014-15, 2015-16 and pooled values. Similarly, Jang et al. (2009) also reported no significant change in protein content among six Korean rice varieties after 12-months storage at 5, 15 and 25 ºC.

Alkali value

Among 3 containers, C_{Pb} recorded the highest ASV (score 3.9) being at par with C_{Gb} (score 3.6), but

Table 7. Effect of storage container and temperature on grain quality of aromatic rice (cv. Lal Badshabhog) during post-harvest period.

Storage container (C)	3 months storage			6 months storage		
	Temperature (T)		Mean	Temperature (T)		Mean
	8±2ºC (T_8)	28±4ºC (T_{28})		8±2ºC (T_8)	28±4ºC (T_{28})	
			Head rice recovery (%)			
Gunny bag (C_{Gb})	56.0	53.9	55.0	55.4	52.8	54.1
Polypropylene bag (C_{Pb})	57.1	54.4	55.7	55.3	52.8	54.0
Earthen pot (C_{Pb})	56.7	55.3	56.0	53.9	53.4	53.7
Mean	56.6	54.6	55.6	54.9	53.0	53.9
Factor	C	T	C × T	C	T	C × T
CD	0.76	0.62	ns	ns	0.73	1.27
			Amylose (%)			
Gunny bag (C_{Gb})	17.6	17.4	17.5	17.3	17.5	17.4
Polypropylene bag (C_{Pb})	17.8	17.4	17.6	17.7	17.8	17.8
Earthen pot (C_{Pb})	17.1	17.0	17.1	17.9	17.4	17.7
Mean	17.5	17.3	17.4	17.6	17.6	17.6
Factor	C	T	C × T	T	T	C × T
CD	0.35	ns	ns	ns	ns	ns
			Protein (%)			
Gunny bag (C_{Gb})	7.2	7.0	7.1	7.1	7.0	7.1
Polypropylene bag (C_{Pb})	7.1	7.0	7.0	7.7	7.5	7.6
Earthen pot (C_{Pb})	7.4	7.3	7.4	7.4	7.1	7.3
Mean	7.2	7.1	7.2	7.4	7.2	7.3
Factor	C	T	C × T	C	T	C × T
CD	0.20	ns	ns	0.29	ns	ns
			Alkali value (score)			
Gunny bag (C_{Gb})	3.3	4.0	3.6	2.9	3.0	3.0
Polypropylene bag (C_{Pb})	3.3	4.5	3.9	3.2	3.1	3.2
Earthen pot (C_{Pb})	2.8	3.4	3.1	2.8	2.8	2.8
Mean	3.1	3.9	3.5	2.9	3.0	3.0
Factor	C	T	C × T	C	T	C × T
CD	ns	0.43	ns	ns	ns	ns
			Elongation ratio			
Gunny bag (C_{Gb})	1.80	1.79	1.80	1.80	1.79	1.79
Polypropylene bag (C_{Pb})	1.80	1.78	1.79	1.83	1.78	1.81
Earthen pot (C_{Ep})	1.77	1.79	1.78	1.79	1.80	1.79
Mean	1.79	1.79	1.79	1.81	1.79	1.80
Factor	C	T	C × T	C	T	C × T
CD	0.01	ns	0.02	ns	0.02	0.03
			Aroma (score)			
Gunny bag (C_{Gb})	1.85	1.69	1.77	1.52	1.52	1.52
Polypropylene bag (C_{Pb})	2.08	1.72	1.90	1.57	1.47	1.52
Earthen pot (C_{Ep})	2.03	1.93	1.98	1.85	1.72	1.78
Mean	1.99	1.78	1.88	1.64	1.57	1.61
Factor	C	T	C × T	C	T	C × T
CD	0.12	0.10	ns	0.16	ns	ns

significantly greater over C_{Ep} (score 3.1) in second experiment (Table 7). Storage temperature had significant influence ($P < 0.05$) on alkali value of 3-m stored Lal Badshabhog rice only in both the years and pooled values. Paddy stored in ambient condition (28±4ºC) for 90 days resulted in higher ASV (score 3.9) indicating near-intermediate GT; while cool storage condition (8±2ºC) recorded lower alkali values (score

3.1, high-intermediate GT). Similarly, Butt et al. (2008) reported slight reduction in ASV score (4.1 vs. 3.9) of two Basmati rice varieties with increase in storage temperature from 5ºC and 45ºC at Faisalabad, Pakistan.

Elongation ratio

The ER of Lal Badshabhog rice did not vary among 3 different types of containers at both 3-m and 6-m storage period, except for 90 d for pooled values only in second experiment (Table 7). Perusal of data revealed that there was notable change in ER from 90 to 180 d storage time during both 2015 and 2016. Storage temperature could not have significant effect on ER of Lal Badshabhog rice after 6-m storage except for the pooled values. Similar type of findings in cooked kernel elongation of *thai* paddy varieties during the ageing process was reported by Kanlayakrit and Maweang (2013).

Aroma

The intensity of aroma was slightly improved with delay in planting from 10 July (score 1.5) to 10 August (score 1.7) in first investigation (Table 4). This might be due to low day-night temperature (32.7/19.4ºC) during grain-filling and ripening period in late planting condition (D_{10A}) compared to early plantings on 10 July (33.7/ 23.2ºC) and 25 July (33.0/21.6ºC) at Kalyani, West Bengal. The discussion could be supported by the fact that low temperature (< 20ºC) during grain filling period (Dutta et al. 1999) as well as cooler temperature (25ºC day/21ºC night) during crop maturity (Juliano 1972) favoured better retention of aroma in Basmati rice. The coefficient of variation in aroma score due to planting times was obtained as 7.4 and 12.6% during first and second year, respectively (data not shown). The ANOVA for the aroma of Lal Badshabhog rice showed that year (Y) and planting time (D) contributed 3.34 and 32.91%, combining about 36.25% for the environment (Y+D) of the total variation in the sum of squares (Table 4). Thus, the variation in aroma induced by different planting dates was greater than the variation due to year. The significant negative correlation (P<0.05) between GDD during 50% flowering to maturity (Table 6) indicated that low air temperature during the ripening stage could favour the development of aroma in grains of Lal Badshabhog rice during the wet season. Planting density and nutrient management had no significant influence on the aroma of the Lal Badshabhog rice grain.

In Experiment 2, the intensity of aroma was decreased from 3-m to 6-m storage period irrespective of container, temperature and year (Table 7). Baradi and Elepano (2012) reported significant effect of storage time on 2AP level in milled rice for paddy (Maligaya Special 6) stored as rough rice at 30ºC ambient temperature, where 2AP began to decrease rapidly starting from second week, which began to slow down on the fifth week to reach an equilibrium on the sixth week. Among containers, C_{Ep} (score 2.03 and 1.73) could favour maximum retention of aroma compared to the other two containers (C_{Pb} and C_{Gb}) during both the years of the experiment. The storage temperature could exert significant influence on aroma of 3-m aged Lal Badshabhog rice during 2016 and pooled over two years, where T_8 resulted in greater retention of aroma than T_{28}. A perusal of data for 6-m storage revealed slightly greater aroma in T_8 than in T_{28} during both years, but the differences were not significant.

Nutrient uptake

N, P and K uptake by Lal Badshabhog rice crop varied significantly among three planting times and three nutrient management practices adopted in the experiment (Table 4). The range of variation in uptake of nutrients among nutrition-based treatments was noted as: 40.4-41.7 kg N ha^{-1}, 15.2-16.1 kg P ha^{-1} and 28.4-31.6 kg K ha^{-1}. The finding indicated that the variety belonging to tall-indica group had low responsiveness to the fertilizers. Besides, N uptake by grain was usually more than double (26.9-27.6 kg ha^{-1}) compared to the uptake by straw (13.5-14.2 kg ha^{-1}) of Lal Badshabhog paddy during *kharif* season (data not shown). The greater replacement of chemical fertilizers by organic manures either integrated (N_{O+CF}) or sole organic nutrient sources (N_O) resulted in lower uptake of N, P and K by Lal Badshabhog paddy in the study.

CONCLUSION

Planting of Lal Badshabhog rice on 10 August (D_{10A}) *i.e.*, slightly delayed from normal planting could have favourable influence on grain yield, amylose content and medium aroma compared to D_{25J} and D_{10J} plantings; while close spacing ($S_{15\times15}$) improved the grain yield by 4.0% over wider spacing ($S_{20\times15}$) due to greater production of panicles per m^2. Application of 100% RD ($N_{50}P_{25}K_{25}$ kg ha^{-1}) through chemical fertilizers resulted in higher grain yield and protein content compared to organic (N_O) or integrated nutrient management (N_{O+CF}). Earthen pot (C_{Ep}) could be used for better HRR, protein content, and medium aroma for 3-m period; while for sustained aroma only during 6-m storage. The cool storage environment (8±2°C) favoured greater head rice recovery and aroma during 3-m storage, while it improved the HRR and ER than ambient condition (28±4°C) during the 6-m post-harvest period.

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Characterization of South Indian rice accessions for physico-chemical properties, starch digestibility and antioxidant properties

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ABSTRACT

Pigmented rice accessions are renowned for their unique colour characteristics and therapeutic applications. This study aims to characterize five popular pigmented rice accessions from South India-Matta (brown), Rakthasali, Njavara, Mappilai samba (red), and Kavuni (black)-by evaluating their physicochemical properties, starch digestibility, and antioxidant properties. The investigation included a detailed analysis of phytochemical concentrations, such as total phenolic content (TPC), total flavonoid content (TFC), and anthocyanins. Antioxidant activities were assessed using the 2,2-Diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay, 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging assay, and ferric-reducing antioxidant potential (FRAP) assay. In terms of starch digestibility and glycemic potential, all rice accessions exhibited an intermediate glycemic index (GI) ranging from 55 to 60. Kavuni rice, with a GI value of 60, showed the highest glycemic potential, likely due to its low amylose concentration (6%). All accessions demonstrated significant phytochemical concentrations and notable antioxidant potential. Specifically, Kavuni rice exhibited high levels of TPC (1.4 mg GAE/g), TFC (9.6 mg QE/g), and anthocyanins (0.7 mg CE/g). The high antioxidant activities observed in Kavuni (DPPH - 86.8%; ABTS - 91%; FRAP - 0.5 mg AAE/g) are attributed to its elevated bioactive compound levels. The findings underscore the health-promoting potential of pigmented rice, especially in managing diabetes and related disorders. Future research should explore the molecular mechanisms underlying the health benefits of pigmented rice, particularly in metabolic disorder management. Additionally, breeding programs could aim to enhance specific phytochemical profiles to maximize therapeutic potential.

