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Society for Advancement of Rice Research

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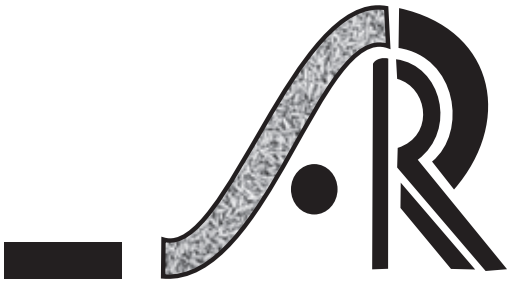

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Morphological and molecular diversity in rice (*Oryza sativa* L.) genotypes

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Abstract

The study was carried out to assess the genetic relatedness of 50 rice genotypes based on morphological and molecular diversity analysis. Data recorded for 19 morphological traits was used for grouping genotypes into different clusters by using Tocher's and Ward's minimum variance methods. The results revealed that the genotypes were grouped into 8 clusters in both the methods. Based on the clustering pattern and inter cluster distance obtained from both morphological diversity analysis studies, it is suggested that BPT 2231, MTU 1032, MTU 1075, RGL 2537 and WGL 20471 may be crossed with BPT 2411, BPT 2605, BPT 2511, BPT 2570, JGL 3844, JGL 17004, JGL 11721, Surya, BPT 2505 and BPT 2571 for obtaining superior transgressive segregants. Total of 20 SSR markers were used for screening these genotypes, in which fifty genotypes were grouped into three major clusters which were divided into 2-3 sub-clusters. The tree obtained from the SSR marker revealed that the marker was more discriminatory, highly polymorphic and thus more informative than the one obtained from morphological data.

Key words: Rice diversity, Morphological markers, SSR markers

Introduction

Although there are large numbers of rice varieties popular and cultivated in Andhra Pradesh, a small fraction of these have been used in practical breeding. Therefore, better understanding of the genetic makeup of underutilized rice germplasm is an important issue for rice breeding. Knowledge regarding the extent of genetic variation and genetic relationships between genotypes are vital for designing effective breeding and conservation strategies. Genetic analysis of rice cultivars collected from different regions helps in understanding the complex interaction between rice diversity and human cultivation practices and culture, as the cultivar structure is shaped by the interplay between adaptation to the local environment and artificial selection imposed by the rice breeders and farmers. Recent advent of molecular and computational tools now enables the estimation of genetic diversity and population yield structure of rice germplasm rather easily (Vanniarajan *et al.*, 2012). Realizing the need to improve the productivity and quality of rice, an attempt was made to study the diversity of 50 rice genotypes for various yield components and quality traits.

Material and methods:

The material for diversity analysis consisted of released varieties and advanced cultures collected from various research stations in Andhra Pradesh. The present investigation was carried out during Kharif, 2012 at Agricultural College Farm, Bapatla, with 50 rice genotypes while the molecular diversity analysis was carried out at Indian Institute of Rice Research, Hyderabad. The experiment was laid out in RBD with two replications. One month old seedlings were transplanted in thoroughly puddled main field. Each experimental unit consisted of 3.0m^2 and the spacing adopted was 20cm between the rows and 15 cm between the plants. All the recommended package of practices were followed during the crop growth and data was collected on ten randomly selected plants in each replication for 19 yield components and quality traits *viz.*, plant height, number of ear bearing tillers per plant, panicle length, days to 50% flowering, number of filled grains per panicle, test weight, kernel length, grain yield/plant, hulling percentage, milling percentage, head rice recovery percentage, kernel breadth, L/B ratio, kernel length after cooking, water uptake, alkali spreading value, amylose content, elongation ratio and gel consistency. The mean data obtained was used to determine the genetic diversity among the genotypes using Mahalanobis D^2statistic as per Rao (1952), Principal component analysis (PCA) as described by Jackson (1991) and cluster analysis as described by Anderberg (1993).

Molecular diversity: The DNA of 50 genotypes for genotyping was isolated from young leaves harvested after 15 days of sowing using C-TAB method as described by Doyle and Doyle (1990). The genomic DNA of these genotypes was subjected to PCR amplification as per the procedure described by Chen *et al*. (1997). PCR was carried out using a programmable thermocycler (Corbett Research, Australia). The PCR reaction mixture containing 2µl DNA, 8.5 µl water, 1.5 µl Taq buffer, 1 µl dNTP, 0.5 µl forward primer, 0.5 µl reverse primer and 1 µl Taq polymerase (15 µl reaction mixture) was subjected to the polymerase chain reaction. 36 microsatellite markers distributed over 12 chromosomes were used to reveal the genetic polymorphism between resistant and susceptible mutants. The profile of the PCR (PCR conditions) was as follow: 94ºC - 5 minutes (Initial denaturation), 94ºC - 30 seconds (denaturation), 55ºC - 1 minute (annealing), 72ºC - 1 minute (extension), and 72 º C - 10 minutes (final extension). A 3% agraose gel was prepared and the PCR product was loaded to check the amplification of SSR markers. After the gel run was over, the gel was visualized under UV light transmitted gel documentation system (Alpha Infotech, USA). The banding pattern was observed and recorded using gel documentation unit. For cluster analysis a neighbour -joining tree with bootstrap values was constructed utilizing unweighted pair group method with arithmetic averages algorithm with the help of DAR win version 5.0.14 (Perrier *et al*., 2003).

Results and Discussion:

The results of D^2 analysis showed the presence of considerable genetic divergence among 50 genotypes studied. The genotypes were grouped into 8 clusters and maximum number of genotypes (11) were clustered in III and IV followed by cluster I (9) and cluster II & VI (7) (Table 1). The pattern of distribution of genotypes into various clusters was at random indicating that geographical origin and genetic diversity were not related and there are forces other than geographical separation which is responsible for diversity.

Table 1: Clustering pattern of genotypes in Tocher's method and Ward's minimum variance method

S. No.	Cluster No.	Name of the genotype clustered in Tocher's method	Name of the genotype clustered in Ward's method
1	I	BPT2507, BPT2571, RGL2538, NLR33892, RGL1414, RGL2332, NLR40024, BPT2575, BPT2604	BPT2295, JGL384, JGL1798,BPT5204, MTU1010,WGL48684
2	II	BPT2411, BPT2605, BPT2511, BPT2570, NLR20084, Kalanamak, Improved Samba Mahsuri	BPT2270,MTU1001,NLR145,NLR3041, WGL13400,MTU3626, Chittimuthyalu
3	III	JGL3844, JGL17004, WGL14377, BPT1768, JGL32100, WGL14, JGL11727, Surya, JGL1798, MTU2077, WGL13400	Kasturi, Pusa Basmathi 1,Pusa 1121
4	IV	BPT2295, JGL384, BPT5204, WGL48684, NLR145, BPT2270, MTU1010, MTU3626, NLR3041, MTU1001, Chittimuthyalu	BPT 2570, NLR20084, BPT2411, BPT2605, BPT2511, BPT2604, BPT2575, MTU2077, Tetep, Kalanamak, Improved Samba Mahsuri
5	V	Kasturi, Pusa Basmathi1,Pusa 1121	WGL14, JGL32100, BPT1768, BPT2507, BPT2571, Surya, JGL11727
6	VI	MTU1032,MTU1061,RGL2537, MTU1075, BPT 2231,WGL20471, Taroari Basmathi	JGL3844, JGL17004, RGL2538, RGL1414, RGL2332, NLR4002, NLR33892, WGL14377
7	VII	Tetep	BPT2231, RGL2537, MTU1075, MTU1032, MTU1061, WGL20471
8	VIII	BPT3291	BPT3291, Taroari Basmathi

Similar results were also reported by Ravindra Babu *et al* (2006), Kar *et al* (2013) and Beevi and Venkatesam (2015). The pattern of group constellations indicated significant variability among the genotypes. Percent contribution of each character is calculated on the basis of occurance of ranks and is presented in Table 2. Out of 19 characters studied, gel consistency (59.51%), alkali spreading value (13.96%), elongation ratio (11.92%), water uptake (5.88%), test weight (3.27%), kernel length after cooking (2.78%) contributed maximum towards divergence.The

observed results find support from studies conducted by Arun Sharma *et al*., (2008) and Subudhi *et al*. (2009) who reported maximum contribution of test weight and kernel elongation ratio respectively. Similarly, Tushara *et al*. (2013) reported 27.49% divergence through alkali spreading value while Kishore *et al*. (2007) elucidated the importance of water uptake in their studies for genetic divergence. The average intra-cluster distances ranged from (cluster VII and VIII) 0.0 to 1650.89 (cluster VI). Maximum inter-cluster distance (10006.45) was found between cluster V and cluster VI suggesting wide diversity between these clusters while minimum distance (833.01) was found between clusters II and VII suggesting that genotypes of these clusters had maximum number of gene complexes (Figure 1).

Table 2: Contribution of different characters towards genetic divergence among 50 genotypes of rice (*Oryza sativa* L.)

Character	% Contribution towards divergence
Plant height (cm)	0.00
Ear bearing tillers/ Plant	0.00
panicle length (cm)	0.00
Days to 50% flowering	0.08
Filled Grains/ Panicle	0.98
Test weight (g)	3.27
Grain yield/ plant	0.08
Hulling (%)	0.00
Milling (%)	0.57
Head Rice Recovery (%)	0.73
Kernel length (mm)	0.08
Kernel breadth (mm)	0.08
L/B ratio	0.08
Kernel Length after Cooking (mm)	2.78
Water uptake (ml)	5.88
Alkali spreading value	13.96
Amylose content (%)	0.00
Elongation Ratio	11.92
Gel consistency (mm)	59.51

By Ward's minimum variance method also, the 50 genotypes studied were grouped into 8 clusters. Among these, cluster IV got maximum number of genotypes (11) followed by cluster VI (8), cluster II and IV (7 each) and cluster I and VII (6 each). The overall composition of clustering pattern showed that genotypes collected from the same geographic origin were distributed into different clusters. Among the eight clusters studied in Wards method,

Figure 1: Tree construction showing genetic relationship among 50 rice genotypes

cluster IV manifested desirable mean values for majority of characters studied *viz.*, ear bearing tillers per plant, filled grains per panicle, semi-dwarf plant stature, early flowering, test weight, head rice recovery %, kernel length, water uptake, amylose content and ultimately recorded highest mean value for grain yield per plant suggesting that the genotypes in this cluster may be utilized for hybridization purpose for getting desirable transgressive seggregants (Table 4). Under D^2 analysis maximum mean value for grain yield/plant (15.72g) was recorded by cluster II followed by cluster VIII (15.4g) and cluster I (14.65g). The genotypes in cluster IV manifested desirable mean value for majority of the characters studied and these genotypes would be utilized in hybridization programme for transfer of desirable genes. Likewise, cluster VI in Tocher's method and cluster VII in Ward's method also recorded desirable mean values for yield and quality traits. The genotypes from cluster VI may be crossed with genotypes grouped in cluster VII as the inter-cluster distance between these two clusters is high. Likewise, the inter cluster distance between cluster IV, V and VII (Ward's method) and cluster V and VI (D^2 analysis) is also high, hence the genotypes from these clusters may be utilized for hybridization.

Under diversity analysis studies i.e, Tocher's and Ward's minimum variance method, all the genotypes were grouped into 8 clusters. In both the methods, majority of the genotypes studied clustered in one group. For example, three basmati varieties *viz.*, Kasturi, Pusa Basmati 1 and Pusa 1121 were grouped in the same cluster. Likewise, majority of the genotypes in cluster VII of Ward's method

were also grouped in cluster VI in Tocher's method. BPT 2295, JGL 384, BPT 5204, WGL 48684 and MTU 1010 were grouped into IV cluster in Tocher's method and in cluster I under Ward's method of clustering pattern. Based on the clustering pattern and inter cluster distance obtained from both morphological diversity analysis studies, it is suggested that BPT 2231, MTU 1032, MTU 1075, RGL 2537 and WGL 20471 may be crossed with BPT 2411, BPT 2605, BPT 2511, BPT 2570, JGL 3844, JGL 17004, JGL 11721, Surya, BPT 2505 and BPT 2571 for obtaining superior transgressive segregants which may ultimately result in isolation of best genotypes for both yield components as well as quality traits.

A total of 20 SSR primer pairs were used for molecular analysis of 50 cultivars collected from different rice research stations of Andhra Pradesh and Telangana (Fig.2&3). All markers showed clear amplification and out of 20 SSRs, 16 were polymorphic (92% polymorphism) while the remaining four markers showed monomorphism. A wide range of amplicon sizes were observed ranging from 50 to 200 bp. The number of alleles detected for each of the 16 SSR loci ranged from 2 to 3 per locus with mean of 2.1 alleles per locus. Similar results were also observed by Jayavardhan (2012). The PIC values for 20 SSR loci in our study varied from 0.0768 (RM 159) to 0.631 (RM 218) with an average of 0.42. The estimated average PIC values are relatively higher than the average PIC values as reported by others (Lu *et al*., 2005; Juneja *et al*., 2006; Joshi *et al*., 2010) and thus might be due to higher genetic diversity present in selected rice genotypes. Moreover, the SSR markers used in the study were selected on the basis of their high PIC values reported earlier. Higher PIC values for some SSRs similar to our findings were also reported in the literature (Juneja *et al.,* 2006; Jayamani *et al.,* 2007). Fifty genotypes were grouped into three clusters I, II and III and again these clusters were divided into 2-3 sub clusters. The distribution of genotypes into three clusters is shown in Fig.3. Jayamani *et al*. (2007) obtained comparable groupings of Portuguese rice accessions by PCA and UPGMA cluster analysis with some deviations. The groupings identified by PCA were very similar to those identified by the UPGMA tree cluster analysis of the 52 Indian aromatic/ quality rice genotypes (Jain *et al*., 2004). Aggarwal *et al*. (2002) observed six clusters in PCA while 3clusters in UPGMA analysis by characterizing Indian Basmati and other elite genotypes using AFLP markers. The present analysis clearly indicated that microsatellite markers are useful in assessing genetic diversity in rice genotypes. All the genotypes analyzed could be distinguished from each other.

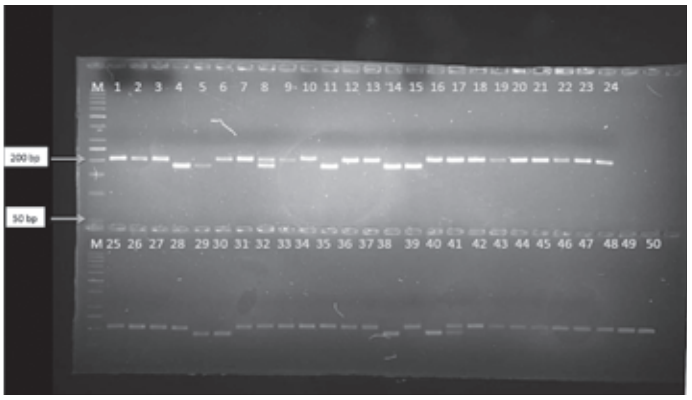

Fig.2: Amplification profile of the DNA of 50 rice genotypes using the primer RM 218

1. BPT-5204	11. Taroari Basmathi	21. MTU-2077	31. WGL-20471	41. NLR-40024
2. BPT-1768	12. BPT-2570	22. MTU-1032	32. WGL-48864	42. NLR-3041
3. BPT-2295	13. BPT-2511	23. MTU-1032	33. WGL-14	43. NLR-20084
4. BPT-2231	14. BPT-3411	24. MTU-3626	34. WGL-32100	44. NLR-33892
5. BPT-2270	15. BPT-2605	25. Improved Samba Mahsuri	35. JGL-384	45. RGL-2538
6. BPT-3291	16. BPT-2604	26. Tetep	36. JGL-3844	46. RGL-2537
7. Surya	17. BPT-2575	27. Kalanamak	37. JGL-1798	47. RGL-1414
8. Pusa-1121	18. MTU-1010	28. Chittimuthyalu	38. JGL-11727	48. RGL-2332
9. Kasturi	19. MTU-1075	29. WGL-13400	39. JGL-17004	49. BPT-2505
10. Pusa Basmathi1	20. MTU-1001	30. WGL-14377	40. NLR-145	50. BPT-2571

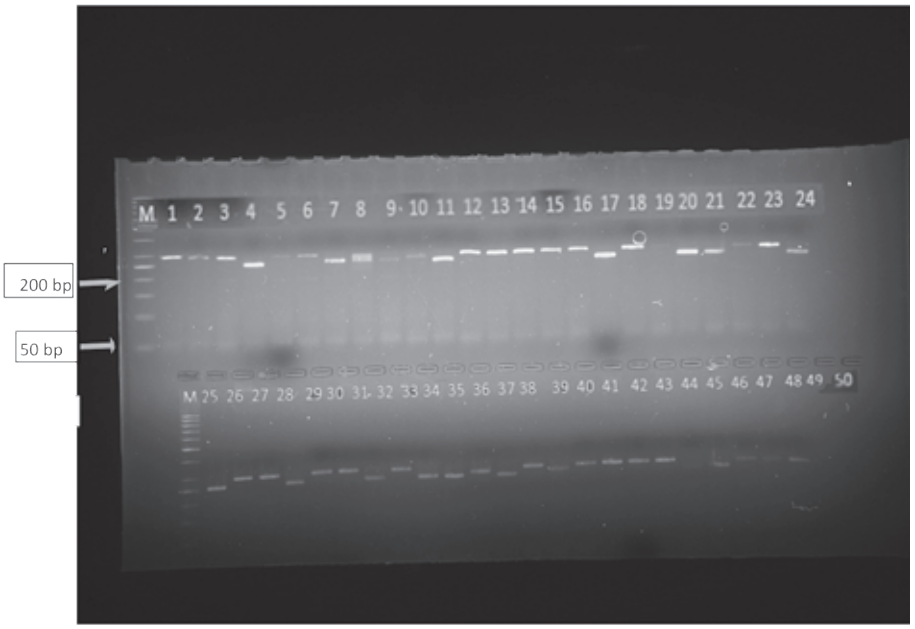

Fig.3: Amplification profile of the DNA of 50 rice genotypes using the primer RM 480

1. BPT-5204	11. Taroari Basmathi	21. MTU-2077	31. WGL-20471	41. NLR-40024
2. BPT-1768	12. BPT-2570	22. MTU-1032	32. WGL-48864	42. NLR-3041
3. BPT-2295	13. BPT-2511	23. MTU-1032	33. WGL-14	43. NLR-20084
4. BPT-2231	14. BPT-3411	24. MTU-3626	34. WGL-32100	44. NLR-33892
5. BPT-2270	15. BPT-2605	25. Improved Samba Mahsuri	35. JGL-384	45. RGL-2538
6. BPT-3291	16. BPT-2604	26. Tetep	36. JGL-3844	46. RGL-2537
7. Surya	17. BPT-2575	27. Kalanamak	37. JGL-1798	47. RGL-1414
8. Pusa-1121	18. MTU-1010	28. Chittimuthyalu	38. JGL-11727	48. RGL-2332
9. Kasturi	19. MTU-1075	29. WGL-13400	39. JGL-17004	49. BPT-2505
10. Pusa Basmathi1	20. MTU-1001	30. WGL-14377	40. NLR-145	50. BPT-2571

In breeding programme, generally parents are selected based on the genetic divergence for obtaining superior transgressive genotypes. Selection of parents from each cluster and crossing them in diallele fashion were proved to be highly rewarding. Different clustering patterns have also been reported by different methods of diversity analysis in previous studies (Seetharam *et al.*, 2009 and Zhang *et al.*, 2010). Molecular studies represent the actual genotypic containments and are independent of environment. When all three methods of diversity analysis were compared, some of the genotypes *viz.*, MTU 1010, NLR 145, BPT 2511 and BPT 5204 which were grouped into cluster IV in both morphological diversity studies clustered in II group under molecular studies. Likewise, under molecular analysis JGL 1798, JGL 11727, MTU 2077, MTU 1032, JGL 384 and JGL 32100 were grouped into cluster III even though they were divided into three sub groups. All the above said genotypes found place in V and VI clusters under Ward's method whereas under D^2 and Tocher's diversity analysis all these varieties were grouped in cluster III. Even though, Pusa Basmati 1, Pusa Basmati 1121 and Kasturi, all three basmati genotypes were grouped into one cluster in both the morphological diversity studies, these three varieties were divided into different sub groups in molecular study. A judicious use of diversity estimate from morphological and molecular data may be required for the selection and identification of diverse parental lines that can be used further for synthesizing experimental hybrids for evaluation of their heterotic potential. Hence, from the present study, it may be concluded that BPT 2505, RGL 2537, WGL 20471, JGL 17004 may be crossed with BPT 2411, BPT 205, BPT 2570, BPT 4538 and JGL 11727 for isolation of superior transgressive segregants from further generations.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Correlation and Path analysis for Grain Yield and Quality Traits in Promising Rice Genotypes (*Oryza Sativa* L.)

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Abstract

A study to obtain information on genetic correlation and inter-relationship of grain yield and associated characters under rainfed conditions was carried out using 54 diverse genotypes. The results of correlation studies indicated that genotypic correlation coefficients were higher in magnitude than their corresponding phenotypic correlation coefficients for most of the traits. The grain yield per plant exhibited highly significant and positive correlation with plant height, productive tillers per plant, number of grains per panicle, hulling per cent, milling per cent and amylose content at genotypic and phenotypic levels. On the contrary, it expressed negative and highly significant genotypic and phenotypic association with panicle length, L/B ratio, protein content and iron content with grain yield per plant. Path coefficient analysis revealed that days to 50 % flowering, plant height, panicle length, grains per panicle and hulling percentage had strong positive direct effects on grain yield per plant whereas, milling percentage, amylose content and zinc content showed moderate to low positive direct effects. On the contrary, negative and low to negligible direct effects were observed for productive tillers per plant and iron content.

Key words: Rice, correlation, path co-efficient, yield, yield components quality traits

Introduction

Rice (*Oryza sativa* L.) is one of the most important food crops in the world, second only to wheat in terms of annual production for human consumption. Rice belongs to family Poaceae and genus *Oryza*. In India rice accounts for about 22 per cent of the total cropped area under cereals, and about 31 per cent of the total area under food grains (Singhal, 2003). Asia is considered to be "Rice Bowl" of the world and produced more calories and carbohydrates per hectare than any other cereal (Lu and Chang 1980). In India, rice is grown in an area of 42.56 million ha of land with a production of 95.33 million tonnes and productivity of 2240 kg. Rice is grown throught India in all most all the states. In Gujarat, rice occupies about five per cent of gross cropped area of the state and it accounts about 14 per cent of total food grain production. In Gujarat, rice is cultivated in 7.88 lakh hectares with production of 16.36 lakh tones and productivity of 2076 kg per hectare (Anon., 2015). At present results of heterosis breeding in rice are not so enthusiastic. Therefore, there is urgent need to evolve short duration, high yielding and best quality genotypes of rice crop best suited to the region. Further, information on correlation co-efficient between grain yield and its component characters is essential for yield improvement, since grain yield in rice is a complex entity and is highly influenced by several component characters. Studies on path co-efficient also provide useful information regarding the direct and indirect effects of different yield component characters on grain yield and thus aid in the identification of effective selection criteria for effective yield improvement.

Material and Methods

The experiment was conducted with 54 diverse genotypes under rainfed lowland condition during *kharif* 2016 at Main Rice Research Centre, Navsari Agricultural University, Navsari (Gujarat). The material was grown in a randomized block design with three replications. Each entry was sown in a double row of three meter length with inter and intra row spacing of 20 cm and 15 cm respectively. Normal crop raised following all recommended cultural practices and plant protection measures. Five plants from each replication were selected at random and observations were recorded on 14 characters *viz.*, days to 50% flowering, plant height (cm), number of productive tillers per plant, panicle length (cm), number of grains per panicle, grain yield per plant (g), L/B ratio (kernel), hulling per cent (%), milling per cent (%), head rice recovery (%), amylose content (%), protein content (%), zinc content (ppm) and iron content (ppm).

The observation on days to 50% flowering was recorded on plot basis. The mean over replication of each character was subjected to statistical analysis. The genotypic and phenotypic correlations were calculated using the formulae suggested by Fisher and Yates (1967), while the direct and indirect contribution of each character for grain yield was estimated by path co-efficient analysis suggested by Wright (1921).

Results and Discussion

The genotypic and phenotypic correlations for yield and yield components are presented in Table 1. The results of correlation coefficients revealed that both genotypic and phenotypic correlations followed the same trend but the genotypic correlations were generally higher than the phenotypic correlations indicating that the phenotypic expression of correlations is reduced under the influence of environment. A perusal of these results revealed that grain yield per plant exhibited highly significant and positive correlation with plant height (0.251), panicle length (0.424), grains per panicle (0.332), hulling per cent (0.203), milling per cent (0.169) and amylose content (0.219) at genotypic levels indicating an increase in grain yield with an increase in these characters. Therefore, priority should be given to these traits, while making selection for yield improvement. The findings are in agreement with the reports of Gande *et al.* (2013), Santipriya *et al.* (2017), Ekka *et al.* (2015) Deepasankar *et al.* (2006), Kishore *et al.* (2015), Nagesh *et al.* (2012), Seyoum *et al.* (2012), Arulmozhi *et al.* (2013) and Alam *et al.* (2014). Furthermore, it expressed negative and highly significant genotypic and phenotypic association with panicle length, L/B ratio, protein content and iron content with grain yield per plant. A negative association between grain yield with productive tillers per plant, L/B ratio, protein content and iron content were finding similar Krishna Naik *et al.* (2005) and Nagesh *et al.* (2012).

In the present study path coefficient analysis has been conducted taking grain yield per plant as dependent variable. The persual of the results revealed that days to 50 % flowering, plant height, panicle length, grains per panicle and hulling percentage had strong positive direct effects on grain yield per plant (Table 2) This may indicate that direct selection of these characters is likely to be effective in increasing grain yield. Similar results obtained

Table 1. Genotypic (rg) and phenotypic (rp) correlation coefficients of thirteen characters in rice.

Character	C	PH	PTP	PL	GP	LBR	HP	MP	HRR	AC	PC	ZC	FC	GY
DF	rg	0.312**	-0.350**	0.227**	0.380**	-0.098	0.012	0.031	-0.076	0.095	-0.112	0.289**	0.054	0.383**
	rp	0.230**	-0.269**	0.204**	0.308**	-0.033	0.000	0.020	-0.068	0.092	-0.103	0.254**	0.049	0.251
PH	rg		0.127	0.504**	0.432**	0.055	-0.118	-0.209**	-0.001	0.123	-0.178*	0.067	-0.066	0.350**
	rp		0.083	0.305**	0.361**	0.045	-0.093	-0.156*	-0.021	0.095	-0.164*	0.052	-0.052	0.253
PTP	rg			0.266**	-0.276**	0.161*	0.069	0.038	-0.199*	-0.197*	-0.016	-0.334**	-0.198*	-0.125
	rp			0.198*	-0.206**	0.120	0.049	0.026	-0.178*	-0.177*	-0.009	-0.302**	-0.176*	-0.081
PL	rg				0.087	-0.094	0.026	0.170	-0.272	-0.270	-0.115	0.001	-0.142	0.424**
	rp				0.066	-0.041	0.059	0.092	-0.200*	-0.193*	-0.076	-0.005	0.090	0.245
GP	rg					-0.153*	0.176*	0.140	0.264**	-0.035	-0.047	0.188*	0.029	0.332**
	rp					-0.137	0.151	0.123	0.280**	-0.028	-0.050	0.177*	0.035	0.272
LBR	rg						0.109	0.018	-0.156*	-0.141	0.057	-0.151*	-0.063	-0.038
	rp						0.083	0.013	-0.139	-0.116	0.056	-0.143	-0.054	-0.038
HP	rg							0.988**	0.110	-0.376**	0.058	-0.030	-0.063	0.203**
	rp							0.827**	0.090	-0.337**	0.024	-0.031	-0.059	0.159
MP	rg								0.124	-0.414**	0.064	-0.049	0.030	0.169*
	rp								0.102	-0.361**	0.047	-0.043	0.025	0.141
HRR	rg									0.027	-0.068	0.036	0.014	0.030
	rp									0.037	-0.073	0.040	0.018	0.024
AC	rg										-0.031	-0.053	0.070	0.219**
	rp										-0.035	-0.050	0.065	0.194
PC	rg											-0.442**	0.238**	-0.089
	rp											-0.404**	0.224**	-0.046
ZC	rg												-0.065	0.133
	rp												-0.064	0.109
FC	rg													-0.248**
	rp													-0.214

*, ** Significant at P=0.05 level and P=0.01 level

DF = Days to 50 % flowering
PH = Plant height (cm)
PTP = Productive tillers per plant
PL = Panicle length (cm)
C = Correlation
GP = Grain per panicle
GY = Grain yield per plant (g)
LBR = L/B ratio (kernel)
HP = Hulling per cent (%)
MP = milling per cent (%)
HRR = Head rice recovery (%)
AC = Amylose content (%)
PC = Protein content (%)
ZC = Zinc content (ppm)
FC = Iron content (ppm)

Table 2: Path coefficient analysis showing direct and indirect effects of thirteen characters on grain yield per plant of rice.

Chara-cters	DF	PH	PTP	PL	GP	LBR	HP	MP	HR	AC	PC	ZC	FC
DF	**0.1194**	0.0373	-0.0418	0.0271	0.0455	-0.0117	0.0015	0.0038	-0.0091	0.0114	-0.0135	0.0345	0.0065
PH	0.0676	**0.2163**	0.0275	0.1092	0.0936	0.0119	-0.0257	-0.0453	-0.0002	0.0267	-0.0387	0.0146	-0.0143
PTP	0.0202	-0.0073	**-0.0577**	-0.0154	0.0159	-0.0093	-0.0040	-0.0022	0.0115	0.0114	0.0009	0.0193	0.0114
PL	0.0468	0.1041	0.0549	**0.2063**	0.0179	-0.0196	0.0054	0.0353	-0.0562	-0.0559	-0.0238	0.0004	-0.0294
GP	0.0649	0.0737	-0.0471	0.0148	**0.1703**	-0.0261	0.0300	0.0239	0.0451	-0.0061	-0.0081	0.0321	0.0050
LBR	-0.0147	0.0082	0.0242	-0.0142	-0.0229	**0.1495**	0.0164	0.0027	-0.0234	-0.0212	0.0086	-0.0227	-0.0092
HP	-0.0113	0.1046	-0.0611	-0.0230	-0.1548	-0.0964	**-0.8798**	-0.8694	-0.0972	0.3312	-0.0510	0.0270	0.0558
MP	0.0388	-0.2561	0.0465	0.2091	0.1718	0.0223	1.2088	**1.2233**	0.1523	-0.5065	0.0787	-0.0600	0.0368
HRR	-0.0003	0.0000	-0.0008	-0.0011	0.0011	-0.0006	0.0004	0.0005	**0.0040**	0.0001	-0.0003	0.0001	0.0001
AC	0.0446	0.0575	-0.0922	-0.1262	-0.0167	-0.0661	-0.1754	-0.1929	0.0127	**0.4659**	-0.0147	-0.0248	0.0330
PC	-0.0140	-0.0221	-0.0020	-0.0142	-0.0059	0.0071	0.0072	0.0079	-0.0085	-0.0039	**0.1236**	-0.0547	0.0295
ZC	0.0416	0.0097	-0.0481	0.0003	0.0271	-0.0218	-0.0044	-0.0070	0.0053	-0.0077	-0.0636	**0.1438**	-0.0094
FC	-0.0200	0.0241	0.0722	0.0519	-0.0108	0.0223	0.0231	-0.0110	-0.0053	-0.0258	-0.0871	0.0240	**-0.3645**
Correlation with GY	0.3837**	0.3500**	-0.1257*	0.4245**	0.3323**	-0.0385	0.2035**	0.1697*	0.0308	0.2197**	-0.0890	0.1397*	**-0.2486****

*, ** Significant at P =0.05 and 0.01 level. Residual effect =0.5011. Bold figures show direct effect

DF = days to 50% flowering
HP = Hulling percent (%)
MP = Milling percent (%).
HRR = Head rice recovery (%).
AC = Amylose content (%).
PC = Protein content (%)
ZC = Zinc content (ppm)
FC = Iron content (ppm)
PL = Panicle length (cm)
PH = Plant height (cm)
GP = Grains per panicle
GY = Grain yield per plant (g)
LBR = L/B ratio (kernel)

by Nagesh *et al.* (2012), Khare *et al.* (2014), Sandhya *et al.* (2014), Ekka *et al.* (2015), Kishore *et al.* (2015), Santipriya *et al.* (2017), Rahman *et al.* (2014), Dhurai *et al.* (2016), Mustafa and Elsheikh (2007) and Naseer *et al.* (2015). Whereas, milling percentage, amylose content and zinc content showed moderate to low positive direct effects as they are quality parameters. But L/B ratio and head rice recovery exhibited negligible positive direct effects. On the contrary, negative and low to negligible direct effects were observed for productive tiller per plant and iron content indicates low influence of these traits on grain yield. The indirect effects of days to 50 % flowering, plant height, panicle length, grains per panicle, hulling percentage, milling percentage, amylose content and zinc content on grain yield per plant were positive and highly significant, whereas, productive tillers per plant and iron content had negative and highly significant indirect effect on grain yield. These findings suggested that, selection pressure on panicle length, grains per panicle, hulling percentage, milling percentage, amylose content and zinc content would be effective for improvement of grain yield in rice.