Key words: *Digestibility, phytochemicals, antioxidants, pigmented rice*

INTRODUCTION

Rice (*Oryza sativa* L.) is consumed as a staple diet in most of the Asian countries. In recent years, there has been a significant increase in the global demand for rice and rice-based products, that may possibly be due to an increased interest in Asian cuisine. Due to the nutritional quality and higher digestibility, rice is considered as the queen of cereals (Anjum et al., 2007). The dehusked and polished white rice is preferred by most of the populations (about 85%) and the remaining 15% is contributed by the pigmented rice. The pigmented rice may or may not contain bran; and based on the pericarp colour, these are grouped as brown, black, red, blue and purple rice varieties. The principal components in rice are carbohydrates specifically starch along with considerable portions of proteins and fats. The rice starch component namely, amylose, plays a vital role in determining its overall cooking quality (Zhou et al. 2022). The rice with high amylose content (25-33% amylose) has appropriate cooking and eating qualities. As starch is closely associated with hyperglycemia, the regular consumption in higher amounts result in adverse effects to the body. The over consumption of carbohydrate rich foods is positively associated with the increased risk of type 2 diabetes (Hu et al., 2012; Ren et al., 2021). Sun et al. (2010) stated that white rice causes a pointedly high glycemic response in body.

Starch digestibility plays a vital role in the nutritional quality by determining glycemic response of rice and is affected by factors such as the amylose-to-amylopectin ratio, granule size, and the amount of resistant starch present. Rice with a higher amylose content generally has lower starch digestibility, which helps to moderate the glycemic response (Kumar et al., 2022). Moreover, processing techniques like cooking and retrogradation can significantly alter starch digestibility by changing the structure of starch granules and their availability to digestive enzymes (Toutounji et al., 2019).

In recent years, the pigmented rice varieties are getting wide acceptance due to its nutritional value and health benefits including antioxidant, anti-glycemic, anti-adipogenic and anti-hyperglycemic properties (Callcott et al., 2018). These advantages are conferred by its phytochemical and micronutrient concentrations. The polyphenols in rice grain could be classified into phenolic acids (the most common secondary metabolites in cereal grains); anthocyanins (the primary functional components of pigmented rice) (Delgado-Vargas et al. 2000) and proanthocyanidins (consist of catechin and epicatechin block units). Together with these, the high proportion of resistant starch were also reported in pigmented rice; (Deepa et al., 2010) therefore, slower digestibility properties. The conversion of pigmented rice to different rice-based products, could be an alternate to preservation and source for novel functional foods. There is a void in the nutritional profiling and digestibility studies of pigmented rice accessions of South India, and providing such information will be instrumental in breeding healthier rice. In view of these, the aim of the study is to evaluate the physicochemical, antioxidant and digestibility characteristics of five popular commercially available pigmented rice accessions in South India.

2. MATERIALS AND METHODS

2.1. Sample collection

The samples including three red pigmented (Njavara, Rakthasali, Mappilai Samba), one each black (Kavuni) and brown (Matta) pigmented, were collected from Rice Research Station, Vyttila and Block Development Office, Kerala, India during the time period of June 2021 to December 2021. All samples were cleaned and stored in air tight containers at room temperature, for further studies.

2.1. Physicochemical properties

2.1.1. Physical parameters

The length, breadth, bulk density (BD), 1000-kernel weight (TKW), and color of the rice accessions were determined according to the method described by Oko et al. (2012). The color values (L*, a*, b*, h*, and c*) of the different rice accessions were measured using a Lovibond colorimeter (Lovibond LC 100 Spectrocolorimeter, UK).

Proximate composition

The moisture, crude fat, crude protein, ash, and crude fiber contents were determined following AOAC (2000) procedures. Carbohydrate content was calculated using the difference method as described by Pehrsson et al. (2015). The concentrations of sodium (Na), potassium (K), and calcium (Ca) were estimated using the Flame Photometer (Elico CL 378, India) by wet ash method.

Alkali spreading value (ASV), Gelatinization Temperature (GT) and amylose content (AC)

The alkali spreading of the rice starch gel was measured by placing milled kernels in a petri plate (9cm) containing 1.7% (w/v) potassium hydroxide (10ml) solution and left for 23 h at 30°C for spreading (Chemutai et al.2018). Based on the degree of kernel disintegration, the results were expressed using a 7-point score card. The GT was estimated accordingly to the ASV as suggested by Unnevehr et al. (1985). A high ASV rating indicates more disintegration and a low gelatinization temperature. Samples with ASV score of <4 had high GT (75-79), those with ASV of <6 had intermediate GT (70-74), and those with ASV of >6 had low GT (55-69). AC was determined using a colorimetric approach based on iodine-binding process (Lauro et al. 1999).

Rapidly digestible starch (RDS), slowly digestible starch (SDS) and resistant starch (RS).

The digestible starch (RDS and SDS) and RS were determined using the method described by Patindol et al. (2010). The cooked samples (0.5g) homogenized in acetate buffer (10ml, 0.1 M, pH 5.2) were incubated at 37^{o}C and treated with 2.5 ml of enzyme cocktail (3800 U/ml pancreatin, 188 U/ml amylase, 13 U/ml amyloglucosidase from Sigma-Aldrich, St. Louis, MO, USA). Aliquots (0.25 ml) were collected after 20 and

120 min for the determination of RDS and SDS, respectively. The residue collected after centrifugation of remaining mix, was washed with absolute alcohol (10ml), treated with 7 M KOH, boiled, and hydrolyzed using amyloglucosidase (50 U/ml) to determine RS (starch fraction that remained unhydrolyzed after 120 min). The amount of glucose released in aliquots was determined using the glucose assay kit (Sigma-Aldrich, St. Louis, MO, USA). The total Starch (TS) was calculated by summing up the digestible starch (DS) and RS.

$$RDS = Glucose_{(20\ min)} \times 0.9$$

$$SDS = (Glucose_{120\ min} - Glucose_{20\ min}) \times 0.9$$

$$RS = Glucose_{residue} \times 0.9$$

$$TS = RDS + SDS + RS$$

Glycemic index (GI) and glycemic load (GL)

The available starch and starch hydrolysis index at 90 minutes (HI 90) was determined according to Goñi et al. (1997). The cooked rice samples (250 mg) were homogenized with distilled water (25ml) and digested using α-amylase (Sigma-Aldrich, India) (200 µl, 15 min at 90 C). The supernatant (1 ml) was incubated with sodium phosphate buffer (pH 4.7, 2ml) and amyloglucosidase (1ml, Sigma-Aldrich, India) at 60°C for 30 min with intermittent stirring. It was then diluted to 10 ml with distilled water. The glucose was estimated by using glucose assay kit (Sigma-Aldrich, St. Louis, MO, USA). The percentage available starch was estimated using the formula:

$$\text{Percent available starch} = \frac{\mu g\ \text{glucose x 25 x 0.9}}{\text{sample weight (mg)}}$$

For the estimation of HI 90, the cooked samples (1600 mg) were homogenized with phosphate buffer (50 ml, 0.1 M, pH 6.9) and digested using pancreatin (1mg/ 100 ml, 37°C, 90 min; Sigma-Aldrich, India). The clear supernatant (0.1 ml) was drawn at 0 min and after 90 min and analyzed for maltose using the DNSA method. The results were expressed as mg maltose released after 90 min of hydrolysis of 1 g sample (dry weight). HI 90 was calculated using the formula:

$$HI90 = 100 \times 0.9 \times (\text{mg maltose})/(\text{mg starch})$$

The GI was calculated using the formula:

$$GI = 39.21 + 0.803 \times HI\ 90$$

The glycemic load (GL), expressed as a percentage, is calculated by multiplying the GI by the available carbohydrate content per portion (Salmerón et al., 1997). The formula is as follows:

$$GL = (\text{Available carbohydrate content per portion in g} \times GI)/100$$

Bioactive compounds and antioxidant properties

2.5.1. Sample extraction

The milled rice samples were extracted using an ultrasonic-assisted extraction method. In brief, 1 g of the sample was mixed with 20 mL of 80% (v/v) methanol and subjected to low-frequency (20-40 kHz) ultrasonication using an ANM Industries USC-200 device (India) at 45°C for 30 minutes. The mixture was then centrifuged using an Eltec Labspin TC 450D centrifuge (India) at 3000 rpm and 37°C for 15 minutes. The supernatant was collected for further analysis.

2.5.2. Total phenols, total flavonoids and anthocyanin content

The total phenolic content (TPC) of the extracts was determined using the Folin-Ciocalteu assay at 750 nm, as described by Bhalodia et al. (2011). In a typical procedure, 1 mL of aliquot was vigorously mixed with 0.5 mL of Folin-Ciocalteu's reagent and 5 mL of distilled water. After 5 minutes, 1.5 mL of 20% sodium carbonate was added, and the volume was made up with distilled water. The samples were kept in the dark at ambient temperature for 2 hours. The absorbance was measured at 750 nm using a UV-visible spectrophotometer (Jasco V-630, Japan), against a blank. Gallic acid (0.01-0.1 mg/mL) was used as the standard. The results were expressed in milligrams of gallic acid equivalents per 100 g of dry mass (mg GAE/ 100 g).

Flavonoid content was determined using the aluminum chloride colorimetric assay, as described by Kalita et al. (2013). Briefly, 1 mL of the extract was thoroughly mixed with 0.3 mL of 5% (w/v) sodium nitrite solution and 4 mL of distilled water. After 5 minutes, 0.3 mL of 10% (w/v) aluminum chloride was added, followed by 2 mL of 1 M sodium hydroxide. The volume was then made up to 10 mL with distilled water. Absorbance was measured at 510 nm using a UV-visible spectrophotometer (Jasco V-630, Japan), with a blank as the reference. Quercetin (0.1-1.0 mg/mL) was used as the standard. Results were reported in milligrams of quercetin equivalents per 100 g of dry mass (mg QE/ 100 g).

The concentration of anthocyanin pigment of the rice accessions was determined by pH differential methods, with slight modifications (Wolfe et al. 2003). Briefly, 0.5 ml of the extract was mixed thoroughly with 0.025M potassium chloride buffer (pH 1, 3.5ml). After 15 min, the absorbance was measured at 515 and 700 nm against distilled water as blank using UV-vis spectrophotometer (Jasco V-630, Japan). The results were expressed as milligram of cyanidin-3-glucoside equivalent/100 g of sample (dry weight).

$$\text{Total anthocyanin content} = \frac{A \times MW \times DF \times 1000 \quad \times C}{E}$$

Where,

A (absorbance) = $[(A_{515}-A_{700})$ pH 1.0-$(A_{515}-A_{700})$ at pH 4.5

MW = 449.2

DF = dilution factor

= molar absorptivity of cyanidin-3-glucoside (26,900)

C = concentration of buffer

2.5.3. Antioxidant properties

2.5.3.1. 2,2-Diphenyl-1-picryl hydrazyl (DPPH) free radical scavenging assay

The DPPH free radical scavenging assay was determined according to Boly et al. (2016) with slight modifications. Briefly, 2 ml of extracts were treated with 0.01% (w/v) methanolic DPPH solution (2 ml) and incubated for 30 min in the dark at ambient temperature. The absorbance was recorded at 540 nm using a spectrophotometer (Jasco V-730, Japan). The DPPH radical scavenging activity (%) was calculated using the formula:

DPPH scavenging activity (%) = $[(Ac-As)/Ac]\times 100$

Where, Ac- absorbance of control [DPPH alone],

As - absorbance of sample [DPPH +extract].

2.5.3.2. 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) free radical scavenging assay

The ABTS radical scavenging assay was determined according to Arnao et al. (2001) with some modifications. Briefly, the ABTS working stock solution was prepared with an absorbance of 0.706 ± 0.01 units. The sample extract (1 ml) and ABTS solution (1 ml) were treated and the absorbance was recorded at 734 nm (Jasco V-730, Japan) after 7 min. The ascorbic acid was considered as standard. The percentage inhibition was calculated using the formula:

ABTS (%) = $(Abs(C) - Abs(S)\times 100) / (Abs(C))$

Where,

$Abs(C)$ = absorbance of ABTS radical in methanol

$Abs(S)$ = absorbance of ABTS radical solution with sample extract or standard.

2.5.3.3. Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed according to Lalhminghlui and Jagetia (2018) with slight modifications. The extracts (500 l) were treated with 3 ml of FRAP reagent (10: 1:1, 300mM acetate buffer, pH 3.6, TPTZ solution and 20 mM FeCl3.6H2O solution, respectively) and incubated at 37?C for 30 min. The absorbance was measured at 593 nm using UV-visible spectrophotometer (Jasco V-730, Japan). The antioxidant activity of the extracts was expressed as gram ferrous sulphate equivalent /100 g of samples on dry basis (mg FeSO4/100g).

2.6. Statistical analysis

The results are expressed as mean standard deviation of triplicate samples. The Analysis of variance (ANOVA) was performed using GRAPES 1.0.0. software (Kerala Agricultural University, India) (Gopinath et al., 2021). Duncan's multiple range tests were conducted for comparison of means at $p<0.05$. Pearson's correlation coefficients were calculated for the relationships between biochemical parameters (carbohydrate, protein, lipid, crude fiber) and food indexes (total starch content, resistant starch, digestible starch).

3. RESULTS

3.1. Physical properties

The physical parameters studied included length, breadth, length-to-breadth ratio, and bulk density (BD), as detailed in Table 1. The colour characteristics of the five rice accessions are presented in Figure 1. The average length was highest for Matta (0.8 cm), followed by Kavuni and Mappilai Samba (0.6 cm each), and Njavara (0.5 cm). The average breadth measurements were 0.23 cm for Mappilai Samba, 0.21 cm for Njavara, 0.20 cm for Kavuni, 0.18 cm for Rakthasali, and 0.15 cm for Matta. Mappilai Samba and Matta exhibited the highest bulk density (0.63 g/cm³), followed by Njavara (0.62 g/cm³), Rakthasali (0.61 g/cm³), and Kavuni (0.60 g/cm³). The a* value, indicative of redness, was highest for Njavara (11.4±0.4) and lowest for Kavuni (7.7±0.3). The b* value, reflecting yellowness, was highest for both Matta and Rakthasali (11.5±0.2 and 11.5±0.1, respectively) and lowest for Kavuni (3.6±2.2). The hue angle (h*) ranged significantly from 53±1.3 for Matta to 25±3.4 for

Table 1. Physicochemical characteristics, proximate composition, phytochemical content and antioxidant potential of pigmented rice varieties.

	Matta	Rakthasali	Njavara	Mappilai Samba	Kavuni
Physical characteristics					
Length (cm)	0.8±0.1^{a*}	0.5±0.1^{c*}	0.5±0.02^{b*}	0.6±0.04^{b*}	0.6±0.03^{bc*}
Breadth (cm)	0.2±0.1	0.2± 0	0.2±0.03	0.2±0.01	0.2±0
L/B** ratio	5.1±0.8^{a*}	2.6±0.3^{bc*}	2.4±0.3^{c*}	2.6±0.3^{bc*}	3±0.3^{b*}
B.D** (g/cm^3)	0.6±0.1^{b*}	0.6±0.4^{b*}	0.62±0.3^{b*}	0.6±0.9^{b*}	0.6±0.8^{a*}
Chemical characteristics					
Moisture (g%)	12.4±0.1^{b*}	11.1±0.1^{c*}	10.2^{d*}	11.3±0.3^{c*}	13.3±0.4^{a*}
Fat (g%)	2.0±0.3^{a*}	1.3±0.5^{c*}	1.8±0.4^{b*}	1.6±0.2^{b*}	1.0±0.1^{d*}
Protein (g%)	8.6±0^{d*}	9.7±0.6^{ab*}	10.5±0.3^{a*}	8.7±0.3^{cd*}	9.8±0.3^{bc*}
Ash (g%)	1.2±0.2	1.3±0.2	1.5±0.2	1.3±0.1	1.6±0.1
Crude fibre (g%)	1.9±0.6^{a*}	1.1±0^{d*}	1.1 ± 0.1^{d*}	1.3±0.3^{c*}	1.4 ± 0.1^{b*}
Carbohydrates (g%)	75.8	76.6±0.1	76.1±0.3	77.2±0.3	74.3±0.7
Mineral content					
Sodium (mg/g)	0.8±0.1^{c*}	1.2±0.1^{b*}	1.0±0.04^{bc*}	0.7±0.1^{a*}	1±0.6^{bc*}
Potassium (mg/g)	2.1±0.3^{d*}	2.5±0.2^{b*}	2.0±0.3^{d*}	2.3±0.8^{c*}	3.1±0.6^{a*}
Calcium (mg/g)	0.2±0.1^{c*}	0.2±0.0^{a*}	0.2±0.2^{b*}	0.2bc*	0.22±0.1^{b*}
Starch characteristics					
ASV**	5^{b*}	5^{b*}	6^{a*}	2^{c*}	6^{a*}
GT**	Intermediate	Intermediate	Low	High	Low
AC** (g%)	19.3±0.3^{a*}	18.0±0.2^{ab*}	19.5±0.9^{a*}	16.6±0.5^{b*}	6.0±0.1^{c*}
Phytochemicals					
TPC**mg GAE#/g	0.8±0.03^{d*}	0.8±0.05^{cd*}	1.2±0.2^{b*}	0.9±0.08^{c*}	1.4±0.1^{a*}
TFC** mg QE#/g	2.6±0.06^{d*}	4.5±0.2^{c*}	9.7±0.1^{b*}	10.1±0.2^{a*}	9.6±0.1^{b*}
Anthocyanins mg CE#/g	0.02±0.6^{d*}	0.1±0.02^{b*}	0.07±0.09^{c*}	0.2±0.1^{d*}	0.7± 0.4^{a*}
Antioxidant Potential					
****DPPH** scavenging Activity (%)	93.3±0.1^{b*}	91.5±0.3^{c*}	93.8±0.1^{a*}	82.9±0.1^{e*}	86.8±0.1^{d*}
****ABTS** scavenging Activity (%)	85.6±0.4^{d*}	89±1.4^{c*}	96±0.7^{b*}	96±0.5^{ab*}	98±0.7^{a*}
****FRAP** mg AAE#/g	1.7±0.4^{e*}	2.6±0.1^{d*}	3.8±0.1^{b*}	3.2±0.7^{c*}	5.4±1.1^{a*}

* Treatments with same letters are not significantly different. **TKW- thousand kernel weight; ASV- alkali spreading value; GT- degree of gelatinization; AC- amylose content; TPC- Total phenolic content; TFC- Total flavonoid content; DPPH- 2,2-Diphenyl-1-picryl hydrazyl; ABTS - 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid); FRAP-Ferric reducing antioxidant power. #GAE- Gallic acid equivalents; QE- Quercetin Equivalent; CE- Cyanidin -3-glucoside equivalent; AAE- Ascorbic acid equivalent.

Kavuni. The highest chroma value (c*) was observed for Rakthasali (15.9±0.4), followed by Njavara (15.8±0.1), Matta (14.5±0.1), Mappilai Samba (9.3±0.4), and Kavuni (8.5±2.9).

3.2 Proximate composition

The proximate composition of the five pigmented rice accessions is detailed in Table 1. Moisture content across the rice accessions ranged from 10.2% to 13.3%. Fat content varied significantly among the accessions, with Kavuni having the lowest at 1% and Matta the highest at 2%. Protein content also differed significantly, ranging from 8.6% in Matta to 10.5% in Njavara. Rakthasali and Kavuni exhibited relatively high protein contents at 11.7% and 10.8%, respectively, while Mappilai Samba had a lower protein content of 8.7%. Ash content varied between 1.2% and 1.6%, with Kavuni showing the highest ash content, followed by Njavara, Rakthasali, Mappilai Samba, and Matta. Carbohydrate content ranged from 74.3% to 77.2%. Significant differences in mineral content were observed ($p<0.05$), with potassium being the most abundant mineral across all accessions, ranging from 2.0 to 3.1 mg/g.

3.3 Gelatinization temperature and amylose content

The Apparent Starch Viscosity (ASV) of the various pigmented rice accessions ranged from 5.0 to 6.0. Based on the observed ASV, the Gelatinization Temperature (GT) of the rice samples is estimated to be between 55°C and 75°C. Significant differences in amylose content were found among the rice accessions, with values of 19.5% for Rakthasali, 19.3% for Matta, 18.0% for Njavara, 16.5% for Mappilai Samba, and 6.0% for Kavuni.

3.4 Starch digestibility and glycemic index

Total starch content is composed of rapidly digestible starch (RDS), slowly digestible starch (SDS), and resistant starch (RS) (Englyst et al., 1992). RDS and SDS contribute to digestible starch (DS). The total starch content across the five rice accessions ranged from 22.4% to 26.07%, with digestible starch ranging from 21.4% to 25.3% (Table 2). Kavuni exhibited the highest RDS content at 15.2%, while Njavara had the lowest at 9.7%. The highest SDS content was found in Rakthasali (13.8%), followed by Mappilai Samba (12.7%) and Njavara (12.3%). RS content ranged from 0.7% to 0.9%. The Glycemic Index (GI) of the rice accessions varied between 57 and 60, with Kavuni at 60, Matta at 58.6, Rakthasali at 57.0, Njavara at 58.7, and Mappilai Samba at 59.1. The Glycemic Load (GL) for all accessions ranged from 28.5 (Rakthasali) to 30.4 (Kavuni).