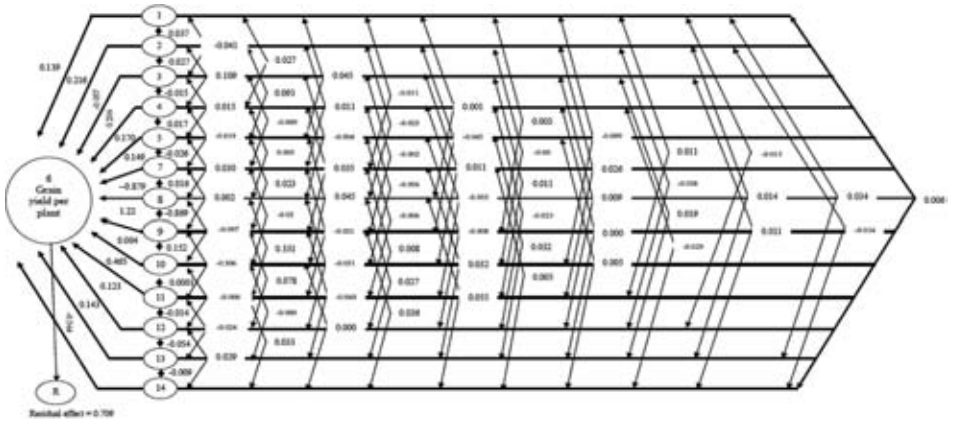

Figure 1: Diagrammatical representation of genotypic path analysis in rice: 1. Days to 50 % flowering 2.Plant height (cm) 3. Productive tillers per plant 4. Panicle length (cm) 5. Grains per panicle 6.Grain yield per plant (g) 7.L/B ratio 8. Hulling percentage (%) 9. Milling percentage (%) 10. Head rice recovery (%) 11. Amylose content (%) 12. Protein content (%) 13. Zinc content (ppm) 14. Iron content (ppm)

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Identification of Restorers and Maintainers for the Development of Rice Hybrid Suitable for Himachal Pradesh

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Abstract

A set of ten traditional/improved varieties adapted to mid hill conditions of Himachal Pradesh were crossed with IR68897A to identify effective maintainers and restorers during *Kharif* 2015 at RWRC Malan. On the basis of spikelet fertility and pollen fertility genotype T 23 was categorized as effective maintainer and two genotypes *viz.* Ranbir Basmati and HPU 741 as effective restorers. Most of the genotypes were found partial restorer and partial maintainer. Three crosses IR68897A x HPR2880, IR68897A x Ranbir Basmati and IR68897A x HPU741 exhibited significant positive heterosis for grain yield per plant. .

Key words: Rice, maintainers, restorers, hybrids, spikelet fertility, pollen fertility

Introduction

To increase rice production, it is essential to improve rice yield because there will be little scope in further expanding the rice area. Hybrid rice is one of such innovation. Hybrid rice can out yield other varieties of rice. It is a key technology that meets the increasing global demand for rice. In Himachal Pradesh, rice occupies 770 thousand hectares and total production of 131.6 thousand metric tons with productivity of 17.05 quintals/hectare which is below the national productivity. So, there is need to advocate hybrid rice technology in Himachal Pradesh.

Rice hybrid for unfavorable environment can be developed using elite parental lines adapted to these environments. In heterosis breeding programme using cytoplasmic male sterility (CMS) system, identification of maintainers and restorers is prerequisite. Restorers for different cytosterile sources will increase the cytoplasmic diversification. The successful exploitation of hybrid vigour in rice using CMS system , identification of maintainers with higher adaptability and restorers with higher combining ability from elite breeding lines and landraces through test crossing and their use in further breeding programme are the initial steps in three-line heterosis breeding (Siddiq ,1996).

Cytoplasmic male sterility and the fertility restoration system have been primarily used to develop heterotic rice hybrids in and outside China. The need of increasing rice productivity and production encouraged rice scientists to develop and standardize the hybrid rice technology using CMS lines.

Materials and Methods

The present investigation was undertaken with the objective to identify different restorers and maintainers for a CMS line from among the local and high yielding rice using a CMS line. The present investigation was conducted at RWRC, Malan during 2015-2016. The experimental material comprised of ten hybrids and their male parents along with IR68897A and IR68897B. The experiment was laid out in a Randomised Block Design (RBD) with three replications. Recommended package of practices were followed during crop growth period. Pollen studies were carried out for their fertility/sterility responses. Five spikelets were selected from each plant before anthesis and fixed in 70% alcohol. Three anthers from each spikelet were placed together on a glass slide and squashed in 1% iodine solution. Slides were examined under microscope for fertile/sterile pollens. Estimates were based on five panicles from bagged hybrid plants. Maintainers and restorers for IR68897A were identified based on pollen and spikelet fertility. The criteria for classifying the parental lines as maintainers and restorers were done according to Virmani et al. (1997).

Results and Discussion

The pollen fertility and spikelet fertility are the important traits which directly influence the ultimate product i.e. grain yield. Pollen fertility and spikelet fertility and heterosis are used to to determine maintainers or restorers. None of the crosses showed 100% pollen sterility except IR 68897A xT 23 (0.00%). Among crosses IR 68897A x HPU 741, IR 68897A x IR 36 and IR 68897A x Ranbir Basmati had pollen fertility more than 90%. Cross IR 68897A x T 23 produced completely sterile F_1 (0.00%) Hybrids IR 68897A x HPU 741, IR 68897A x Ranbir Basmati, IR 68897A x IR 36, IR 68897A x Sabarmati and IR 68897A x Sukara Red had spikelet fertility above 75 %.

Hybrids exhibited higher mean values as compared to those of parents. This indicates the presence of heterosis for these characters. Heterosis expressed as per cent increase or decrease in the mean values of F_1 hybrid over better parent and standard check were observed for various characters. IR68897A x HPR 2880, IR68897A x Ranbir Basmati, IR68897A x HPU 741 showed significant positive heterosis for grain yield over better parent. Two crosses IR 68897A x Sukara red (43.33%) and IR 68897A x Ranbir Basmati (51.33%) showed positive heterosis for grain yield over the standard check

Hybrid rice is produced by crossing two-parental lines with very distinct genetic back ground. The success of hybrid rice programme depends upon the magnitude and direction of heterosis. A good hybrid should manifest high heterosis for commercial exploitation. Hybrid rice breeding programme exploits the phenomenon of heterosis. Hybrid rice development technology is different from those used for inbred rice varieties. Inbred line breeding accumulates productivity genes that perform well under homozygous conditions while hybrid breeding assembles genes under heterozygous conditios from the two parents. Heterosis for grain yield has been reported by Parihar and Pathak (2008), Roy et al. (2009), Tiwari et al. (2011) and Kumar et al. (2012). Both restorer crosses and maintainer cross showed resistance reaction to leaf and neck blast disease at RWRC, Malan under natural epiphytotic conditions. New CMS lines in diverse genetic backgrounds can be developed in locally adapted germplasm that is prerequisite for hybrid rice breeding. Restorer genes found in exotic genotypes can be transferred to elite high yielding and desirable genotypes through appropriate backcross breeding programme to develop new restorer lines.

Table 1: Mean performance of parents and crosses for yield and yield component traits

Parents/Crosses	Pollen fertility (%)	Spikelet fertility (%)	Grains/ panicle (Nos.)	Grain yield/plant (gram)
IR 68897A	0.00	0.00	-	-
IR 68897B	96.00	63.27	89.33	29.19
IR68897A X Sukara Red	53.33	75.53	144.53	25.41
Sukara Red	93.33	83.53	76.00	18.27
IR68897A X Sabarmati	66.33	75.74	145.60	23.29
Sabarmati	96.33	83.73	168.53	25.23
IR68897A X HPR 2880	75.00	70.50	109.97	21.84
HPR 2880	97.00	81.27	83.60	17.73
IR68897A X HPR 2656	56.00	51.87	70.57	15.07
HPR 2656	97.33	88.67	170.87	23.63
IR68897A X Ranbir Basmati	96.00	88.09	160.37	26.87
Ranbir Basmati	96.00	76.13	98.53	18.01
IR68897A X T 23	0.00	0.00	-	-
T 23	96.33	85.20	199.47	21.03
IR68897A X HPR 894	55.67	50.53	73.27	9.10
HPR 894	96.00	74.67	110.00	21.39
IR68897A X HPU 741	91.67	90.68	78.11	14.81
HPU 741	95.33	75.47	113.37	9.64
IR68897A X IR 36	92.00	85.20	124.47	18.88
IR 36	96.67	89.00	232.13	18.06
IR68897A X HPR 2373	53.33	43.03	57.50	11.45
HPR 2373	95.00	82.47	93.07	18.85
S.E (m)	2.39	4.39	12.09	2.02

Table 2: Magnitude of F_1 heterosis (%) over standard check (SC) with respect to yield and its components

Crosses	Grains/ panicle (Nos.)	Grain yield/plant (gram)
	SC	SC
IR68897A × HPR 2880	31.54	23.16
IR68897A × Ranbir Basmati	91.83*	51.53*
IR68897A × HPU 741	-6.57	-16.45
S.E	17.10	2.86

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Association studies for physiological parameters and early vigour traits under anaerobic condition in rice (*Oryza sativa* L.)

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Abstract

Forty eight high yielding rice genotypes were studied to determine the association of traits that contribute for germination and growth of seedlings under anaerobic conditions. The character association studies revealed that germination percentage under 14 days of submergence showed significant and positive correlation with all other parameters under study. Free amino acid concentration manifested positive and significant relationship with shoot length at 7 and 14 days under anaerobic conditions, while total sugars exhibited significant positive relationship with shoot length, dry matter production, germination percentage and vigour index at 7 days under submergence. The studies of path analysis revealed maximum direct effect for vigour index at 14 days of submergence followed by root length and number of leaves at 14 days of submergence.

Key words: Anaerobic germination, Rice, Path analysis, Correlation.

Introduction

Direct seeding of rice is being adopted in irrigated low land ecosystem also because it reduces labour costs in addition to other benefits. Wide adoption of direct seeded rice practice has been hindered by poorly levelled fields, heavy rainfall and poor drainage system which cause accumulation of water in the fields shortly after sowing leading to poor crop establishment. This is due to the inability of most rice varieties to germinate and reach the water surface under complete submergence. Hence, tolerance of anaerobic condition during germination is an essential trait for direct seeded rice cultivation both in rainfed as well as in irrigated ecosystems. Varieties that can germinate in flooded soils could be beneficial for direct seeded systems not only in low land areas but also for intensive irrigated systems where early flooding can suppress weeds (Ismail *et al.*, 2012). This will consequently result in enormous savings in production costs as opposed to when rice is transplanted. It can also reduce the cost of manual or mechanical weeding or the use of hazardous chemicals for weed control. The literature pertaining to the association of various characters on anaerobic germination is very limited. Hence an attempt was made to determine the direct and indirect effects of various traits and association of different vigour related characters on anaerobic germination ability of rice genotypes.

Materials and Methods

The experiment was carried out with 48 rice genotypes during *kharif,* 2016 at Regional Agricultural Research Station, Maruteru, Andhra Pradesh and material used are presented in Table 1.

Table 1: Material Used In The Experiment

1 -	MTU4870	13 -	NLR20084	25 -	MTU2067	37 -	BPT4358
2 -	NLR33892	14 -	BPT2590	26 -	JGL17004	38 -	NLR4002
3 -	MTU5249	15 -	BPT2593	27 -	RGL1414	39 -	MTU1001
4 -	MTU7029	16 -	MTU2077	28 -	BPT1768	40 -	BPT2675
5 -	BPT2571	17 -	MTU3626	29 -	MTU1064	41 -	BPT2270
6 -	BPT2660	18 -	IR85961-23-1-2-1	30 -	MTU2716	42 -	AC39416A
7 -	BINADHAN11	19 -	NLR4001	31 -	BPT3291	43 -	MTU1140
8 -	BPT2644	20 -	AC39397	32 -	BPT2231	44 -	BPT2573
9 -	BPT2295	21 -	JGL3828	33 -	BPT5204	45 -	MTU1166
10 -	BPT2782	22 -	BPT2411	34 -	MTU1061	46 -	BPT2740
11 -	RGL2537	23 -	BPT2595	35 -	MTU1075	47 -	MTU5293
12 -	BPT2673	24 -	BPT2507	36 -	MTU5182	48 -	BPT2743

Screening for tolerance to anaerobic germination was conducted as per the method delineated by Reddy *et al*.(2015). Sterilized seeds were placed in petri dishes with moistened filter papers and incubated at 30^0C for 48 hrs for germination. After 3 days of sowing fifteen pre-germinated seeds were kept in seedling trays (35.5×10×4.5cm) at about 1 cm soil depth in two replications per treatment in Completely Randomized Design. Each tray consisted of three rows and 10 holes (2.5 cm) in each row. After sowing, the trays were submerged carefully in concrete tanks filled with 10 cm depth of water.The water level was maintained at 10cm depth above the soil surface in the trays for 7 &14 days. Data was recorded on five randomly selected seedlings per genotype per replication for root length at 7 & 14 days after submergence, shoot length at 7 & 14 days after submergence, number of leaves at 7 & 14 days after submergence, dry matter at 7 & 14 days after submergence by following standard methods (Reddy *et al*. 2015). Vigour index was calculated at 7 and 14 days of submergence by following the method suggested by Vijay *et al*. (2010).Number of plants survived after 7 & 14 days of submergence were counted and germination percentage was calculated as per standard procedure. Sugar concentration in normal seeds was estimated by using anthrone method described by Hedge and Hofreiter (1962) while amino acid concentration in seeds was estimated by using ninhydrin method described by Moore and Stein (1948). Phenotypic and genotypic correlations were worked out by using the formulae suggested by Falconer (1964). Path coefficient analysis suggested by Wright (1921) and elaborated by Dewey and Lu (1959) was used to calculate the direct and indirect contribution of various traits to anaerobic germination.

Results and Discussion

The aim of correlation studies is primarily to know the suitability of various characters for indirect selection because any particular trait may bring about changes in other associated characters (Singh, 1998). In the present investigation, the results of character association studies revealed that germination percentage at 14 days of submergence showed significant and positive association with all characters under study *viz.*,shoot length at7 & 14 days after submergence, root length at7 & 14 days after submergence, dry matter at 7 & 14 days after submergence, vigour index at 7 & 14 days after submergence, germination percentage at 7 days of submergence,number of leaves at 7 & 14 days after submergence, total soluble sugars (0.377& 0.384)and free amino acids(0.261& 0.267) at both genotypicand phenotypic levels respectively (Table 2). The results indicated that genotypes with high anaerobic germination percentage also possessed longer shoot and root length, more number of leaves, high vigour index,higher amount of free aminoacids and total soluble sugars. The cumulative effect of all these traits ultimately resulted in inducing tolerance to submergence at seedling stage under direct sowing. Among the early vigour traits, dry matter at 7& 14 days of submergence manifested positive association with all other characters under study while root length at 7 & 14 days of submergence exhibited positive relationship with all other characters under study except with free aminoacids.This suggests that the presence of free amino acids in the endosperm will enhance coleoptile elongation or germination of genotypes which was previously reported by Atwell *et al*.(1982). Free aminoacids manifested positive and significant association with shoot length under 7 days after submergence (0.118 &0.117) &14 days after submergence (0.2050& 0.202) both at phenotypic and genotypic levels.Vigour index at 7 & 14 days of submergence and total soluble sugars also exhibited positive relationship with all other characters under study. Soluble sugar concentration in seed and its transport from the endosperm to the coleoptile are reported to be closely associated with coleoptile elongation under anoxia (Furuhata *et al*. 2006; Kato-Noguchi *et al*.2010).

The observed correlation between the vigour related traits and its component character is the net result of the direct and indirect effects of the component character through other vigour attributes. The correlation coefficients observed among vigour traits may sometimes be misleading since, it may be over or under estimate because of its association with other characters. Hence, the correlation coefficient needs to be split into direct and indirect effects, using path coefficient analysis for critical evaluation. Thus, the correlation and path analysis in combination, can give a better insight, into cause and effect relationship between different pairs of characters. Studies of path analysis revealed that vigour index at 14 days of submergence (0.5885& 0.5674) manifested maximum positive direct effect followed by root length at 14 days of submergence (0.5701& 0.3857), number of leaves at 14 days after submergence (0.3240 &0.2435) at genotypic and phenotypic levels respectively (Table 3). These traits also exhibited positive correlation coefficients indicating the true relationship and selection through these traits will be effective for improvement of anaerobic germination. These findings are in agreement with the results reported

by Pavan Shankar (2015). Shoot length at 14 days after submergence (-0.1881 and -0.4192) and number of leaves at 7 days of submergence (-0.1339 and -0.2191) manifested negative direct effect but manifested positive association with anaerobic germination percentage at 14 days of submergence. Hence, the direct effects may be the casual factors for positive correlation and the indirect casual factors are to be considered simultaneously while exercising selection to improve anaerobic germination.

Table 2: Estimates of correlation coefficients among early vigour traits in rice (*Oryza sativa* L.)

Characters		1	2	3	4	5	6	7	8	9	10	11	12	13	14
Shoot length at 7 daysof submergence (1)	P	1.000	0.197	0.540**	0.015	0.397**	0.076	0.219*	0.16	0.282**	0.346**	0.191	0.118	0.291**	0.205*
	G	1.000	0.190	0.529**	0.016	0.369**	0.067	0.18	0.153	0.259*	0.289**	0.175	0.117	0.283**	0.194
Shoot length at 14 days of submergence (2)	P		1.000	0.378**	0.760**	0.476**	0.682**	0.281**	0.706**	0.381**	0.329**	0.459**	0.205*	0.226*	0.545**
	G		1.000	0.349**	0.708**	0.398**	0.650**	0.249*	0.672**	0.348**	0.293**	0.419**	0.202*	0.221*	0.515**
Root length at 7 days of submergence (3)	P			1.000	0.478**	0.078	0.034	0.369**	0.286**	0.183	0.042	0.042	-0.134	0.128	0.271**
	G			1.000	0.447**	0.078	0.023	0.300**	0.270**	0.173	0.03	0.029	-0.132	0.124	0.255*
Root length at 14 days of submergence (4)	P				1.000	0.270**	0.573**	0.369**	0.669**	0.471**	0.214*	0.250*	-0.082	0.122	0.596**
	G				1.000	0.242*	0.519**	0.303**	0.620**	0.429**	0.18	0.217*	-0.079	0.117	0.557**
Dry matter at 7 days of submergence (5)	P					1.000	0.593**	0.373**	0.226*	0.580**	0.558**	0.587**	0.338**	0.412**	0.469**
	G					1.000	0.530**	0.305**	0.197	0.497**	0.494**	0.549**	0.309**	0.376**	0.429**
Dry matter at 14 days of submergence (6)	P						1.000	0.263**	0.628**	0.558**	0.770**	0.463**	0.075	0.18	0.525**
	G						1.000	0.223*	0.589**	0.525**	0.731**	0.440**	0.072	0.171	0.491**
Number of leaves at 7 days of submergence (7)	P							1.000	0.431**	0.446**	0.231*	0.254*	0.127	0.072	0.275**
	G							1.000	0.383**	0.401**	0.243*	0.225*	0.113	0.064	0.244*
Number of leaves at 14 days of submergence (8)	P								1.000	0.501**	0.384**	0.344**	0.033	0.162	0.577**
	G								1.000	0.475**	0.347**	0.309**	0.032	0.16	0.557**
Germination percentage at 7 days of submergence (9)	P									1.000	0.580**	0.571**	0.265**	0.432**	0.685**
	G									1.000	0.566**	0.497**	0.253*	0.417**	0.637**
Vigour index at 14 days of submergence (10)	P										1.000	0.372**	0.14	0.341**	0.400**
	G										1.000	0.336**	0.127	0.316**	0.364**
Vigour index at 7 days of submergence (11)	P											1.000	0.449**	0.346**	0.733**
	G											1.000	0.426**	0.326**	0.713**
Free amino acids (12)	P												1.000	0.388**	0.267**
	G												1.000	0.387**	0.261*
Total soluble sugars (13)	P													1.000	0.384**
	G													1.000	0.377**
Germination percentage under 14 days of ubmergence (14)	P														1.000
	G														1.000

*Significance at 5% level , **significance at 1% level

Table 3: Direct and indirect effects of vigour related traits on germination percentage at 14 days under submergence among 48 genotypes of rice (*Oryza sativa* L.)

Sl. No	Character		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)
1	Shoot Length 7 DAS under anaerobic conditions	P	**0.0002**	-0.0008	-0.0001	-0.0000	-0.0001	-0.0001	-0.0001	-0.0002	-0.0004	0.0004	-0.0003	-0.0002	-0.0004
		G	**0.0059**	0.0011	0.0032	0.0009	0.0023	0.0004	0.0013	0.0009	0.0016	0.0020	0.0011	0.0007	0.0017
2	Shoot Length 14 DAS under anaerobic conditions	P	-0.0358	**-0.1881**	-0.0065	-0.1337	-0.0752	-0.1228	-0.0471	-0.1268	-0.0656	-0.0552	-0.0791	-0.0381	-0.0417
		G	-0.0827	**-0.4192**	-0.1586	-0.3185	-0.1993	-0.2858	-0.1180	-0.2959	-0.1599	-0.1378	-0.1926	-0.0858	-0.0946
3	Root Length 7 DAS under anaerobic conditions	P	0.0412	0.0271	**0.0779**	0.0347	0.0060	0.0017	0.0233	0.0210	0.0135	0.0023	0.0022	-0.0102	0.0096
		G	0.0563	0.0395	**0.1044**	0.0499	0.0081	0.0035	0.0385	0.0298	0.0191	0.0043	0.0043	-0.0140	0.0133
4	Root Length 14 DAS under anaerobic conditions	P	0.0065	0.2724	0.1717	**0.3847**	0.0929	0.1995	0.1165	0.2385	0.1648	0.0692	0.0834	-0.0303	0.0449
		G	0.0086	0.4331	0.2727	**0.5701**	0.1541	0.3267	0.2101	0.3813	0.2683	0.1222	0.1425	-0.0467	0.0693
5	Dry Matter7 DAS under anaerobic conditions	P	-0.0053	-0.0057	-0.0011	-0.0035	**-0.0145**	-0.0077	-0.0044	-0.0028	-0.0072	-0.0071	-0.0079	-0.0044	-0.0054
		G	0.0392	0.0470	0.0077	0.0267	**0.0989**	0.0586	0.0369	0.0223	0.0573	0.0550	0.0580	0.0334	0.0407
6.	Dry matter 14 DAS under anaerobic conditions	P	-0.0055	-0.0536	-0.0018	-0.0427	-0.0437	**-0.0824**	-0.0183	-0.0485	-0.0432	-0.0602	-0.0363	-0.0059	-0.0141
		G	-0.0084	-0.0761	-0.0038	-0.0640	-0.0662	**-0.1116**	-0.0294	-0.0700	-0.0623	-0.0860	-0.0517	-0.0084	-0.0200
7	No.of Leaves 7 DAS under anaerobic conditions	P	-0.0241	-0.0334	-0.0401	-0.0405	-0.0405	-0.0298	**-0.1339**	-0.0513	-0.0536	-0.0325	-0.0301	-0.0151	-0.0085
		G	-0.0480	-0.0616	-0.0808	-0.0807	-0.0817	-0.0577	**-0.2191**	-0.0943	-0.0978	-0.0506	-0.0556	-0.0277	-0.0157
8	No of Leaves 14 DAS under anaerobic conditions	P	0.0371	0.1636	0.0658	0.1510	0.0479	0.1433	0.0934	**0.2435**	0.1156	0.0846	0.0752	0.0078	0.0390
		G	0.0517	0.2286	0.0926	0.2167	0.0732	0.2033	0.1395	**0.3240**	0.1624	0.1243	0.1115	0.0106	0.0526
9	Germination percentage 7 DAS under anaerobic conditions	P	0.0417	0.0560	0.0279	0.0691	0.0801	0.0846	0.0646	0.0765	**0.1612**	0.0912	0.0800	0.0408	0.0673
		G	0.0207	0.0280	0.0134	0.0346	0.0426	0.0411	0.0328	0.0369	**0.0736**	0.0426	0.0420	0.0195	0.0318
10	Vigour Index 7 DAS under anaerobic conditions	P	0.0156	0.0158	0.0016	0.0097	0.0267	0.0396	0.0131	0.0188	0.0306	**0.0541**	0.0182	0.0068	0.0171
		G	0.0270	0.0257	0.0032	0.0168	0.0437	0.0603	0.0181	0.0300	0.0454	**0.0783**	0.0291	0.0109	0.0267
11	Vigour Index 14 DAS under anaerobic conditions	P	0.0990	0.2377	0.0163	0.1230	0.3114	0.2499	0.1277	0.1753	0.2818	0.1905	**0.5674**	0.2420	0.1849
		G	0.1124	0.2703	0.0244	0.1471	0.3455	0.2724	0.1494	0.2025	0.3358	0.2187	**0.5885**	0.2642	0.2038
12	Free Amino Acids	P	0.0048	0.0084	-0.0054	-0.0032	0.0128	0.0029	0.0047	0.0013	0.0105	0.0052	0.0177	**0.0415**	0.0160
		G	0.0113	0.0197	-0.0129	-0.0078	0.0324	0.0072	0.0121	0.0031	0.0255	0.0134	0.0432	**0.0962**	0.0373
13	Total Soluble Sugars	P	0.0191	0.0150	0.0084	0.0079	0.0255	0.0116	0.0040	0.0108	0.0283	0.0214	0.0221	0.0262	**0.0678**
		G	0.0108	0.0083	0.0047	0.0045	0.0153	0.0066	0.0026	0.0060	0.0160	0.0126	0.0128	0.0144	**0.0371**
14	Germination percentage 14 DAS under anaerobic condition	P	0.1945*	0.5151**	0.3146**	0.5565**	0.4293**	0.4903**	0.2435**	0.5562**	0.6366**	0.3635**	0.7127**	0.2610**	0.3768**
		G	0.2048*	0.5444**	0.2702**	0.5954**	0.4689**	0.5250**	0.2748**	0.5766**	0.6850**	0.3990**	0.7331**	0.2673**	0.3840**

*Significance at 5% level , **significance at 1% level

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Grain quality characteristics of two-line rice hybrids in Kerala

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Abstract

A study was conducted to analyse the quality characteristics of promising two line rice hybrids at College of Horticulture Vellanikkara, Kerala Agricultural University, Thrissur. The Hulling percentage ranged from 55.56 (TGMS 74S x Aiswarya) to 83.33 (TGMS 82S x Prathyasa). The milling percentage exhibited a range of 48.00(TGMS 74S x Aiswarya) to 68.96(TGMS 81S x Makom) for hybrids with a mean value of 62.16. Cooking quality analysis revealed intermediate amylose and alkali spreading value for the hybrids TGMS 91S x Makom, TGMS 91S x Kanchana , TGMS 81S x Makom and TGMS 81S x Aiswarya. Sensory quality characters of all two line rice hybrids exhibited red streaks (pericarp) after cooking and well separated moderately soft and desirable taste.

Key words: Rice quality, Physical properties, cooking quality, milling quality

Introduction

Rice (*Oryza sativa* L.) is the most important staple food for about 50 per cent of the world's population. Ninety per cent of the world's rice is grown and consumed in Asia. Rice provides about 29.4 per cent of total calories/capita/day in Asian countries (FAO 2006). However, increase in rice production is not commensurate with population growth. The total global rice production is declining gradually even with the extensive use of the high yielding modern varieties and hybrids. In India rice is grown in area of 45.54 million ha with a production of 99.18 million tonnes and a productivity of 2178 kg ha^{-1}. In Kerala, rice is cultivated in an area of 0.21 million ha with a production of 0.47 million tonnes and a productivity of 2238 kg ha^{-1}.

In countries where rice is consumed, traits of grain quality dictate market value and have a pivotal role in the adoption of new varieties (Juliano, 2003 and Fitzgerald *et al.*, 2008). Quality traits encompass physical appearance, cooking and sensory properties and more recently, nutritional value. The value of each trait, for example the length of the grain, varies according to local cuisine and culture. Physical properties include yield of edible and marketable polished grain, uniform shape, whiteness and, in most countries, translucence. These traits are immediately obvious to consumers and so are major factors defining market value. Cooking and sensory qualities typically include: cooking time (Juliano *et al*., 2003) textural properties of cooked rice aroma and its retention after cooking and the ability to remain soft for several hours after cooking (Philpot *et al.*, 2006).

Yield of rice hybrids by itself would not make hybrid rice technology acceptable. They must also have acceptable grain quality. Only limited efforts have been made to improve the grain quality of hybrid rice (Khush *et al.,* 1986). Since rice hybrids have entered the country recently there is a need to look into the quality aspects so that hybrid rice can be developed coupled with improved quality characteristics. Rice grain quality is mainly determined by the combination of many physical as well as chemical characters. Physical quality characters include kernel size, shape, hulling, milling percentage and head rice recovery. Chemical quality is mainly determined by amylose content, gelatinization temperature, gel consistency. High volume expansion and greater length wise expansion of kernel during cooking decide the consumer preference. (Mahalingam *et al*., 2012). Rice with soft to medium gel consistency, intermediate amylose content and gelatinization temperature is a preferred level for the consumer which determines the eating and cooking quality of rice grains (Bao et al., 2002). So far 102 rice hybrids were released for cultivation in India are based on three line (CGMS) systems, however

no hybrids released based on two line system. The present study was, therefore, aimed to identify high yielding two line cross combinations with acceptable grain yield and cooking quality parameters. The best performing two line hybrids based on mean performance for grain yield, along with its parents were analyzed for physical and cooking quality characters.