3.5 Phytochemical concentration

The total phenolic, flavonoid, and anthocyanin contents of the rice accessions are detailed in Table 1. The black rice accession Kavuni exhibited the highest total phenolic content at 1.4 mg GAE/g, followed by the red rice accession Njavara with 1.2 mg GAE/g. The phenolic content in other samples was as follows: Mappilai Samba had 0.9 mg GAE/g, and Rakthasali and Matta each had 0.8 mg GAE/g. The total flavonoid content ranged from 2.6 mg QE/g to 10.14 mg QE/g, with Matta showing the lowest and Mappilai Samba the highest levels. Kavuni also had the highest anthocyanin content at 0.7 mg/g, while the other accessions had significantly lower anthocyanin levels, ranging from 0.02 to 0.2 mg/g. The lowest anthocyanin content was observed in Mappilai Samba and Matta,

Table 2. Starch digestibility and Glycemic Index of cooked pigmented rice samples.

Samples	Matta	Rakthasali	Njavara	Mappilai Samba	Kavuni
RDS**	$11.4±0.2^{bc*}$	$10.7±0.5^{c*}$	$9.7±0.5^{d*}$	$11.8±0.3^{b*}$	$15.2±0.7^{a*}$
SDS**	$10.04±0.5^{e*}$	$13.8±0.6^{a*}$	$12.3±0.3^{c*}$	$12.7±0.7^{b*}$	$10.1±0.9^{d*}$
DS**	21.4±0.7	24.5±0.9	22±0.8	24.5±0.6	25.3±0.7
RS**	0.9±0.02	0.8±0.04	0.7±0.02	0.9±0.05	0.7±0.03
TS **	$22.4±0.7^{b*}$	$25.3±0.9^{a*}$	$22.6±0.8^{b*}$	$25.5±0.6^{a*}$	$26±0.7^{a*}$
GI**	$58.6±1.2^{bc*}$	$57.02±0.7^{b*}$	$58.7±0.8^{c*}$	$59.1±0.8^{b*}$	$60.8±0.7^{a*}$
GL**	29.3±0.5	28.5±0.7	29.4±0.2	29.6±0.3	30.4±0.3

*Treatments with same letters are not significantly different. **RDS - Readily digestible starch; SDS - slowly digestible starch; DS - Digestible starch; RS - Resistant starch; TS - Total starch; GI - Glycemic index; GL- Glycemic load.

both at 0.02 mg/g.

3.6 Antioxidant properties

The antioxidant potential of the various rice accessions is detailed in Table 1. The highest DPPH radical scavenging activity was observed in Njavara rice (93.8%), while Mappilai Samba exhibited the lowest activity (82.9%). In the ABTS scavenging assay, all samples demonstrated increased inhibition, with Kavuni showing the highest activity at 98%, followed by Njavara and Mappilai Samba, both at 96%, Rakthasali at 89%, and Matta at 85.6%. The Ferric Reducing Antioxidant Power (FRAP) value was highest for Kavuni (5.4 mg AAE/g) and lowest for Matta (1.7 mg AAE/g).

4. DISCUSSIONS

4.1. Physicochemical characteristics

Wide variations were observed in the physicochemical properties of the rice accessions. Based on the observed length of kernels, the rice accessions were classified as, long (Matta and Kavuni), medium (Rakthasali and Mappilai Samba) and short grains (Njavara). The Length/Breadth (L/B) ratio also emphasized the theory of categorization. The rice accessions with larger size have low BD and it greatly influences cooking time (Singh et al. 2005). The colour variables, lightness (L*), redness (a*) and yellowness (b*) indicated the extend of rice pigmentation and the lowest hue angle (h*) designates the sample with darkest external appearance. Chroma (c*) is the vividness of colour. From the observations as described in Fig. 1; the Kavuni variety showed a greatest pigmentation with lowest L*, a*, b*, c* and h* values due to the black pericarp of the kernel.

Statistical analysis indicated significant differences ($p \geq 0.05$) in the proximate composition of the rice accessions. All Samples were sufficiently dried since the moisture content observed under the value of 14%, which is the recommended maximum moisture content in rice for storage (Tahir et al., 2023). Harvesting seasons and post-harvest operations affect the moisture content of rice samples (Ibukun, 2008; Devraj et al., 2020). Juliano (2003) reported that the moisture content plays a key role in keeping quality, milling properties and cooking and processing conditions of rice. The degree of milling and polishing greatly influences fat, ash, and fiber contents in rice as these elements are mainly concentrated in the germ as well

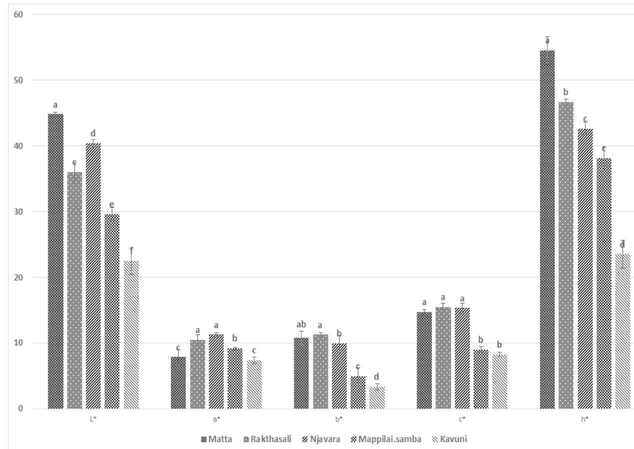

Fig. 1. Colour distribution of pigmented rice samples. L*- lightness; a*- redness; b*-yellowness; c- chroma and h- hue angle (o).

as bran layer of the grain (Eyarkai et al., 2017; Reddy et al., 2017). The mineral content in a food relies on its total ash content. The observed protein content stood in a higher side to the values obtained by Devraj et al., (2020) when they analyzed the same on selected traditional and white rice accessions. The results in proximate composition were like earlier reports and near about the desired range (Verma and Srivastav, 2017; Wang et al., 2021). The Carbohydrate content is attributed to the concentration of other components present in rice.

The ASV (from 1 to 6) of rice samples implies the variance in gelatinization characteristics of rice accessions. From the values observed, the collected rice samples possess with low, medium, intermediate, and high gelatinization properties. The observed ASV showed all rice accessions fall under a low to intermediate gelatinization temperatures. da Rosa et al. (2010) classified amylose content of rice starches as high amylose (31.6%), average amylose (23.4%) and low amylose (6.9%). Thus, the rice accessions in the study belonged to average amylose except Kavuni. The amylose content is responsible for the texture and determines the quality characteristics of rice. Goddard et al., (1984) found that high amylose rice slows down digestibility and hence it has a positive impact in reduction of blood glucose levels.

4.2 Starch digestibility, GI and GL

The *in vitro* method for assessing starch hydrolysis rates is both cost-effective and less time-consuming compared to the *in vivo* method. Cooking in excess water causes starch granules to swell and disintegrate, exposing starch chains and making them more susceptible to enzymatic digestion (Deepa et al., 2010). Various physical factors, such as stirring, water-starch ratio, and cooking and cooling regimes, influence the formation of resistant starch (RS) (Deepa et al., 2010). Additionally, the amylose-amylopectin ratio impacts RS formation (Frei et al., 2003). The easier digestibility of pigmented rice accessions can be attributed to their smaller granule size and greater surface area relative to starch, facilitating enzymatic hydrolysis (Deepa et al., 2010). The glycemic index (GI) measures the rise in blood sugar levels two hours after consuming a particular food, while the glycemic load (GL) indicates the rise in blood glucose levels per gram of carbohydrate in the food. Foods are classified based on their GI values as high (≥70%), medium (56-69%), and low (≤55%) (FAO and WHO, 1998). Similarly, GL values classify foods as low (GL ≤10), medium (GL >10 to <20), and high (GL ≥20) (Sethupathy et al., 2020). In the present study, all samples except Kavuni exhibited intermediate glycemic potential according to GI, whereas all samples had a high GL. The high GI and GL values in Kavuni rice could be due to its low amylose concentration and high RDS content.

Numerous studies have shown that low-GI and low-GL meals can help reduce blood glucose levels (Brand-Miller et al., 2003; Zafar et al., 2019). Low-GI diets have also been shown to help prevent cardiovascular diseases (CVD) (Aston, 2006; Maki et al., 2007). Although low-GI foods are preferred for diabetes management, moderate consumption of rice accessions with intermediate *in vitro* GI values can be suitable for glycemic control in diabetic conditions. The correlogram (Fig. 2) displayed a strong negative correlation between amylose content (AC) and RDS with GI and GL, and a significant negative correlation between AC and SDS content.

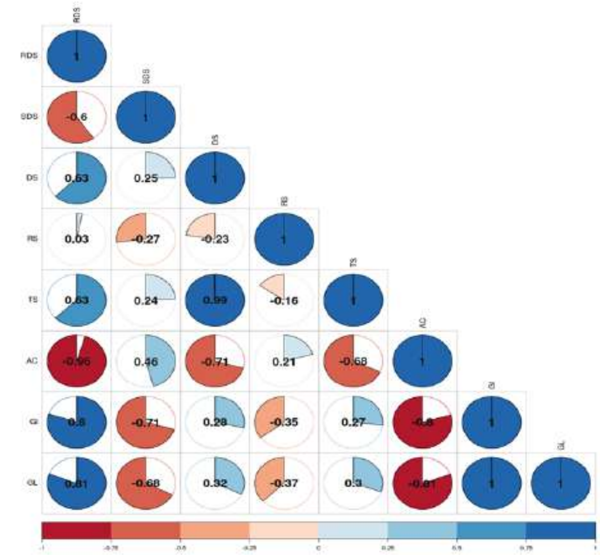

Fig. 2. Correlogram showing the relationship between Starch digestibility, AC, GI and GL ($p<0.05$). RDS- Readily digestible starch; SDS- slowly digestible starch; DS- Digestible starch; RS- Resistant starch; TS- Total starch; AC- Amylose content; GI- Glycemic index; GL- Glycemic Load.

Bioactive components and antioxidant properies

Rice extracts are effective antioxidants owing to their metal ion chelation, reducing capacity, free radical scavenging, and lipid peroxidation prevention. Phenolics with one or more hydroxyl groups form aromatic rings, and their concentration is linked to antioxidant properties hence, they are considered to be desirable for human health (Van Hung, 2016). Similar studies with different coloured pericarp rice accessions have reported that black and red rice accessions have higher total phenolic content (Fracassetti et al., 2020). The flavonoid content in pigmented rice contrasts with the results reported by Tyagi et al. (2022), as total flavonoids are higher in brown rice accessions. Flavonoids and phenolics are covalently linked to cell wall structures via ester linkages that reach the colon undigested. Gut microbes break these, releasing bound phenolics for beneficial biological actions. Therefore, free and bound phenolics and flavonoids are absorbed by the gut lining and have positive health effects. Anthocyanins are the most abundant hydrophilic flavonoids in cereal grains. Anthocyanin consumption has been linked to neuroprotection, glycemic control, anticancer, antihypertensive, and immune response enhancement.