Materials and methods

The materials used in the study comprised of 10 promising two line rice hybrids produced in the Department of Plant Breeding and Genetics, College of Horticulture Vellanikkara, Kerala Agricultural University Thrissur. The grain quality parameters were carried out

Table 1: List of hybrids selected for analysis of quality parameters

S. No.	Details of the hybrids
1	TGMS 91S x Samyuktha
2	TGMS 81S x Matta Triveni
3	TGMS 74S x Kanchana
4	TGMS 74S x Kairali
5	TGMS 91S x Makom
6	TGMS 82S x Prathyasa
7	TGMS 81S x Makom
8	TGMS 81S x Kanchana
9	TGMS 81S x Aiswarya
10	TGMS 74S x Aiswarya

Grain and milling quality characters:

Kernel colour: Kernel colour was identified as red or white.

Kernel length (mm): Length of 10 unbroken brown rice, in three sets was measured and the mean was expressed in mm and the grains were categorized as follows:

Size category	Length (mm)
Extra long	>7.50
Long	6.61-7.50
Medium	5.51-6.60
Short	< 5.50

Kernel width (SES-IRRI, 1996): Breadth of 10 unbroken brown rice was measured using vernier caliper in three sets and the mean was expressed in mm

Milling traits: Milling recovery of rough rice is an estimation of the quantity of head rice and total milled rice that can be produced from a unit of rough rice. It is generally expressed as percentage (Khush *et al.*, 1979) as given below:

$$\text{Hulling percentage} = \frac{\text{Total hulled rice}}{\text{Total rough rice}} \times 100$$

$$\text{Milling percentage} = \frac{\text{Total milled rice}}{\text{Total rough rice}} \times 100$$

$$\text{Head rice recovery} \quad \frac{\text{Total head rice}}{\text{Total rough rice}} \times 100$$

Amylose content: Samples were weighed about 100 mg ± 0.5 mg three times for each sample and these samples were placed into 100 ml volumetric flasks. To this, 1 ml of ethanol was added by using pipette to wash down any of the flour adhering to the side of the flask. These contents were shaked well in order to wet the entire sample. To this, 9.0 ml of NaOH solution (1M) was added and mixed it well until the starch was completely dissolved by standing overnight. The test solutions were allowed to cool at room temperature and made up the volume with the distilled water. The blank solution was prepared without the sample in the 1000 ml volumetric flask, from the prepared test solutions, 0.5 ml aliquot was pipetted out into two test tubes. To this, 5.0ml of water, 0.1 ml of acetic acid and 0.20 ml of iodine were added, to make up the volume to 10.0 ml. These contents were mixed well by using vortex mixer. The test chemicals were measured the absorbance at 720 nm against the blank solution using the spectrophotometer. Based on the amylose content the rice was categorized as waxy (< 2%), very low (2 – 8%), low (8- 19%), intermediate (20 - 25%), and high (>25%) suggested (IRRI, 1972)

Alkali spreading value: Ten milled rice kernels were placed in 10.0 ml of 1.7 per cent KOH in shallow container (petriplate) .The kernels were so arranged that they did not touch each other. They were allowed to stand for 23 hours at 30^0 C. The appearance and disintegration of the kernels were usually after incubation based on the following numerical scale. A rating of 1 to 2 was classified as high final gelatinization temperature, 3 as high intermediate, 4 to 5 as intermediate(70-74^{0}C) and 6 to 7 as low final gelatinization temperature (<70^{0}C)

Numerical scale for rating kernels for alkali spreading value:

Description	Score
Grain not affected	1
Grain swollen	2
Grain swollen, collar incomplete or narrow	3
Grain swollen, collar complete and wide	4
Grain split or segmented, collar complete and wide	5
Grain dispersed, merging with collar	6
Grain completely dispersed and intermingled	7

Cooking quality characters:

Volume expansion ratio: The volume of raw rice as well as cooked rice was determined by water displacement using a measuring cylinder (Onate and Del Mundo, 1966):

$$\text{Volume expansion ratio} = \frac{\text{Volume of cooked rice}}{\text{Volume of raw rice}}$$

Kernel elongation ratio:Kernel elongation was determined as described by Azeez and Shafi (1966). Ten raw and ten cooked kernels were taken at random and their length was measured as given below:

$$\text{Kernel elongation ratio} = \frac{\text{Mean length of cooked kernel}}{\text{Mean length of raw kernel}}$$

Sensory characters:

Appearance after cooking: 5g rice samples were taken in a test tube.15 ml of water added and soaked for 10 min. Rice samples were cooked in a water bath for 15 min and transferred in to a petridish and scored as per panel test performance like white, creamish white, red streaks, white with brown streaks and white with black streaks

Cohesiveness: 5g rice samples were taken in a test tube.15 ml of water added and soaked for 10 min. Rice samples were cooked in a water bath for 15 min and transferred in to a petridish and scored as per panel test performance like well separated, partially separated, slightly separated, moderately separated and very sticky.

Tenderness to touch: 5g rice samples were taken in a test tube.15 ml of water added and soaked for 10 min. Rice samples were cooked in a water bath for 15 min and transferred in to a petridish and scored as per panel test performance like soft, moderately soft, moderately hard, hard and very soft.

Tenderness on chewing: 5g rice samples were taken in a test tube.15 ml of water was added and soaked for 10 min. Rice samples were cooked in a water bath for 15 minutes and transferred in to a petridish and scored as per panel test performance like soft, moderately soft, moderately hard, hard and very soft.

Taste: 5g rice samples were taken in a test tube.15 ml of water added and soaked for 10 min. Rice samples were cooked in a water bath for 15 min and transferred in to a petridish and scored as per panel test performance like good, desirable, tasteless and undesirable.

Results and Discussion

Quality analysis

The physical and cooking quality characters of superior two line rice hybrids produced in the study and their parents are presented in Table 2, 3 and Table 4. The female parents having white kernels and the male parents had red kernels. But all the promising hybrids exhibited red kernels. The length of kernels ranged from 4.34 (TGMS 81S x Aiswarya) to 6.51 (TGMS 91Sx Kanchana) for hybrids and 3.41 (Prathyasa) to 4.94 (Matta Triveni) for parental average being 5.36 and 4.49 for hybrids and parents. Kernel width ranged from (TGMS 81S x Kanchana) to 2.43 (TGMS 91S x Samyuktha) for hybrids and 1.08 (Kairali) to 1.86 (Prathyasa) for parents average being 1.04 and 1.22 for hybrids and concerned parents taken for the study. The hulling percentage ranged from 55.56 (TGMS 74S x Aiswarya) to 83.33(TGMS 82S x Prathyasa) for hybrids and 70.5 (Aiswarya) to 80 (Prathyasa) for parents average being 72.83 and 75.21 for hybrids and parents.

The milling percentage exhibited a range of 48.00 (TGMS 74S x Aiswarya) to 68.96 (TGMS 81S x Makom) for hybrids with a mean value of 62.16. maximum milling percentage was recorded by the parent TGMS 81S (68.88) and Kairali (50.1) showed the minimum value average being 62.82. Amylose content of promising two line hybrids ranged from 21.2 per cent (TGMS 74S x Kairali) to 26.2 per cent (TGMS 74S x Aiswarya) and the mean amylase content was 24.04 per cent. Highest amylose content was observed in the pollinator parent Aiswarya (27.5 per cent) and the TGMS 91S recorded the minimum value of 21.5 per cent average being 24.56. The two line hybrid TGMS 74S x Kairali was found to be have a mean alkali spreading value of 5.0 where as the lowest value of 1.9 was recorded by the hybrid TGMS 81S x Matta Triveni. The average alkali spreading value for hybrids was 3.51. Among the parents TGMS 74S and Kanchana (4.3) recorded a maximum alkali spreading value and Matta Triveni (1.0) recorded the minimum alkali spreading value with a mean value of 3.17. The two line rice hybrid TGMS 82S x Prathyasa reported the highest volume expansion ratio (2.50) and the lowest by 2.00 (TGMS 81S x Aiswarya) with a mean value of 2.20. Volume expansion ratio of the parents ranged from 2.11 (Makom) to 2.50 (Prathyasa) and mean was 2.19.

The mean kernel elongation ratio exhibited a range of 1.13 (TGMS 91S x Makom) to 1.32 (TGMS 74S x Kairali) with a mean value of 1.25. Maximum kernel elongation ratio was recorded by Matta Triveni (1.32) and Samyuktha (1.15) showed the minimum value average being 1.24. Abdominal white is absent in all two line rice hybrids and endosperm type showed intermediate type. Sensory quality characters all two line rice hybrids exhibited red streaks after cooking and well separated, moderately soft and desirable taste.

Better understanding of the factors that contribute to the overall grain quality of rice will lay the foundation for developing new breeding and selection strategies for combining high quality,with high yield. It is necessary to meet the growing global demand for high quality rice while offering producing countries additional opportunities for generating higher export revenues.

Keralities have a dietary preference towards red kernelled rice. All the promising two line hybrids have red kernels. Milling recovery defined the recovery of milled rice from the paddy, Milling recovery of two line rice hybrids reflected less loss of paddy on milling (Fig. 1). The cooking quality of rice depends mainly on amylose content and gelatinization temperature. Amylose content determines the stickiness of cooked rice. Intermediate amylase content is preferred by Keralities. Low amylose content show low water absorption ,expansion on cooking and the grains become sticky. The variety with high amylase amylose content cooks dry and fluffy but becomes hard on cooking and hence, intermediate amylose content is preferred is preferred in Kerala. Intermediate amylose content was noted for the hybrids, TGMS 91S x Samyuktha, TGMS 81S x Matta Triveni, TGMS 91S x Kanchana, TGMS 91S x Makom, TGMS 81S x Makom , TGMS 81S x Kairali and TGMS 81S x Aiswarya.

Gelatinization temperature is the temperature at which starch grains swell irreversibly when boiled in water. It ranges from 56-79^{0}C, depending on the hardness of starch granules. The higher the gelatinization temperature of rice, the more water and time are needed to cook. Gelatinization temperature is assayed as alkali digestion value. Intermediate gelatinization temperature is preferred (70-74^{0}C). The two line hybrids TGMS 91S x Kanchana, TGMS 74S x Kairali, TGMS 91S x Makom, TGMS 82S x Prathyasa, TGMS 81S x Makom, TGMS 81S x Kanchana, TGMS 81S x Aiswarya and TGMS 74S x Aiswarya exhibited intermediate gelatinization temperature.

Table 2: Grain and Milling quality characteristics of Two line hybrids and parents

Sl. No	Two line rice hybrids	Kernel colour	Kernel length (mm)	Kernel width (mm)	Hulling %	Milling %	Head rice Recovery	Abdominal white	Amylose content	Alkali spreading value
1	TGMS 91S x Samyuktha	Red	5.70	2.43	76.74	68.60	58.17	Absent	24.5	2.6
2	TGMS 81S x Matta triveni	Red	5.99	1.14	72.32	58.82	31.13	Absent	23.0	1.9
3	TGMS 91S x Kanchana	Red	6.51	0.51	75.43	61.40	45.33	Absent	25.0	3.1
4	TGMS 74S x Kairali	Red	4.35	1.07	71.76	64.71	59.71	Absent	21.2	5.0
5	TGMS91S x Makom	Red	5.64	1.03	79.17	66.67	45.33	Absent	23.6	3.9
6	TGMS 82S x Prathyasa	Red	5.19	0.22	83.33	53.66	46.15	Absent	25.8	4.3
7	TGMS 81S x Makom	Red	4.93	1.09	72.41	68.96	56.00	Absent	24.6	3.7
8	TGMS 81Sx Kanchana	Red	6.34	0.48	66.67	63.32	47.17	Absent	22.0	3.1
9	TGMS 81S x Aiswarya	Red	4.34	1.26	75.00	67.50	51.92	Absent	24.5	3.6
10	TGMS 74S x Aiswarya	Red	4.62	1.17	55.56	48.00	37.49	Absent	26.2	3.9
11	Aisawarya	Red	4.85	1.10	70.5	62.3	52.34	Absent	27.5	3.8
12	Kanchana	Red	4.71	1.12	71.5	64.9	56.00	Absent	26.5	4.3
13	Kairali	Red	3.73	1.08	77.8	50.1	37.49	Absent	24.5	2.7
14	Matta triveni	Red	4.94	1.15	71.7	63.1	52.01	Absent	23.6	1.0
15	Makom	Red	4.65	1.25	77.2	57.1	36.43	Absent	27.4	3.3
16	Prathyasa	Red	3.41	1.86	80.00	65.65	46.16	Absent	24.5	3.4
17	Samyuktha	Red	4.43	1.52	78.56	67.65	52.34	Absent	25.6	3.2
18	TGMS 74S	White	4.61	1.16	73.42	67.62	42.50	Slightly present	22.3	4.3
19	TGMS 81S	White	5.35	1.19	75.52	68.68	47.17	Slightly present	21.6	3.9
20	TGMS 82S	White	4.92	1.08	74.38	61.35	52.19	Slightly present	23.3	2.1
21	TGMS 91S	White	5.02	1.15	76.45	63.10	59.61	Slightly present	21.5	2.5

Table 3: Cooking quality characterstics of two line rice hybrids and parents

Sl. No	Two line rice hybrids	Volume expansion	Elongation ratio	Endosperm type
1	TGMS 91S x Samyuktha	2.13	1.25	Intermediate
2	TGMS 81S x Matta triveni	2.30	1.28	Intermediate
3	TGMS 91S x Kanchana	2.11	1.32	Intermediate
4	TGMS 74S x Kairali	2.16	1.32	Intermediate
5	TGMS91S x Makom	2.23	1.13	Intermediate
6	TGMS 82S x Prathyasa	2.50	1.23	Intermediate
7	TGMS 81S x Makom	2.29	1.27	Intermediate
8	TGMS 81Sx Kanchana	2.14	1.31	Intermediate
9	TGMS 81S x Aiswarya	2.00	1.28	Intermediate
10	TGMS 74S x Aiswarya	2.32	1.15	Intermediate
11	Aisawarya	2.13	1.20	Non waxy
12	Kanchana	2.35	1.30	Non waxy
13	Kairali	2.21	1.13	Non waxy
14	Matta triveni	2.15	1.32	Non waxy
15	Makom	2.00	1.27	Non waxy
16	Prathyasa	2.50	1.22	Non waxy
17	Samyuktha	2.20	1.15	Non waxy
18	TGMS 74S	2.26	1.25	Waxy
19	TGMS 81S	2.31	1.27	Waxy
20	TGMS 82S	2.09	1.29	Waxy
21	TGMS 91S	2.10	1.28	Waxy

Table 4: Sensory quality characteristics of selected materials

Sl. No	Two line rice hybrids	Appearance after cooking	Cohesiveness	Tenderness in touching	Tenderness on chewing	Taste
1	TGMS91S x Samyuktha	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
2	TGMS 81S x Matta triveni	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
3	TGMS 91S x Kanchana	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
4	TGMS 74S x Kairali	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
5	TGMS91S x Makom	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
6	TGMS 82S x Prathyasa	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
7	TGMS 81S x Makom	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
8	TGMS 81Sx Kanchana	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
9	TGMS 81S x Aiswarya	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
10	TGMS 74S x Aiswarya	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
11	Aisawarya	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable

Sl. No	Two line rice hybrids	Appearance after cooking	Cohesiveness	Tenderness in touching	Tenderness on chewing	Taste
12	Kanchana	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
13	Kairali	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
14	Matta triveni	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
15	Makom	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
16	Prathyasa	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
17	Samyuktha	Red streaks	Well separated	Moderately soft	Moderately soft	Desirable
18	TGMS 74S	White	Partially separated	soft	soft	Desirable
19	TGMS 81S	White	Partially separated	soft	soft	Desirable
20	TGMS 82S	White	Partially separated	soft	soft	Desirable
21	TGMS 91S	White	Partially separated	soft	soft	Desirable