The DPPH, ABTS and FRAP assay examine the antioxidant property of samples; the former is based on hydrogen and electron transfer reactions and the latter is solely on electron transfer reactions (Tyagi et al., 2022). The antioxidant properties may be more pronounced in the case of Kavuni rice accessions which

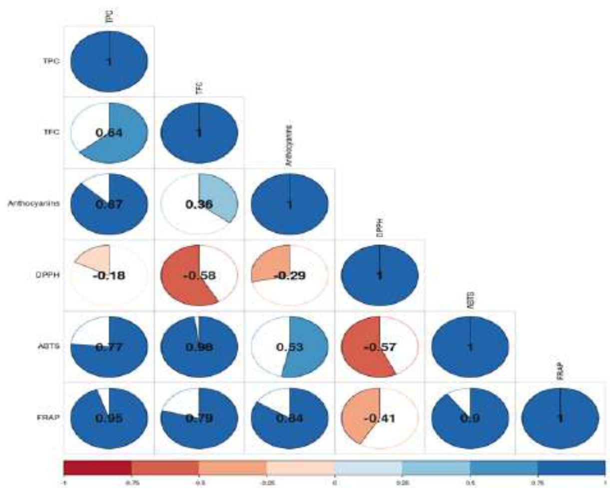

Fig. 3. Correlogram showing relationship between total phenolics, flavonoids and anthocyanin with antioxidants ($p<0.05$). TPC- Total phenolic content; TFC- Total flavonoid content; DPPH- 2,2-Diphenyl-1-picryl hydrazyl free radical scavenging activity; ABTS- 2,2-Azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) free radical scavenging activity; FRAP- Ferric-reducing antioxidant potential (FRAP).

could be attributed to the inhibitory compounds of anthocyanins and phenolics. This property of Kavuni rice accession could reduce the low-density lipoprotein and nitric oxide formation (Sholikhah et al., 2021). Therefore, it could minimize the risk of cardiovascular diseases.

The coloured rice exhibits more antioxidants than white rice, which is more commonly consumed. Loss of the outer bran layer during milling could be one of the factors contributing to the loss of antioxidants in white rice. The correlogram (Fig. 3) showed a significant positive correlation between FRAP and TPC, along with ABTS and TFC. The anthocyanin content exhibited a substantial positive association with TPC, ABTS radical scavenging activity, and FRAP values. These observations clearly demonstrated that pigmented rice is a potential source of antioxidants. These findings indicate that pigmented rice contains a high concentration of phenolics, flavonoids, anthocyanins and other phytochemicals that may improve human health and also promote its use in developing food products.

CONCLUSION

This study provides a comprehensive characterization of five pigmented rice accessions from South India-Matta, Rakthasali, Njavara, Mappilai Samba, and Kavuni-highlighting their distinct physicochemical properties, starch digestibility, and antioxidant capacities. All accessions, except Kavuni, demonstrated intermediate glycemic indices, making them suitable for managing glycemic response with moderate consumption. All samples showed remarkable concentrations of bioactive compounds, including phenols, flavonoids, and anthocyanins. These compounds conferred rice extracts with effective antioxidant properties. These plant bioactives have been linked to neuroprotection, glycemic control, anticancer properties, anti-hypertension, and immune response enhancement. These findings reinforce the therapeutic potential of pigmented rice in managing diabetes and related metabolic disorders. Future research should delve into the molecular mechanisms driving these health benefits and consider breeding strategies to optimize phytochemical profiles for enhanced therapeutic applications.

AUTHORS' CONTRIBUTIONS

The first author contributed to the data collection, analysis, interpretation, manuscript preparation, and revision. The second author was responsible for study design, data interpretation, supervising, manuscript preparation, and revision.

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Economic and institutional analysis of hybrid rice seed value chain in India: Evidence from Telangana

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ABSTRACT

This study analysed the institutional and economic dynamics of the hybrid rice seed industry in Telangana, India, during the 2021-22 summer season, with a focus on contractual arrangements in production and benefit distribution among stakeholders in hybrid rice seed value chain. Primary data from 196 seed growers, 60 organizers, and 10 companies across 30 seed villages revealed that hybrid rice seed enterprises predominantly operated under a centralized contract farming model. Of the 196 seed growers, 96.43% engaged in contracts, with 55.55% relying on verbal and 44.45% on written contracts, the latter proving more effective due to well-defined terms. Institutional support played a pivotal role in enhancing productivity, with technical staff visits, contracts with multinational corporations, and written agreements positively influencing hybrid rice seed yield. Training participation had the most significant impact, underscoring the importance of capacity-building interventions in improving production efficiency. An analysis of benefit distribution indicated that seed companies captured the largest share (53.64%) of total economic gains, followed by seed dealers (20.23%), seed distributors (13.53%), seed growers (10.26%), and organizers (2.08%). The study recommends the establishment of a legal framework to formalize and enforce contracts, promoting written agreements for greater transparency in pricing and payment terms. Additionally, policies should mandate price transparency and equitable benefit-sharing mechanisms to ensure fair compensation for seed growers while optimizing costs and improving value distribution within the hybrid rice seed value chain.

Key words: *Hybrid rice, seed system, contract farming and value chain analysis*

INTRODUCTION

Rice is a vital agricultural crop globally, particularly in Asia, where it plays a pivotal role in economic development, poverty reduction, and food security. In India, rice ensures the food security of over 65% of the population, accounting for nearly 40% of total food grain production and providing a major share of caloric intake (Pathak et al., 2020). For millions of smallholder farmers, rice cultivation is a primary source of income and food self-sufficiency. To meet the demands of a growing population and improve farm incomes, enhancing rice productivity had become a national priority. This imperative led the Indian government to promote high-yielding rice varieties in the 1960s, which subsequently triggered the Green Revolution (Pingali et al., 1997; Pingali and Hossain, 1999). As a result, rice production had increased at an average rate of 2.8% per annum between 1968 and 2000, peaking at around 4% during the 1980s (Janaiah and Xie, 2010). However, by the early 1990s, the rate of yield improvement in India's irrigated rice systems had begun to decline, raising concerns over the long-term stability of rice supply and food security (Janaiah et al., 2005; Janaiah et al., 2006).

This emerging stagnation in yield gains had prompted researchers and policymakers to explore alternative technologies to sustain rice productivity, particularly in favourable agro-climatic regions (Janaiah, 2000). Inspired by China's successful experience in commercializing hybrid rice, the Indian Council of Agricultural Research (ICAR) had initiated a focused research programme to develop hybrid rice varieties. In 1994, the first hybrid rice variety was approved for commercial cultivation, following its initial release during the *rabi* season of 1993-94 in Andhra Pradesh. This marked a significant milestone, making India the second country after China to adopt hybrid rice technology on a commercial scale (Janaiah, 2002).

Since then, India has developed 117 hybrid rice varieties, offering a 15-20% yield advantage over traditional inbred varieties and spanning durations of 115-150 days to suit various ecosystems (Rout et al., 2020). With rice cultivated on approximately 45 million hectares, the crop remains a cornerstone of India's agricultural economy (Agricultural Statistics at a Glance, 2021, MOA, GOI). The commercial introduction of hybrids attracted private sector investment in the rice seed market (Janaiah, 2002). However, adoption remained sluggish until 2004 due to grain quality concerns, high seed costs, and limited price premiums (Virmani and Ish Kumar, 2004; Spielman et al., 2013; Chengappa et al., 2003). But, between 2008 and 2020, hybrid rice acreage expanded from 1.4 to 3.5 million hectares, particularly in northern and eastern India, where hybrids outperformed high-yielding varieties (HYVs) (Janaiah and Xie, 2010; Verma et al., 2020). This expansion triggered greater demand for hybrid seeds, contributing to a 12% share of India's ₹10,000 crore hybrid seed industry (Kaviraju et al., 2022; NSAI, 2020).

Hybrid rice cultivation emerged as a strategic intervention to enhance productivity and ensure food security in densely populated and land-constrained regions. However, its widespread adoption depends largely on the availability of affordable and high-quality hybrid seeds, as observed in other crops such as maize, pearl millet, sorghum, and sunflower (Trip, 1998). The affordability and accessibility of hybrid seed are influenced by the costs incurred across various stages of the seed value chain and the institutional arrangements that govern interactions among different stakeholders. Despite the growing significance of hybrid rice in India's agricultural development, comprehensive empirical data on the economic dynamics and institutional mechanisms of the hybrid seed sector remain limited. Addressing these knowledge gaps is crucial to improving the efficiency, equity, and sustainability of the hybrid rice seed system. In this context, the present study has been undertaken to examine the institutional framework of hybrid rice seed production, assess the role of contractual arrangements in seed production, and analyse the benefit-sharing mechanisms among key stakeholders, including seed growers, organizers, seed enterprises, distributors, and dealers.

The subsequent sections of this paper present the methodological framework, empirical results and discussion, and finally, conclusions along with policy implications.

MATAREIALS AND METHODS

Telangana contributes 85% of India's hybrid rice seed production, with Hanamkonda and Karimnagar districts accounting (Fig. 1) for nearly two-thirds of the state's output due to favourable Agro-climatic conditions, reliable irrigation, and the presence of R&D-driven private sector firms (Pal & Tripp, 2000; Janaiah & Xie, 2010; Janaiah and Behura, 2014; Nirmala, 2015). Given the significant role of hybrid seed production in Telangana's agricultural economy, this study was conducted in 30 villages from Hanamkonda and Karimnagar districts (15 villages from each district) (Table 1). In these villages, 80-85% of farmers are

Table 1. Sampling framework of study area.

S. no.	Characteristics of study area (Telangana)	Particulars	
1	Selected districts	Hanamkonda	Karimnagar
2	Number of Mandals (Blocks) form each district	3	3
3	Number of villages	15	15
4	Method of sampling	Purposive	Purposive
5	Sample seed growers	94	102
6	Seed organisers (middlemen)	30	30
7	Seed companies' representatives	10	
6	Crop year	2021-22 DS*	2021-22 DS*

*Note: Dry season (*rabi*)/ summer season.

predominantly involved in rice seed production for public and private seed agencies (Kaviraju et al., 2022).

This study, based on the author's doctoral research, surveyed 300 rice seed growers from above said 30 villages (10 hybrid and inbred rice seed growers per village), comprising 196 hybrid rice growers and 104 inbred rice growers. Data for this paper were specifically derived from 196 hybrid rice seed growers collected during the 2021-22 Summer/Dry Season (DS), including 94 growers from Hanamkonda and 102 from Karimnagar (Table 1). A purposive sampling approach, in consultation with local stakeholders, was employed to select experienced hybrid rice seed growers. Additionally, 60 seed organizers (two per village) and representatives from 10 seed companies were surveyed to offer a comprehensive perspective on the hybrid rice seed value chain (Table 1).