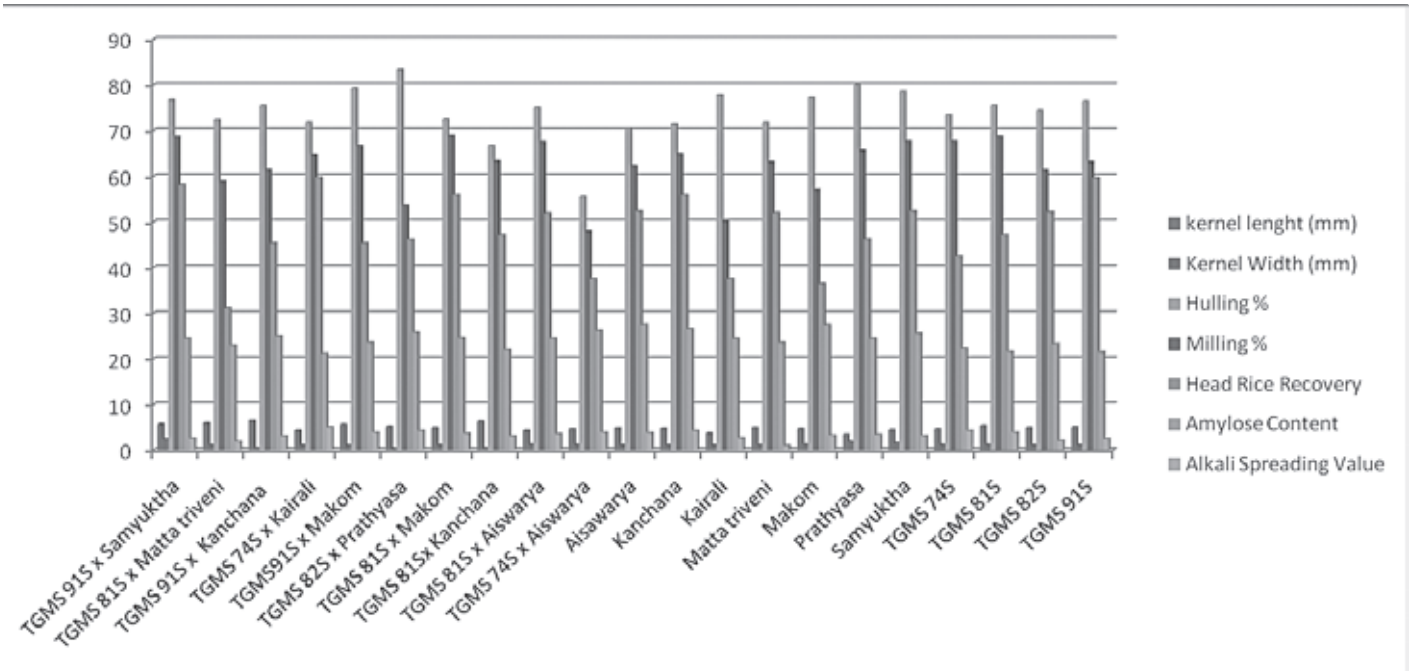

Figure 1: Grain and milling quality characters of promising two line hybrids and parents

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Genetic Analysis and Character Association of Yield Traits in Rainfed Rice (*Oryza sativa* L.)

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Abstract

The experiment comprised with 38 improved rice cultures obtained from different research organizations. The analysis of variance revealed that all the treatments are highly significant for various characters under studied *i.e.* days to 50% flowering, plant height, number of productive tillers per plant, number of productive tillers per square metre plot area, panicle length, filled grains per panicle, grain yield and straw yield. The higher magnitude of PCV and GCV were recorded for number of productive tillers per square metre plot area, number of filled grains per panicle and straw yield. Days to 50% flowering, number of productive tillers per square metre, panicle length, number of filled grains per panicle and grain yield showed high broad sense heritability. High heritability coupled with high genetic advance was recorded for number of productive tillers per square metre, number of filled grains per panicle, straw yield and grain yield indicated the major role of additive gene action in the inheritance of these characters. The trait number of productive tillers per plant expressed high direct effect and straw yield and days to 50% flowering had moderate direct effect on grain yield. Thus, these characters may serve as effective selection parameters during breeding programme in the rainfed rice ecosystem.

Key words: variability, heritability, genetic advance, association, rainfed rice.

Introduction

Rice (*Oryza sativa* L.) is the major food crop of more than half of the global population and will continue to occupy the pivotal place in global food and livelihood security systems. In India, rice is grown in an area of 43.5 million ha (23% of gross cropped area) with an annual production of 110 million tons. The population growth in most of the Asian countries, except China, continues to be around 2% per year. Hence, it is very pertinent to critically consider whether the rice production can be further increased to keep pace with population growth. With the current green revolution technologies, it is estimated that by 2020 at least 115-120 million tons of milled rice is to be produced in India to maintain the present level of self-sufficiency. Yield is a complex character, which is highly influenced by the environment, hence direct selection for yield alone limit the selection efficiency and ultimately results in limited success in yield improvement. Genetic variability studies are important in selection of parents for hybridization because crop improvement depends upon magnitude of genetic variability in base population (Adebisi *et al.*, 2001). Once genetic variability has been ascertained, crop improvement is possible through the use of appropriate selection method and increasing total yield would be made easier by selecting for yield components because they are more often easily inherited than total yield itself. An idea on the extent of association between traits conferring higher yield will be much helpful to decide upon the traits to be given importance in selection process. Path coefficient analysis assists plant breeders in identifying traits on which selection pressure should be given for improving yield. An attempt was made in the present investigation to assess the variability, heritability, genetic advance and association of some quantitative characters in rainfed rice.

Materials and methods

The experimental material comprised with 38 improved rice cultures received from various research organizations which were evaluated in a randomized block design with three replications at Agricultural Research Station, Tamil Nadu Agricultural University, Paramakudi during Rabi 2017-18. The experimental site is located at 9" 21' N latitude, 78" 22' E longitudes and an altitude of 242 m above mean sea level with average annual rainfall of 840 mm. This site has clay loam soil texture with pH of 8.0. Each genotype was raised in 5x2 m plot keeping 15 x 10 cm spacing. The recommended agronomic practices followed to raise good crop stand. The data were recorded on 10 randomly selected plants from each replication for various

quantitative traits studied *viz*, days to 50% flowering, plant height (cm), number of productive tillers per plant, number of productive tillers per square metre plot area, panicle length (cm), filled grains per panicle, grain yield (t/ha) and straw yield (t/ha). Mean values were subjected to analysis of variance to test the significance for each character as per methodology advocated by Panse & Sukhatme (1967). Phenotypic coefficient of variation (PCV) and genotypic coefficient of variation (GCV) were calculated by the formula given by Burton (1952), heritability in broad sense and genetic advance were calculated as per Johnson *et al.*, (1955).

The genotypic correlation coefficients between yield and yield components as well as among the yield components were worked out. From the analysis of variance and covariance tables, the corresponding genotypic variances and co-variances were calculated by using the mean square values and mean sum of products as suggested by Al-Jibouri *et al*. (1958). The relative influence of seven components on yield by themselves (direct effects) and through other traits (indirect effects) was evaluated by the method of path coefficient analysis as suggested by Dewey and Lu (1959). The simple correlation coefficients already estimated at genotypic level were utilized for this purpose. By keeping yield as dependent variable and other eight traits as independent variables, simultaneous equations which express the basic relationship between path coefficients were solved to estimate the direct and indirect effects. The direct and indirect effects were classified based on the scale given by Lenka and Misra (1973).

Results and Discussion

The analysis of variance (Table 1.) revealed that for treatments are highly significant for various characters under studied. This result was in conformity with the earlier findings of Bakele *et al.* (2013) and Rashid *et al*. (2017). The perusal of coefficient of variability indicated that wide range of variability was present at both phenotypic and genotypic levels for all the characters under studied. The magnitude of phenotypic coefficient of variation (PCV) was higher than genotypic coefficient of variation (GCV) for all the traits which may be due to higher degree of interaction of genotypes with the environment (Kavitha and Reddy, 2002). The differences in the magnitude of PCV and GCV for number of productive tillers per square metre plot area and straw yield were of high order. The higher magnitude of PCV and GCV were recorded for number of productive tillers per square metre plot area, number of filled grains per panicle and straw yield (Table 2.); indicating the minimal influence of environment and presence of high genetic variability for these traits in the experimental material. Hence, selection on the basis of phenotype can also be effective for improvement of these traits. Coefficients of variability for various characters observed in the present study were in agreement with the findings of Babu *et al*. (2012) and Sameera *et al* (2016).

Table 1: Analysis of variance for different traits in rainfed rice

Source of variation	Degrees of freedom	Days to 50% flowering	Plant height	Productive tillers per plant	Productive tillers per square metre	Panicle length	Filled grains per panicle	Straw yield per plot	Grain yield per plot
Replication	2	20.17	148.29	2.17	272.60	2.28	76.88	0.41	0.04
Treatment	37	122.58*	218.31*	2.73*	668.31*	11.14*	148.41*	5.15*	0.63*
Error	74	2.73	42.80	1.23	49.79	1.69	64.69	1.05	0.01

*significant at P=0.05 level

Table 2: Estimates of mean, variability, heritability and genetic advance in rainfed rice

Traits	Mean ± SE	PCV	GCV	h^2	GA	GA as % of mean
Days to 50% flowering	82.41 ± 0.95	7.92	7.67	93.61	12.59	15.29
Plant height	67.33 ± 3.78	14.95	11.36	57.75	11.97	17.78
Productive tillers per plant	6.56 ± 0.63	18.83	8.35	19.67	0.50	7.63
Productive tillers per square metre	177.73 ± 12.88	28.46	25.55	80.55	83.95	47.23
Panicle length	19.22 ± 0.75	11.45	9.23	65.03	2.95	15.34
Filled grains per panicle	98.89 ± 4.64	23.45	21.99	87.97	42.03	42.50
Straw yield per plot	5.32 ± 0.59	29.22	21.94	56.35	1.81	33.92
Grain yield per plot	2.39 ± 0.07	19.62	18.87	92.50	0.90	37.38

SE=Standard Error; PCV=Phenotypic Co-efficient of Variation; GCV=Genotypic Co-efficient of Variation; h^2=Heritability (Broad sense); GA=Genetic Advance

High heritability is not enough to make efficient selection in the advanced generations unless it is accompanied by substantial amount of genetic advance. Burton (1952) pointed out that the heritability in combination with intensity of selection and amount of variability present in the population influences gains to be obtained from selection. Thus, genetic advance is yet another important selection parameter which although independent, represents the expected genetic advance under selection. It measures the differences between the mean genotypic values of the selected lines and the mean genotypic values of original population from which these were selected. According to Panse (1957) if a character is governed by non-additive gene action, it may give high heritability but low genetic advance, whereas, if it is governed by additive gene action, high heritability along with high genetic advance provided good scope for further improvement. In the present study, high broad sense heritability estimates were obtained for days to 50% flowering, number of productive tillers per square metre, panicle length, number of filled grains per panicle and grain yield (Table 2.), indicating the major role of additive gene action in inheritance of these traits. The broad sense heritability of these characters is in accordance with those of Babu *et al*. (2012), Sameera *et al* (2016) and Rashid *et al*. (2017).

High heritability alone does not guarantee large gain from selection unless sufficient genetic advance (GA) attributed to additive gene action is present. High heritability coupled with high genetic advance was recorded for number of productive tillers per square metre, number of filled grains per panicle, straw yield and grain yield (Table 2.). It indicates that most likely the heritability is due to additive gene effects and selection may be effective. This finding is in close agreement with the findings of Bakele *et al.* (2013) Sameera *et al* (2016) and Rashid *et al*. (2017). Low heritability coupled with low genetic advance was recorded for number of productive tillers per plant (Table 2.). It indicates that this trait is highly influenced by environmental effects and selection would be ineffective.

In the present investigation, grain yield exhibited positive and significant association with productive tillers per plant, productive tillers per square metre and straw yield (Table 3) indicating an increase in grain yield with an increase in these characters. Therefore, priority should be given to these traits, while making selection for yield improvement. This was in conformity with the findings of Babu *et al*. (2012), Vanisree *et al.* (2013) , Islam *et al*. (2015) and Sameera *et al* (2016). Knowledge on inter relationship between yield traits may facilitate breeder to decide upon the intensity and direction of selection pressure to be given on related traits for the simultaneous improvement of these traits. In the present study, straw yield had positive and significant association with plant height, number of panicles per square metre and panicle length; suggesting that selection based on straw yield is highly fruitful in developing high yielding genotypes under rainfed condition, as it will bring simultaneous improvement of these traits. Similarly positive and significant relationship was observed between number of filled grains per panicle, days to 50% flowering and panicle length. The traits, panicle length and number of productive tillers per square metre showed positive and significant association with plant height and number of productive tillers per plant respectively. Similar results were reported by Vanisree *et al.* (2013), Islam *et al*. (2015) and Sameera *et al* (2016). Number of filled grains per panicle had nagative and significant correlation with number of productive tillers per square metre. This was in conformity with the findings of Vanisree *et al.* (2013) and Sameera *et al* (2016).

Path analysis gives an idea about how a trait influences grain yield directly and indirectly *via* other traits. This is very important in giving due weightage to major yield contributing traits while selection. In the present investigation, the trait number of productive tillers per plant expressed high direct effect and straw yield and days to 50% flowering had moderate direct effect on grain yield (Table 4). This was in conformity with the findings of Babu *et al*. (2012), Vanisree *et al.* (2013), Islam *et al*. (2015) and Sameera *et al* (2016). Genotypic residual effect (0.82) indicates that traits under study contribute 18% to the variability in grain yield. It indicates that many other traits which have not been studied here, need to be included to account fully for the variation in grain yield.

Table 3: Genotypic correlation coefficients for yield related traits and grain yield.

Characters	Days to 50% flowering	Plant Height	Productive tillers per plant	Productive tillers per square metre	Panicle length	Filled grains per panicle	Straw yield per plot	Grain yield per plot
Days to 50% flowering	1.000	-0.220	-0.034	-0.176	-0.084	0.464**	-0.227	0.049
Plant Height		1.000	-0.010	-0.128	0.679**	0.144	0.446**	0.107
Productive tillers per plant			1.000	0.273*	-0.188	-0.179	0.064	0.404**
Productive tillers per square metre				1.000	-0.162	-0.264*	0.431**	0.342*
Panicle length					1.000	0.333*	0.276*	-0.030
Filled grains per panicle						1.000	-0.118	-0.146
Straw yield per plot							1.000	0.363**
Grain yield per plot								1.000

*Significance at 5% level; ** Significance at 1 % level

Table 4: Direct and indirect effects of yield related traits on grain yield

Characters	Days to 50% flowering	Plant Height	Productive tillers per plant	Productive tillers per square metre	Panicle length	Filled grains per panicle	Straw yield per plot	Grain yield per plot
Days to 50% flowering	**0.227**	-0.018	-0.011	-0.025	0.001	-0.059	-0.064	0.049
Plant Height	-0.050	**0.084**	-0.003	-0.018	-0.013	-0.018	0.126	0.107
Productive tillers per plant	-0.007	-0.001	**0.328**	0.039	0.003	0.022	0.018	0.404**
Productive tillers per square metre	-0.040	-0.010	0.089	**0.144**	0.003	0.033	0.122	0.342*
Panicle length	-0.019	0.057	-0.062	-0.023	**-0.019**	-0.042	0.078	-0.030
Filled grains per panicle	0.105	0.012	-0.058	-0.038	-0.006	**-0.127**	-0.033	-0.146
Straw yield per plot	-0.051	0.037	0.021	0.062	-0.005	0.015	**0.283**	0.363**

*Significance at 5% level; ** Significance at 1 % level; Residual effect = 0.82; Diagonal values (in bold) denote the direct effects

A perusal of the results thus emphasized the need for selection based on number of productive tillers per plant, number of productive tillers per square metre, number of filled grains per panicle, straw yield for improvement of grain yield in rainfed rice eco system.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Combining Ability Analysis for Yield and Quality Traits in Rice (*Oryza Sativa* L.)

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Abstract

Seven parents were crossed in a diallel manner to study the combining ability for yield and quality attributes in rice at Agricultural Research Station, Nellore, ANGRAU, Andhra Pradesh. The preponderance of additive gene action was observed for plant height, ear bearing tillers per plant, number of secondary branches per panicle, number of filled grains per panicle, test weight and kernel L/B ratio. Non additive component was observed for grain yield and most of the quality traits. The parents Swarnamukhi, Bharani, NLR 33637, Samba Mahsuri and PR 164 were found to be the good general combiners and could be utilized to generate desirable segregants for future breeding programme. Whereas, Samba Mahsuri x NLR 33637, Swarnamukhi x Bharani and Erramallelu x Samba Mahsuri were found to be the best specific combiners for most of the traits studied.

Key words: Rice, gca, sca, yield, quality traits

Introduction

Rice is the staple food for more than half of the world's population and second most widely grown crop in the world. It is the most extensively and largest grown crop in India having an average of about 43.95 m ha and is grown in almost all parts of the country. In Andhra Pradesh rice is the major cereal crop grown in kharif, rabi and in some areas during summer season accounting in an area of about 3.80 m ha, with a production of 11.57 m.tonnes and a productivity of 2856 kg/ha during 2014-15.

With the enhanced income levels and changing food habits, breeding rice varieties with preferred grain quality features has become the second most important objective after yield. Although emphasis is having laid on improving rice grain quality, combining yield potential with good grain quality is challenging. Physical properties include yield of edible and marketable polished grain, uniform shape, whiteness and translucence. These traits are immediately obvious to consumers and so are major factors defining market value. The traits that exert major effects on the eating and cooking qualities are related to the physico-chemical properties of rice grains such as amylose content, gelatinization temperature, gel consistency, aroma and kernel length after cooking (Shobha Rani *et. al.,* 2008). Predictable expression of these traits across seasons and years gives reputation to a variety. Besides good milling quality and cooking quality traits, nutritional quality improvement in the cereals is the important factor to be considered in breeding. Recently, hidden hunger (micronutrient deficiency) has been recognized in developing countries, where rice is the staple food. Rice is consumed as a polished grain. Nutritional components such as minerals and vitamins are either absent or present at low levels in polished grains. So a modest increase in these levels in rice would provide a significant nutritional boost to the hundreds of millions of people who depend on it. Hence, there is an imperative need for a shift in emphasis towards development of nutritionally high quality rice.

Success of any breeding programme mainly depends on the choice of appropriate parents in the hybridization. The combining ability studies helps in selecting the parents for hybridization and provides information on additive and dominance variance (Thakare *et al* 2010) as well as breeding procedure to be followed to select desirable segregants (Salgotra *et al* 2009). Diallel analysis provides information about general combining ability (gca) and specific combining ability (sca) effects of parents and this method helps to compare the combining ability of parents where parents themselves are used as testers. The present investigation was undertaken to get an idea of the combining ability for yield and quality related traits in rice to identify good combiners for effective breeding programme.

Materials and Methods

The experimental material comprised of twenty one hybrids derived from seven parents in a diallel method II, model I of Griffing (1956). The parents used for hybridization are Erramallelu, IR 72, Samba Mahsuri, PR 164, Swarnamukhi, Bharani and NLR 33637. All the parents and hybrids were grown in a randomized block design (RBD) with three replications at Agricultural Research Station, Nellore, ANGRAU, Andhra Pradesh. The standard agronomic practices were followed to raise a good crop. The seedlings were planted with a spacing of 15 x 10 cm and a plot size of 3 rows of 3 m length with single plant per hill. The observations were recorded on ten randomly selected plants per replication per treatment by avoiding border rows and the mean values were expressed on per plant basis per plant basis for quantitative traits and days to flowering on per plot basis. The traits studied in the present experiment were, days to 50% flowering, plant height (cm), number of ear bearing tillers, panicle length (cm), primaries per panicle, secondaries per panicle, filled grains per panicle, unfilled grains per panicle, test weight (g), grain yield per plant (g), kernel length (mm), kernel breadth (mm), kernel L/B ratio, hulling percentage, milling percentage, head rice recovery, chalkiness percentage, gelatinization temperature score (GT score), water uptake, volume expansion, kernel length after cooking (KLAC), kernal breadth after cooking (KBAC), amylose and protein content. The mean values were taken from the replicated data and utilized for analysis by following the method given by Griffing (1956).

Results and Discussion:

The analysis of variance (Table 1) revealed significant genotypic effects for all the traits under study indicating the wider variability for the respective characters among the seven parents. The mean sum of squares due to sca was also significant for all the characters except number of secondaries, number of filled grains per panicle and unfilled grains per panicle suggesting that there was considerable variation among the crosses for the characters under study indicating the possibility for improvement of yield through yield contributing characters. The *perse* performance was closely associated with the gca of the parents and sca of the crosses in majority of the traits studied. Similar findings were also reported by Singh *et al* (1996).

The general combining ability (gca) identifies superior parental genotypes while specific combining ability (sca) helps in identification of good hybrid combinations. The parents with significant negative general combining ability estimates for days to 50% flowering and plant height and with significant positive gca effects for the remaining characters are considered as good general combiners. The parents with significant positive gca effects for days to 50% flowering and plant height are considered as poor general combiners. The parents with non significant gca estimates for all the characters were considered as average general combiners.

The estimates of gca effects of parents (Table 2) revealed that the parent, Swarnamukhi was found to be the best general combiner among all the seven parents studied and it recorded high mean values coupled with high gca for ten characters *viz*., effective bearing tillers per plant, primary branches per panicle, secondary branches per panicle, grain yield, kernel length, kernel breadth, kernel L/B ratio, gelatinization temperature score, amylose content and kernel breadth after cooking. The next best parent was Bharani which exhibited high mean and gca for nine traits *viz*., plant height, days to 50% flowering, less ill filled grains per panicle, test weight, hulling percentage, head rice recovery, gelatinization temperature score, and kernel length after cooking. NLR 33637 was good for seven characters *viz*., panicle length, number of primaries per panicle and number of secondary branches per panicle, number of filled grains per panicle, milling percentage, water uptake, kernel length after cooking followed by Sambamahsuri which was found good for secondary branches per panicle, less ill filled grains per panicle, lower test weight, less kernel breadth, gelatinization temperature score, volume expansion and kernel length after cooking. However, good general combiners may not necessarily produce good specific combinations for different traits. Similar results were reported by Ramalingam *et al* (1997) and Aditya and Anuradha (2015). It could be mentioned that the parents with significant and positive GCA values might be contributed positive alleles in their hybrids due to its additive nature of gene action for the respective traits. The crosses involving these parents might produce good progenies for the respective traits.

Specific combining ability (sca) of a cross is the estimation and the understanding of the effect of non additive gene action for the trait which is an indicator for the selection of a hybrid combination (Akter *et al* 2010). Therefore the highly significant sca effect is desirable for a successful hybrid breeding programme. Specific combining ability effects were estimated for all the twenty one hybrids and for all the twenty four traits (Table 3). The estimates of sca

effects revealed that none of the hybrids were consistently superior for all the traits. In the present study, positive significant sca effects for grain yield was exhibited by the cross PR 164 x NLR 33637. The high sca effects may be associated with high hybrid vigour (Saidaiah *et al* (2010).

When the sca effects were considered based on the performance among the 21 cross combinations, Samba Mahsuri x NLR 33637 was the best specific combiner for most of the characters *viz*., days to 50 % flowering, primary branches per panicle, filled grains per panicle, low chalkiness percentage, head rice recovery, intermediate gelatinization temperature score and protein content. The next best cross was, Swarnamukhi x Bharani for ear bearing tillers per plant, kernel breadth (high), hulling percentage, kernel breadth after cooking (low), amylose content and protein content. For plant height, less number of ill filled grains per panicle, milling percentage, head rice recovery, KLAC, GT score and protein content, Erramallelu x Samba Mahsuri was the best specific combiner. Swarnamukhi x NLR 33637 was the best cross for less number of ill filled grains, milling percentage, head rice recovery, KBAC (high) and amylose content. Erramallelu x Swarnamukhi was the best combiner for KBAC, amylose content and protein content. For the traits, head rice recovery, volume expansion, and water uptake Samba Mahsuri x Swarnamukhi was the best specific combiner. IR 72/ NLR 33637 was the only good cross combination for grain yield but, this was not found good any other character except for low kernal breadth. The cross combination IR 72 x PR 164 was not found good for any one of the characters studied.

Table 1: Analysis of variance for various yield and quality characters in rice

S.No	Character	Mean sum of squares			σ^2gca	σ^2sca	σ^2gca / σ^2sca+ σ^2gca
		gca	sca	error			
1	Plant height	104.469**	22.655**	3.035	11.270	20.620	0.520
2	Days to 50% flowering	122.896**	27.541**	0.708	13.576	26.830	0.503
3	Ear bearing tillers per plant	5.562**	1.165**	0.646	0.546	0.519	0.678
4	Panicle length	4.037**	0.815**	0.360	0.408	0.455	0.642
5	Primary branches per panicle	3.357**	0.255**	0.055	0.367	0.200	0.786
6	Secondary branches per panicle	48.402**	5.241**	2.367	5.115	2.874	0.781
7	Filled grains per panicle	1093.454**	95.049**	33.254	117.800	61.795	0.790
8	Ill filled grains per panicle	48.199**	32.458**	5.555	4.738	26.903	0.260
9	Test weight	19.836**	0.891**	0.139	2.188	0.752	0.850
10	Grain yield per plant	7.633**	3.063	2.614	0.557	0.449	0.356
11	Kernal length	0.462**	0.129**	0.004	0.051	0.125	0.449
12	Kernel breadth	0.034**	0.026**	0.001	0.004	0.025	0.242
13	Kernal L/B ratio	0.113**	0.007**	0.003	0.012	0.004	0.873
14	Chalkiness percentage	7.025**	31.123**	1.899	0.569	29.224	0.037
15	Hulling percentage	3.489**	0.716**	0.037	0.383	0.679	0.530
16	Milling percentage	6.890**	3.377**	0.455	0.715	2.922	0.598
17	Head rice recovery	49.304**	68.910**	1.438	5.318	67.417	0.136
18	GT score	3.693**	0.397**	0.068	0.403	0.329	0.710
19	Amylose content	14.181**	8.008**	0.823	1.484	7.185	0.292
20	Water uptake	1226.665**	996.655**	195.830	114.537	800.825	0.222
21	Volume expansion	0.067	0.149	0.130	-0.006	0.029	-0.693
22	KLAC	0.056**	0.025**	0.001	0.006	0.024	0.339
23	KLBC	0.016**	0.032**	0.004	0.001	0.028	0.088
24	Protein content	0.964**	1.938**	0.011	0.106	1.927	0.099

**: Significant at 1% level

Table 2: General combining ability effects for various yield and quality characters in rice

S.No	Character	Erramallelu	IR 72	PR 164	Sambamahsuri	Swarnamukhi	Bharani	NLR 33637	SE(gi)
1	Plant height	-3.858**	-2.384**	1.921**	-0.492	0.749*	-2.895**	6.958**	0.388
2	Days to 50% flowering	-5.947**	-0.947*	-0.910*	4.312**	3.942**	-0.984*	0.534	0.350
3	Ear bearing tillers per plant	1.060*	-0.483	-0.413	0.079	0.886*	-0.227	-0.902*	0.430
4	Panicle length	-0.471**	-0.261	0.379*	-0.790**	0.014	-0.408*	1.537**	0.165
5	Primary branches per panicle	-0.830**	-0.458**	-0.750**	0.689**	0.702**	0.067	0.581**	0.114
6	Secondary branches per panicle	-1.467**	-2.003**	-2.037**	2.598**	2.115**	-2.404**	3.198**	0.579
7	Filled grains per panicle	-0.889	-11.092**	-13.856**	16.410**	-0.181	-10.359**	19.-968**	2.680
8	Ill filled grains per panicle	-2.795	1.160	-3.978*	0.211	6.054**	-1.357	0.704	1.622
9	Test weight	-1.057**	1.076**	1.675**	-3.338**	0.372**	1.011*	0.261**	0.113
10	Grain yield per plant	0.803	-1.844	-1.509	-0.335	2.395*	-1.468	1.956	1.041
11	Kernal length	0.004	0.163*	0.347**	-0.423**	0.030*	-0.043*	-0.077**	0.0346
12	Kernel breadth	-0.004	-0.023	0.013	-0.008	-0.047*	0.019	0.050*	0.013
13	Kernal L/B ratio	-0.011	0.184**	0.217**	-0.294**	0.150**	-0.066	-0.179**	0.047
14	Chalkiness percentage	-0.254	3.001**	-2.802**	-3.959**	2.043**	2.673**	-0.702*	0.393
15	Hulling percentage	-0.903**	1.638**	-0.197	-0.333**	-1.623**	0.548**	0.870**	0.116
16	Milling percentage	1.368**	-0.238**	-0.570**	0.181*	-1.340**	0.109	0.491**	0.076
17	Head rice recovery	-3.049**	-0.397**	2.370**	-0.243	-5.769**	3.957**	3.133**	0.084
18	GT score	1.974**	-0.026	0.048	-0.804**	-0.804**	-0.249*	0.139	0.098
19	Amylose content	0.106	0.682**	-2.490**	-0.243	-0.620**	1.708**	0.857**	0.195
20	Water uptake	-3.968	-11.228*	13.624**	14.921**	-12.746**	2.894	2.291	2.563
21	Volume expansion	-0.058	-0.203**	-0.073	0.139*	0.113	0.098	-0.016	0.060
22	KLAC	-0.023**	-0.075**	0.027**	0.093**	-0.072**	0.021*	0.030**	0.009
23	KLBC	-0.177**	-0.022	0.074**	0.019	0.053**	-0.004	0.057**	0.012
24	Protein content	-0.385**	-0.503**	0.286**	0.028	-0.391**	0.433**	0532**	0.053

***:** Significant at 5% level, ** :Significant at 1% level of significance

The results indicated that the gca variances were higher than the sca variances for the traits *viz*., plant height, ear bearing tillers per plant, number of secondary branches per panicle, number of filled grains per panicle, test weight, kernel L/B ratio suggesting that these traits were under the control of additive gene action and these traits can be improved through simple selection methods in segregating generations. Similar results were already reported by Aditya and Anuradha (2015) for plant height and ear bearing tillers per plant, Ramalingam and Jebaraj (2013) for filled grains per panicle, Tushara *et al* (2013) for test weight, Gnanamalar and Vivekanandan (2013) for kernel L/B ratio. Preponderance of non additive gene action was observed in the rest of the traits indicating that these characters can be improved by repeated back crossing besides biparental mating in the early generations followed by selection. These results were in close agreement with the earlier findings of Satheesh kumar and Saravanan (2013) for days to 50% flowering, Satya and Jebaraj (2015) for panicle length, Ramalingam and Jebaraj (2013) for ill filled grains per panicle, Mallikarjuna *et al* (2014) for grain yield per plant, Upadhyaya and Jaiswal (2015) for kernal length and kernel breadth, Showkat *et al* (2015) for hulling%, Milling %, head rice recovery, Malini *et al* (2014) for amylose content and Audilakshmi and Upendra *et al* (2014) for water uptake and volume expansion ratio.