Three sets of primary data were collected from key sources: seed growers, seed organizers, and seed company representatives engaged in hybrid seed production, processing and distribution during the 2021-22 DS/Summer Season. The personal interview method was used to collect data from these sources using pretested and structured schedules. Additionally, secondary data on the value of the seed business and the share of Telangana in hybrid rice seed production were also used to complement the primary data.

Seed growers: Data on household profiles, asset endowment, cropping patterns, seed production details, contractual arrangements, and payment structures.

Seed organizers: Data on their role in seed production, business scale, procurement pricing, and contractual agreements.

Seed companies: Data on seed processing, storage, distribution, and marketing.

i. Correlation analysis

The possible linear association between yield and price of hybrid rice seed varieties for different seed enterprises was assessed through Pearson correlation coefficient. A high positive correlation implies that two variables move in the same direction, whereas a negative correlation indicates an inverse relationship between them. In this study, a negative correlation was observed between seed yield and prices, which is theoretically expected-higher yields typically lead to greater market supply, often resulting in lower prices, and vice versa.

$$r = \frac{n\left(\sum xy\right) - \left(\sum x\right)\left(\sum y\right)}{\sqrt{\left[n\sum x^2 - \left(\sum x^2\right)\right]\left[n\sum y^2 - \left(\sum y^2\right)\right]}}$$

r = correlation coefficient

n = size of the sample

ii. Multiple linear regression model

The multiple linear regression analysis was used to understand the factors influencing the hybrid rice seed yield based on the primary data. Formula for computing multiple linear regression is as follows

$$Y = b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_5X_5 + b_6X_6 + b_7X_7 + b_8X_8 \text{ ----------} + bn\,Xn + e$$

Where,

Y = Yield of hybrid rice seed variety

b_0 = Intercept

b_1 to b_7 = Beta coefficients

X_1 = Age (years)

X_2 = Family size (number)

X_3 = Size of farm (acres)

X_4 = Number of Technical Staff visits

X_5 = MNC (MNC 1, otherwise 0)

X_6 = Written agreement (written 1, otherwise 0)

X_7 = Training (Trained 1, Otherwise 0)

RESULTS AND DISCUSSION

A) Institutional framework of the hybrid rice seed industry

The hybrid rice seed industry has constituted a complex institutional framework involving key stakeholders such as seed growers, seed organizers, seed companies, seed distributors, and seed retailers (Fig. 2). Seed growers, operating under contractual arrangements, have specialized in multiplying parental seed lines (A-line and R-line) provided by seed companies to produce hybrid rice seeds while adhering to strict genetic purity

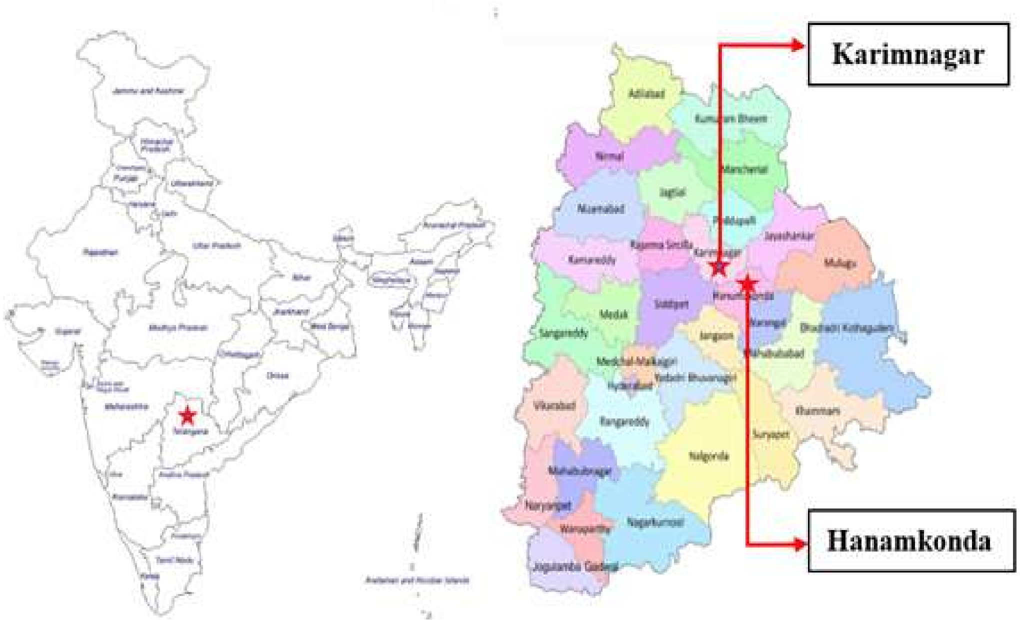

Fig. 1. Geographical representation of the study area in Telangana, India (red coloured star).

standards. The production process has predominantly been organized under a centralized contract farming model, wherein seed companies have formalized agreements with seed growers through intermediaries, typically referred to as seed organizers. In some instances, seed companies have established direct contractual relationships with both seed growers and organizers separately. However, these contracts have largely remained informal, lacking legal enforceability, which has undermined their ability to effectively address transaction risks (Fig. 2).

The contractual arrangements between seed companies and seed growers have outlined the supply of parental lines (A and R lines), along with essential inputs such as pesticides, fertilizers, and growth

Hybrid Rice Seed Value Chain	Identification of Seed Grower	Seed Production	Seed Processing	Seed Marketing & Distribution
Actors	Private seed companies and seed organisers	Contract seed growers, seed organisers and technical staff from seed companies	Private seed processors and seed companies	Wholesale distributors and Retail dealers
Output	Supply prenatal lines, fertilizers, pesticides and growth regulators	Hybrid rice seed produced and sent to processing centres from seed villages	Processing and packaging	Truthfully labelled seed made available for hybrid rice cultivators
Key players in industry	Private Seed Companies, Seed Organisers, Seed Growers, Wholesale Distributors and and Retail dealers			

Fig. 2. Functional system of hybrid rice seed industry.

regulators like gibberellic acid (GA_3), which have been critical for hybrid rice seed production. Furthermore, these contracts have stipulated stringent quality standards, pre-determined procurement prices for hybrid seeds, and the provision of technical support by seed companies. Risk mitigation mechanisms, such as compensation provisions in the event of crop failure, have also been embedded in these contracts to address production uncertainties.

Seed organizers have served as pivotal institutional intermediaries in the hybrid rice seed value chain by coordinating and enforcing contractual obligations. They have played a critical role in identifying skilled growers with reliable irrigation access, supplying inputs, and facilitating credit arrangements, including advances for pre-season production expenses. Organizers have overseen compliance with production protocols by conducting regular field inspections and mediating interactions between growers and the technical staff of seed companies. Their operational responsibilities have extended to monitoring adherence to production standards, ensuring timely communication, and providing logistical support during harvest. For their services, organizers have received commissions, typically based on the quantity of hybrid rice seeds produced, measured in tonnes, quintals, or kilograms (Fig. 2).

Post-harvest, hybrid rice seeds have been procured by seed companies from contracted growers at pre-agreed prices. Organizers have managed the transportation and logistics, ensuring the timely delivery of harvested seeds to the seed company's processing facilities. Upon arrival, the seeds have undergone rigorous quality and germination testing to confirm compliance with contractual standards. Only after these tests have been completed, and the seeds have met the required specifications, seed growers received payments for their contributions to the production process.

To uphold quality assurance, seed companies have adopted institutional mechanisms such as Truthful Labelling (TFL), a self-certification process guaranteeing the genetic and physical purity of hybrid seeds. The TFL seeds have subsequently been integrated into the company's distribution network and supplied to the broader market (Fig. 2). This distribution network has comprised seed distributors and dealers, where distributors have acted as wholesale intermediaries by acquiring seeds from the seed company and selling them to dealers (retailers) and cultivators. The distribution process has involved a credit system, where payment to the seed company has been made only upon the sale of seeds by the distributor, and any unsold surplus has been returned to the seed company for storage until the next crop season (Fig. 2).

This functional system illustrates the interplay of governance, risk-sharing, and coordination mechanisms within the hybrid rice seed production value chain. It underscores the role of institutional intermediaries in reducing transaction costs, ensuring production efficiency, and maintaining product quality, all of which are critical for the sustainable development of the hybrid rice seed industry.

B) Key players in hybrid rice seed production

The study revealed that a total of 374.5 hectares were allocated to hybrid rice seed production by sample growers during the 2021-22 Summer season/DS. Over 15 private companies were actively engaged in hybrid rice seed production within the study area. Among these, Kaveri Seeds and Metahelix emerged as the leading firms, collectively accounting for approximately 27% of the cultivated area and producing a more extensive range of hybrid rice seed varieties compared to other companies. A notable aspect of these firms' operations was the use of coded nomenclature, which preserved the confidentiality of their seed varieties by concealing their commercial identities. This strategy minimized the risk of unauthorized cross-purchasing or dissemination of seed varieties among competitors (Table 2). The study area lacked the involvement of any public agency in hybrid rice seed production, primarily due to the capital-intensive nature and the intricate institutional framework associated with the hybrid rice seed system.

While nearly 97% of the seed growers were aware of the company with which they had contracted, only 40% were familiar with the specific coded name of the hybrid rice variety they produced. This finding highlighted the significance of proprietary safeguards in sustaining competitive integrity within the hybrid rice seed market (Fig. 3).

Table 2. Seed Companies' hybrid rice varieties: area share, average yield, and price.

S. no.	Name of company engaged in hybrid rice seed production	Name of hybrid rice seed varieties	Area under the varieties (hectares)	Average yield (tonne/ hectare)	Price paid to seed grower (₹/ tonne)
1	Kaveri seeds Pvt Ltd.	Kaveri-906, Kaveri-909, Kaveri-6, Kaveri-1, Kaveri-476, Kaveri-701 and Kaveri-901	51.2 (13.67)	2.47	79,560
2	Metahelix Pvt Ltd.	Dhanya-1, Dhanya-6, Dhanya-8, Dhanya-12 and Dhanya-20	49.8 (13.30)	2.73	81,560
3	Bayer crop science	Bayer-766	33.12 (8.84)	2.13	86,500
4	Syngenta	Syngenta-200 and Syngenta-11	27.1 (7.24)	3.28	53,660
5	Mahindra Agri business Pvt Ltd.	M-276 and MP-3030	23.0 (6.14)	3.32	59,000
6	Pioneer	Pioneer-77, Pioneer-95, Pioneer-44 and Pioneer-009	19.0 (5.07)	3.35	57,000
7	Ajith seeds Pvt Ltd.	Ajith-816, Ajith-813 and Ajith-859	15.6 (4.17)	2.33	70,000
8	JK AGRI Genetics Ltd.	JK-2082, JK-2005 and JK-1220	15.2 (4.06)	2.75	56,800
9	VNR Seeds Pvt Ltd.	VNR-3	12.8 (3.42)	2.28	72,400
10	NU Genes Pvt Ltd.	N-5079	12.4 (3.31)	2.55	71,330
11	US Agri Seeds	Usagri-1	11.6 (3.10)	2.56	80,000
12	Advanta India Pvt Ltd.	Upl-60 and UPL-204	10.6 (2.83)	2.95	64,330
13	Nuziveedu seeds Pvt Ltd.	Nuziveedu-15, Nuziveedu-476	9.8 (2.62)	3.00	68,000
14	Ankur Seeds Pvt Ltd.	Ankur-135 and Akur-12	4.8 (1.28)	2.63	65,500
15	Rasi seeds Pvt Ltd.	Rasi-2 and Rasi-4	4.6 (1.23)	2.25	80,000
16	Others	-	73.9 (19.72)	2.25	67,400
	Total		374.5 (100)	2.68	69,600

Source: Compiled from authors own survey data, 2021-22 DS. Note: Figures in the parenthesis are per cent to total.

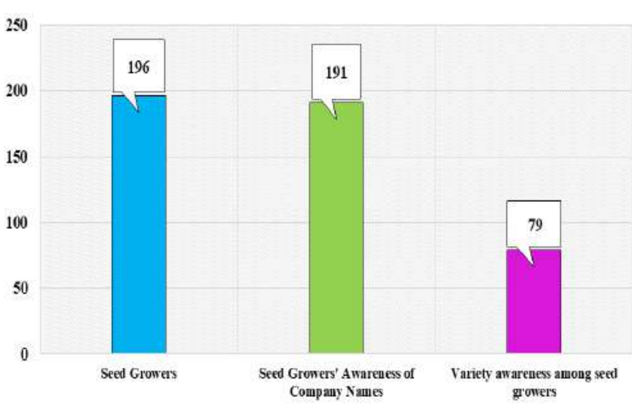

Fig. 3. Awareness of seed company and variety among seed growers.

The study further indicated that, on average, seed growers in the study area produced 2.68 tonnes of hybrid rice seed per hectare, selling the seeds at an average price of ₹69,600 per tonne to the contracted seed companies. During the 2021-22 DS, companies such as Bayer Crop Science, Metahelix, Rasi Seeds, Kaveri Seeds, and US Agri Seeds offered higher procurement prices of ₹80,000 per tonne. This observation was noteworthy, as it appeared that seed companies with higher-yielding hybrid varieties tended to offer lower prices to the sample seed growers, likely leveraging economies of scale (Table 2). To analyse the relationship between price and yield, a Pearson

Table 3. Relation between price and yield of hybrid rice seed varieties.

Pearson Correlation	Yield	Price
Yield	1	-0.568**
Price	-0.568**	1

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

correlation coefficient analysis was performed. The results indicated a strong, negative, and statistically significant correlation between the two variables, confirming that an increase in yield was associated with a decrease in price, and vice versa (Table 3). This finding suggested that the economic benefits from enhanced yields were predominantly captured by the private seed companies. As a result, seed growers who achieved higher yields received lower prices for the hybrid rice seed varieties they produced in the study area.

C) Contractual arrangements and their impact on seed yield

i. Institutional variations in contracting: verbal vs. written agreements

The study identified two primary contracting mechanisms in hybrid rice seed production: verbal and written agreements, which reflected variations in transaction governance. These contracts, typically spanning one season or year, specified terms such as procurement price, supply of parental line seeds and

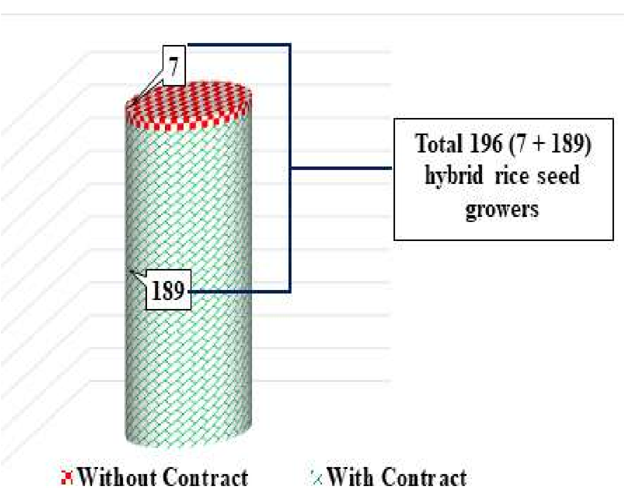

Fig. 4. Contracted and non-contracted seed growers.

Table 4. Contractual specifications in hybrid rice seed production: Verbal vs. Written Agreements

S. no.	Specifications in agreement	Verbal	Written
1	Contract type (No)	100	89
2	Price specification	71 (71.00)	74 (83.14)
3	Quality to be maintained	44 (44.00)	67 (75.28)
4	Supply of parental lines	36 (36.00)	54 (60.67)
5	Information on risk allowance	17 (17.00)	38 (42.70)
6	Supply of GA3	25 (25.00)	16 (17.98)

Source: Estimated from author's own survey data, 2020-21 DS

gibberellic acid (GA3), quality standards, and risk allowances for crop failure (Table 4). Out of the 196 seed growers selected for the study, 189 (96%) had contracts: 100 (55.55%) with verbal agreements and 89 (44.45%) with formal written contracts (Fig. 4 and Table 4). Seven growers (4%) operated without contracts (Fig 1).

Verbal agreements, predominantly employed by domestic seed companies, relied on oral communication of terms by seed organizers to growers. Although this informal approach minimized administrative costs, it often resulted in inefficiencies due to ambiguity and lack of enforceability. In contrast, written agreements, extensively utilized by multinational companies (MNCs), provided a formalized structure by explicitly outlining terms such as pricing, input supply (*e.g.*, GA3 and parental lines), quality standards, and risk-sharing mechanisms. These contracts mitigated transaction costs by reducing information asymmetry and fostering operational transparency, aligning with Williamson's (1985) transaction cost economics framework. The preference for written agreements by MNCs reflected their emphasis on governance efficiency and adherence to global best practices, aligning with findings from Napasintuwong (2015) study in Thailand, where formal written contracts in maize seed production enhanced grower confidence and participation. This dichotomy between verbal and written agreements underscored the institutional heterogeneity in the hybrid rice seed sector, influenced by factors such as asset specificity, risk, and transaction complexity. Encouraging the broader adoption of formalized agreements could have strengthened governance mechanisms, reduced disputes, and enhanced the overall efficiency of the hybrid rice seed production value chain.

ii. Impact of socio-economic and contractual arrangements on hybrid rice seed production

The multiple linear regression (MLR) model was employed to examine the institutional and socio-economic factors influencing hybrid rice seed yield, integrating key explanatory variables that reflect contractual arrangements and governance structures within the hybrid rice seed value chain. The model explained 85% of the variation in seed yield, indicating that institutional mechanisms and contractual relationships play a pivotal role in determining production outcomes (Table 5).

Among the explanatory variables, the age of the seed grower had a significant negative impact on yield (β = -0.0658, $p < 0.01$), indicating that labour-intensive hybrid rice seed production poses challenges for older farmers. This can be attributed to the physically demanding nature of key tasks involved in hybrid seed production, such as supplementary pollination using bib ropes, synchronization of flowering, and timely rouging, all of which require substantial manual effort, agility, and precision. Due to age-related physical limitations, older farmers may find it difficult to perform these tasks effectively, leading to reduced involvement or increased dependence on hired labour. This, in turn, may affect operational efficiency and overall yield performance. Similarly, farm size showed a negative but insignificant coefficient, suggesting that as farm size increases, growers may encounter operational inefficiencies. These inefficiencies likely stem from labour constraints, which impede the timely execution of critical agronomic practices essential for maintaining genetic purity and optimizing seed yield (Table 5).

In contrast, Institutional factors played a crucial role in enhancing productivity. Technical staff visits had a strong positive effect (β = 0.2021, $p < 0.01$), reinforcing the role of extension services in mitigating information asymmetry and ensuring compliance with production protocols. Contractual arrangements with multinational corporations (MNCs) significantly increased yields (β = 1.3895, $p < 0.01$), likely due to the robust institutional support mechanisms provided by these entities, including superior input provision, risk-sharing frameworks, and strict adherence to production protocols. Furthermore, written contracts were positively associated with seed yield (β = 1.4320, $p < 0.01$), suggesting that formalized agreements enhance contract enforceability, reduce opportunistic behaviour, and improve coordination between seed growers and firms. Similarly, training participation had the highest positive impact on seed yield (β = 1.6734, $p < 0.01$), underscoring the critical role of capacity-building interventions in improving technical efficiency and ensuring compliance with hybrid seed production protocols (Table 5). These findings highlight the institutional embeddedness of hybrid rice seed production, where structured contracts, extension services, and MNCs engagement reduce transaction risks and improve productivity. Strengthening formal agreements, extension networks, and farmer training is essential for enhancing seed sector efficiency and sustainability.

Table 5. Regression results of hybrid rice seed yield function.

S. no.	Variables	Coefficient (b)	Standard Error (SE)	t-statistic
1)	Intercept	9.5304***	1.0512	9.0714
2)	Age (years)	-0.0658***	0.0153	-4.295
3)	Family size (number)	0.3756***	0.1524	2.4633
4)	Size of farm (acres)	-0.0351	0.0521	-0.6737
5)	Number of technical staff visits	0.2021***	0.0582	3.4739
6)	Contracted company (MNC 1, otherwise 0)	1.3895***	0.3546	3.9147
7)	Type of agreement (Written 1, otherwise 0)	1.4320 ***	0.3641	3.9346
8)	Training (Trained 1, other wise 0)	1.6734 ***	0.3695	4.5301
	R^2		84.57%	
	F-statistic		63.29	

Note: *** significant at 1 per cent level,

D) Cost and return profile across stakeholders in the hybrid rice seed value chain

The following section outlines the computation and presentation of the cost and return profile at each stage of the hybrid rice seed value chain. Several studies have highlighted the high cost of hybrid rice seeds as a significant barrier to their adoption in cultivation (Janaiah, 2000; Chengappa et al., 2003; Spielman et al., 2013; Negi et al., 2020). Analysing this information provides valuable insights into the factors that contribute to the elevated prices of hybrid rice seeds. By understanding the cost and return dynamics at each stage of the value chain, stakeholders can gain a clearer understanding of the underlying factors influencing seed pricing. This knowledge can, in turn, support informed decision-making and facilitate the development of strategies for cost optimization or price adjustments within the value chain.

i. Cost and returns profile of seed grower

The cost structure for hybrid rice seed production on sample seed farms is presented in Supplementary Table 1. The total input cost averaged at ₹69,444 per hectare. Labour costs emerged as the largest component, accounting for 52% of total cost. This high proportion reflects the additional labour-intensive operations required for hybrid rice production, such as rouging, pollination, and leaf clipping, which are not necessary in conventional rice cultivation. A similar cost pattern was noted in maize seed production, as reported by Kumar et al. (2017), highlighting the significant employment opportunities in regions engaged in large-scale hybrid rice seed production.

Under contractual arrangements, seed companies provided parental lines of hybrid rice and GA3 (gibberellic acid) to seed growers at no cost. However, in some instances, only one of these inputs was provided free of charge, leading to partial cost-sharing by the growers. Following labour costs, machinery usage accounted for 17% of total production costs. The remaining 30% of the expenses were attributed to the procurement of organic manure, chemical fertilizers, and other material inputs. This cost distribution highlights the significant role of both human and material resources in hybrid rice seed production, making it a more resource-intensive process than conventional rice cultivation (Supplementary Table 1 1).

Supplementary Table 2 presents the average seed yield and returns from hybrid rice seed production on sample seed farms. The economic outcomes of hybrid rice seed production are represented by the A-line (cytoplasmic male sterile line/female parental line), which was procured by seed companies, and the R-line (restore line/male parental line), which was sold as grain in the commercial market. The average yield of hybrid rice seed (F1 seed) was 2.68 tonnes per hectare, surpassing the national average of 2.39 tonnes per hectare (Janaiah and Behura, 2014). This higher yield was attributed to the favourable climate conditions in the study area for seed production. Further, seed companies offered a procurement price of ₹69,600 per tonne for the hybrid rice seed sold by growers, while the operational cost of production was ₹49,220 per tonne. This resulted in a net profit of ₹20,380 per tonne for seed growers (Supplementary Table 2 and Fig. 5). When considering both the main product (hybrid rice seed) and the by-product (R-line male parent seed), the total gross returns from hybrid rice seed production were estimated at ₹2,12,784 per hectare. The total input cost incurred by sample seed farms was ₹1,31,912 per hectare, leading to a net return of ₹80,872 per hectare (Supplementary Table 2).

ii. Cost and returns profile of seed organiser

The study revealed that, on average, seed organizers received ₹75,600 per tonne from seed companies for procuring hybrid rice seed. Of this amount, ₹69,600 per tonne was paid to seed growers, while seed organizers retained a gross margin of ₹6,000 per tonne as commission for their intermediary services. The operational costs incurred by the organizers were estimated at ₹2,000 per tonne, resulting in a net benefit of ₹4,000 per tonne (Fig. 5).

iii. Cost and return profile of seed enterprises/ companies

The cost and return analysis conducted at the seed company level demonstrated that the total production cost amounted to ₹91,600 per tonne (Supplementary Table 3). This included ₹75,600 per tonne for the procurement of hybrid rice seeds from contracted growers, facilitated through seed organizers, ₹9,000 per

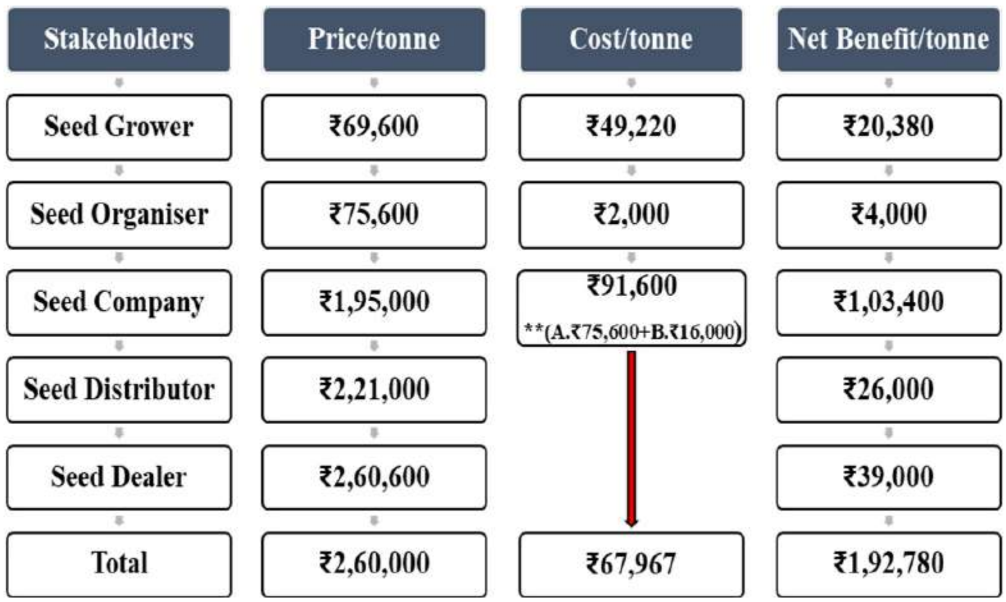

Fig. 5. Hybrid rice seed industry value chain: Stakeholder-wise profitability
**Note: The total cost at the end of the value chain (₹67,967) accounted for only non-procurement expenses incurred at the seed company level, including processing, packaging, transportation, and storage costs (B.₹16,000) (Supplementary Table 3). The procurement cost (A.₹75,600) was excluded to prevent double counting, as it had already internalized the production costs and net margins at both the seed grower and seed organizer levels.

tonne for post-harvest processing and packaging, ₹4,000 per tonne for transportation (transportation from villages to seed processing units and from seed companies to distributors and dealers), and ₹3,000 per tonne for storage and inventory management (Supplementary Table 3). After undergoing processing and packaging, the hybrid rice seeds were transferred to downstream stakeholders, specifically seed distributors, at a sale price of ₹1,95,000 per tonne. The resulting net profit of ₹1,03,400 per tonne was calculated by deducting the total production costs from the revenue generated from the distributor's purchase price (Fig. 5).

iv. Cost and return profile of seed distributor/ wholesaler

The seed distributor purchased seeds from the company at ₹1,95,000 per tonne and sold them to seed retailers at ₹2,21,000 per tonne, generating a net benefit of ₹26,000 per tonne (Fig. 5). Seed dealers, who were in direct contact with cultivators, received ₹2,60,000 per tonne from them and paid ₹2,21,000 per tonne to the distributor, yielding a net benefit of ₹39,000 per tonne (Fig. 5). The higher margin for seed dealers has been attributed to their proximity to cultivators and their active role in promoting hybrid rice varieties on behalf of the seed companies they are associated with through distribution networks. This tendency is consistent with findings by Kulkarni et al. (2017), which indicated that seed dealers in the hybrid maize sector earned a higher margin (15%) than distributors (10%). It was noted that seed distributors and dealers did not bear any direct operational costs, as these were absorbed by the seed industry during processing, packaging, and distribution stages. This cost structure highlights the significant role played by seed companies in bearing initial expenses and ensuring the smooth flow of hybrid rice seeds to cultivators, thereby enhancing accessibility and market penetration.

D) Distribution of benefits among different stakeholders in hybrid rice seed value chain

The distribution of benefits within the hybrid rice seed value chain plays a critical role in fostering economic equity, enhancing stakeholder participation, and ensuring the financial sustainability of the actors involved, while also contributing to the optimization of the value chain and the formulation of evidence-based agricultural policies. This study quantitatively assessed the

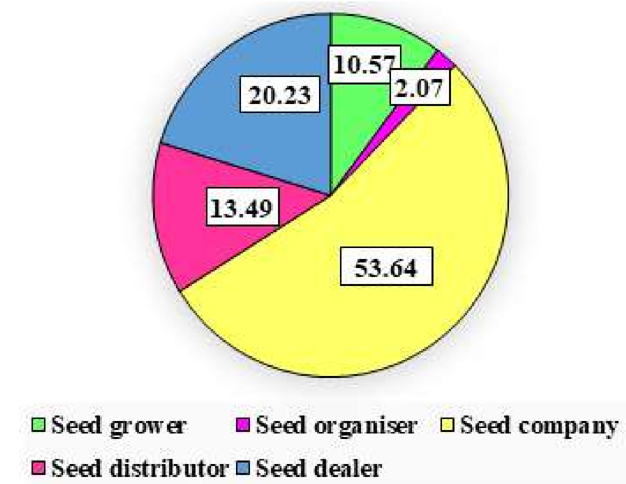

Fig. 6. Percent share of various stakeholders in total benefits of hybrid rice seed chain.

distribution of benefits among stakeholders to provide a comprehensive understanding of value chain dynamics.

The gross price realized at the endpoint of the value chain was ₹2,60,000 per tonne, with cumulative stakeholder costs estimated at ₹67,220 per tonne (Fig. 5). Consequently, the net benefits across the value chain were calculated as ₹1,92,780 per tonne. Of these total benefits, the seed company retained the largest share (53.64%), followed by seed dealers (20.23%), seed distributors (13.53%), seed growers (10.26%), and organizers (2.08%) (Fig. 6). While the seed company received the highest share, it also faced substantial costs related to R&D, advertising, marketing, employee salaries, and establishing processing units, which need to be recovered from these benefits.

CONCLUSION

This study revealed that seed companies predominantly operated under a centralized contract farming model, where verbal agreements were common despite the effectiveness of written contracts. It was observed that Multinational Companies (MNCs) favoured written contracts, ensuring greater transparency in terms of pricing, payment, and input provisions, which enhanced seed growers' confidence. This approach was reflected in the results of the hybrid rice seed yield function, where contracted seed growers working with MNCs achieved more yields. In contrast, domestic companies often relied on informal, verbal agreements, which concealed pricing and led to unfair margins for intermediaries (seed organisers). Additionally, companies offering higher-yielding hybrid rice seed varieties tended to provide lower prices to sample seed growers, leveraging economies of scale. Institutional support significantly influenced productivity, with technical staff visits, formal contracts, and training participation playing key roles in enhancing seed yield. The analysis of benefit distribution within the hybrid rice seed value chain revealed that the seed companies captured the largest share of the benefits, followed by seed dealers, seed distributors, seed growers, and organizers, with growers receiving only a small portion of the benefits despite their essential role. The findings emphasized the need for improved contractual transparency, fair pricing mechanisms, and more equitable benefit-sharing within the value chain.

This study recommends establishing a legal framework to formalize and enforce contracts in hybrid rice seed production, promoting written contracts to enhance transparency in pricing and payment terms. Policies should mandate price transparency and establish fair pricing mechanisms to ensure just compensation for seed growers. Additionally, equitable benefit-sharing mechanisms should be introduced to ensure a fair distribution of economic gains among all stakeholders, this also enables cost optimization and price adjustments within the hybrid rice seed value chain.

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