Table 3: Specific combining ability effects for various yield and quality characters in rice

S. No	Cross	Plant height	Days to 50% flowering	Ear bearing tillers per plant	Panicle length	Primary branches per panicle	Secondary branches per panicle	Filled grains per panicle	Ill filled grains per panicle	Test weight	Grain yield per plant	Kernel length	Kernal breadth
1	1x2	1.595	-3.130**	1.215	0.309	0.139	-1.584	-8.646	-6.054	1.770**	2.361	0.157	0.040
2	1x3	-1.400	-1.833**	-1.112	-1.081**	0.147	-0.310	-0.809	-1.739	0.347	-0.321	-0.107	0.024
3	1x4	1.906**	-3.722**	1.336	0.408	-0.108	-2.041	9.199	-6.655	-0.319	3.355	0.220*	0.019
4	1x5	1.669	-2.019*	0.862	1.197**	-0.208	1.322	11.286	2.858	0.250	3.763	-0.253**	-0.139**
5	1x6	1.327	1.574	-2.121*	0.036	-0.123	1.166	-5.842	9.623*	1.315**	-2.999	0.266**	-0.048
6	1x7	0.297	4.056**	0.503	0.141	-0.070	3.308*	18.384**	2.872	-0.102	3.894	0.320**	0.008
7	2x3	3.550**	0.167	-0.912	0.163	0.219	-0.631	-3.162	-1.114	0.135	-0.834	0.375**	-0.010
8	2x4	0.329	-7.056**	0.117	-0.245	0.014	1.297	-2.241	-1.887	-1.612**	0.845	0.568**	-0.048
9	2x5	-2.968**	1.315	-2.884**	1.674**	-0.116	0.797	-6.324	0.636	1.461**	-3.864	0.055	-0.270**
10	2x6	-0.941	6.241**	-5.021**	0.442	-0.888**	1.009	7.661	-2.209	-0.650*	-7.528**	0.141	-0.079*
11	2x7	5.779**	1.389	4.787**	-0.012	-0.115	1.583	-1.149	3.254	0.052	7.891**	0.185*	0.000
12	3x4	0.158	-5.426**	0.283	0.252	-0.294	0.062	-0.918	6.848	-0.158	0.567	0.878*	0.142**
13	3x5	-4.747**	-0.389	-2.761**	-0.739	-0.611*	0.298	-8.260	-1.215	-0.585*	-6.129*	0.038	-0.006
14	3x6	5.915**	4.870**	1.352	0.666	0.330	1.106	0.088	-2.291	1.477**	3.637	0.411**	-0.205**
15	3x7	-1.165	3.685**	0.416	0.465	0.153	-0.382	-6.842	2.922	0.800**	0.396	-0.039	-0.219**
16	4x5	1.486	3.389*	-1.446	0.357	0.127	-0.770	-1.573	2.986	-0.135	-3.746	-0.325**	0.049
17	4x6	4.124**	-1.019	-0.626	1.609**	0.635*	-1.685	-3.284	1730	0.270	-1.200	-0.669**	-0.114**
18	4x7	1.064	-4.204**	-1.425	0.544	1.512**	1.836	19.065**	2.926	0.976**	-0.201	-0.225**	-0.041
19	5x6	2.893**	-1.981*	2.620*	0.757	0.552	3.688*	7.893	15.967**	-0.730**	-5.524*	0.348**	0.088**
20	5x7	-1.244	-2.500**	1.551	-0.277	-0.532	-0.014	7.633	-0.984	0.169	5.543*	0.222**	0.024
21	6x7	-4.539**	-1.574	-0.456	0.058	2.193**	-0.002	-5.819	-0.423	0.197	1.422	0.221**	0.132**
		0.960	0.865	1.064	0.408	0.283	1.685	6.633	0.280	0.280	2.577	0.101	0.033

1:Erramallelu, 2: IR 72, 3: PR 164, 4: Sambamahsuri, 5: Swarnamukhi, 6: Bharani, 7:NLR 33637

S. No	Cross	Kernal L/B ratio	Chalkiness percentage	Hulling percentage	Milling percentage	Head rice recovery	GT score	Amylose content	Water uptake	Volume expansion	KLAC	KLBC	Protein content
1	1x2	-0.035	-5.488**	2.701**	-0.840**	-7.394**	0.065	-2.692**	18.648**	0.246	-0.136**	-0.113**	-0.948**
2	1x3	-0.171	2.235**	-4.274**	-1.683**	-0.551**	-0.009	0.007	-13.204**	1.315**	0.275**	-0.121**	0.150
3	1x4	0.126	1.109*	0.011	2.763**	11.582**	0.843**	-3.5373**	-22.167**	-0.713**	0.183**	-0.067**	1.744**
4	1x5	0.231*	15.107**	-1.592**	0.591**	-2.982**	1.176**	6.857**	19.167**	-1.231**	-0.229**	-0.121**	2.386**
5	1x6	0.288*	10.253	-1.670**	0.956**	5.896**	0.620*	2.113**	-46.352**	-0.952**	-0.135**	0.009	0.559**
6	1x7	0.174	-4.396**	-2.528**	-4.593**	-4.068**	0.509*	3.916**	-14.204*	-0.639**	0.072**	-0.325**	1.240**
7	2x3	-0.297*	-2.133*	-0.102	-4.652**	-7.053**	-1.349**	-2.462**	-45.275**	-0.379*	-0.102**	-0.017	0.990**
8	2x4	0.534**	12.557**	-0.016	0.914**	1.877**	-1.157**	-2.008**	15.093*	-0.928**	-0.081**	0.035	-0.305*
9	2x5	0.976**	8.549**	0.040	0.736**	6.089**	0.509*	1.857**	-35.907**	-0.575**	-0.126**	-0.290**	0.224
10	2x6	0.266*	13.335**	0.862**	3.216**	1.360**	0.954**	-3.723**	7.907	-0.897**	-0.226**	-0.203**	1.290**
11	2x7	0.069	5.017**	-0.326	0.404*	1.520**	-0.491*	0.577	-26.944**	1.004**	-0.082**	0.457**	0.858**
12	3x4	0.178	3.657**	2.365**	2.551**	4.076**	1.102**	-1.817**	14.907*	0.815**	-0.130**	0.506**	0.070
13	3x5	-0.017	-0.141	-3.189**	-1.260**	0.326	0.102	-3.017**	48.574**	-1.226**	0.048*	-0.068*	-1.845**
14	3x6	0.953**	8.215**	0.571*	1.537**	0.293	1.546**	-1.211*	-8.611	-1.207**	-0.261**	-0.054	-1.832**
15	3x7	0.613**	-3.207**	0.840**	0.922**	2.490**	-1.231**	-2.258**	-14.130*	-0.913**	-0.277**	-0.302**	1.623**
16	4x5	-0.359**	-6.588**	-0.516*	-1.841**	6.082**	-0.713**	-2.097**	35.278**	1.502**	-0.094**	-0.150**	-0.360**
17	4x6	-0.196	-5.622**	-1.770**	-2.454**	-1.297**	-1.269**	-0.913	34.426**	-0.983**	-0.034	0.070*	1.976**
18	4x7	-0.046	-2.707**	0.725*	0.671**	4.540**	-1.380**	-2.523**	-57.426**	-1.072**	-0.246**	-0.117**	2.660**
19	5x6	-0.057	0.246	1.176**	-0.302	-1.148**		-5.114**	-71.907**	0.130	-0.052*	0.121**	2.012**
20	5x7	0.039	4.694**	2.291**	2.716**	11.182**	-1.046**	-6.531**	12.907*	-0.050	-0.055*	0.512**	2.466**
21	6x7	-0.194	17.627**	-0.753**	0.263	-3.704**	-0.935**	-3.485**	42.056**	0.692**	-0.021	0.148**	-2.714**
	SE(sij)	0.117	0.686	0.287	0.187	0.209	0.242	0.483	6.344	0.021	0.021	0.029	0.130

1:Erramallelu, 2: IR 72, 3: PR 164, 4: Samba Mahsuri, 5: Swarnamukhi, 6: Bharani, 7:NLR 33637

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Development of gain of function mutation in rice by tetrad enhancer elements of Cauliflower Mosaic Virus 35S promoter

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Abstract

Rice is a primary staple crop for majority of world's population, yet the crop is prone to various biotic and abiotic stresses. Combating these stresses require novel strategies especially for those traits where resistance is not available in the gene pool. Activation tagging (AT), involving random insertion of enhancer elements, is one of the methods to generate mutant lines enhanced for multiple traits. An attempt has been made to develop AT lines in rice by using activation-tagging T-DNA vector (pDEB) carrying a non-autonomous *Dissociation* (*Ds*) element having 4X *CaMV* 35S enhancers and bar gene is used as a plant selection marker. Transformation of popular indica rice cultivar BPT5204 was done using *Agrobacterium*. The putative transformants were selected on (Murashige and Skoog) medium containing phosphinothricin (PPT). The tagged plants (T_1 and T_2) were confirmed by PCR with enhancer and bar gene specific primers. These tagged homozygous plants were advanced to identification of mutants with gain of function for important traits.

Key words: Activation tagging, mutant lines, *Ds* element.

Introduction

Cultivation of high yielding rice varieties and improved cultural practices have imparted to global increase in rice production. The current scenario demands increase in rice production almost to double by the mid-21st century to fulfill the needs of the exponentially increasing world population, expected to cross the mark of 7.7 billion by 2020 (http://www.worldometers.info/).

The main constraints dampening the efforts of improving rice productivity by biotic and abiotic stresses include outbreak of diseases due to infection by various pests, salinity stress, heat stress, scarcity of water, poor irrigation habits, inferior soil quality (Allara et. al., 2012; Deepa Sankar et. al., 2011; Redman et. al., 2011; Peng et. al., 2004).

Rice plant is model crop for functional genomics. Because of its small genome size and genomic resources eventually guided its way to be the first monocot species to undergo whole genome sequence. The release of rice genome sequence has made way to the identification of gene functions (Li et. al., 2018; Zhang et. al., 2017; Singh et. al., 2016; Kim et. al., 2014). Mutant population serves as essential tools for analyzing plant gene functions. Mutants can be generated by chemical, physical as well as biological methods such as ethyl methanesulfonate application, fast neutron irradiation, T-DNA and transposon insertion (Shuyan Wan et. al., 2009). However, these approaches usually cause loss-of-function mutations and not applicable to dissecting the function of redundant genes. Also, it is difficult to conclude the gene function if loss of function mutation results in early embryogenic or gametophytic lethality owing to the involvement of the genes in multiple stages of life cycle (Jeong et. al., 2002; Weigel et. al., 2000).

Out of the various strategies which have surfaced to deal with the aforesaid shortcomings, the enhanced expression of genes through activation tagging system, which provides gain-of-function phenotype, has proved to be a productive identification strategy. *Ds* element has no transposition activity because of absence of transposase enzyme. *Ds* elements are internal deletion derivatives of the *Ac* element. The first ever direct gain-of-function mutation in plants, utilized the enhancer element from

the cauliflower mosaic virus (CaMV) 35S gene (Odell et. al., 1985). T-DNA vectors bearing four copies of this CaMV 35S enhancer element were utilized successfully for generation of activation-tagged lines and mediating transcriptional activation of nearby genes in Arabidopsis and identify a number of novel functional genes (Borevitz et. al., 2000; Li et. al., 2001, 2002; Neff et. al., 1999). This technology is being deployed in diverse plant species such as petunia (Zubko et. al., 2002); tomato (Mathews et. al., 2003) and rice (Jeong et. al., 2002). In this study, an attempt was made towards development of activation tagged lines in elite indica rice variety BPT 5204 by deploying the *Agrobacterium* mediated transformation.

Materials and Methods

Plant materials: Mature seeds of popular indica rice cultivar BPT 5204 were obtained from the Seed Research and Technology Centre (SRTC), Professor Jayashankar Telangana State Agricultural University (PJTSAU), Rajendranagar, Hyderabad.

Transformation vectors: The Activation Tagging vector pDEB was provided by Dr. K. V. Rao (Centre for Plant Molecular Biology Osmania University, Hyderabad). The pDEB vector carried a non-autonomous *Dissociator* (*Ds*) element, bar gene and tetramer of the transcriptional enhancer of *CaMV* 35S (4XEn) were located between the 5′ and 3′ *Ds* termini. The T-DNA was in the back bone of pCAMBIA-2300 (Fig.1).

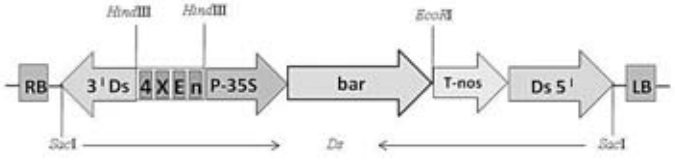

Figure 1: T-DNA of the activation tagging vector pDEB.
RB and LB, Right and Left borders of the T-DNA; Bar gene is selection marker for *Ds* element; 4x En, a tetramer of CaMV35S enhancers; *Hind*III, *E.co*RI and *Sac*I are restriction sites. The T-DNA was in the back bone of pCAMBIA-2300.

Plant Transformation: Regeneration and *Agrobacterium* mediated genetic transformation protocols were described by Manimaran et al., 2013 with some modifications. Mature seeds of BPT5204 were cultured on MS medium supplemented with 2 mg L^{-1} 2,4-D and 0.5 mg L^{-1} Kinetin for highest callus induction. Proliferated calli was transferred onto MS medium containing 4 mg L^{-1} ABA which produced actively growing embryogenic calli which were used as explants for transformation. After co-cultivation, the calli were rinsed in cefotoxime (250 mg L^{-1}) for 5 min followed by 4 washes with sterile distilled water to remove excess of *Agrobacterium* from calli. Then calli were blot dried and transferred on to MS medium supplemented with 5 mg L^{-1} PPT. After one week, surviving calli were further transferred to regeneration medium (MS containing 2 mg L^{-1} BAP) under light for 2 to 3 weeks which lead to induction of shoots. Shoots were sub-cultured again in the same medium and sufficiently grown shoots transferred to rooting medium (1/2 MS basal salt, 15 g L^{-1} sucrose) to induce roots. Fully rooted plants were transferred to trays contain sterile vermiculate for two weeks, then these plants were transferred to transgenic greenhouse.

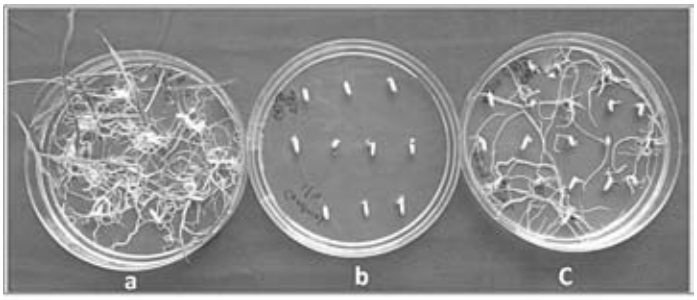

Figure 2: Putative transgenic seeds on selection media containing PPT 5 mg L^{-1} concentration

a. Untransformed control seed without PPT, b. Untransformed control seed with PPT, C. Putative transgenic seed with PPT.

DNA isolation and PCR analysis of transformed plants: Genomic DNA was extracted from young leaves of putative transformants and untransformed control rice plants by using CTAB method (Doyle and Doyle, 1990). The DNA was later analyzed by PCR with the gene specific primers. The set of primers for enhancer (En) element and bar gene were; enhancer: En F 5' CAAAGGGTAATATCGGGAAACC 3' and En R 5' TCACATCAATCCACTTGCTT 3' bar F: 5' CGAGACAAGCACGGTCAACTTC 3' and bar R: 5' AACCCACGTCATGCCAGTTC 3'. PCR reaction was set up a total volume of 15 µl containing, 1x PCR Buffer, 0.25 mM dNTPs each, 2.5 mM $MgCl_2$, 0.1 µM forward primer, 0.1 µM reverse primer, 1U *Taq* DNA polymerase and 2 µl of template DNA (50 ng). Thermal cycling conditions were: initial denaturation at 94°C for 5 min then 35 cycles of denaturation at 94°C for 30 sec, annealing at 58° C for 30 sec, extension at 72°C for 1 min and final extension at 72°C for 7 min. The plasmid DNA was used as positive control and non transformed rice plant genomic DNA was used as negative control for the PCR reaction. The PCR amplified products were electrophoresed on 1.5% agarose gel and analyzed on Gel Documentation system (Bio-RAD).

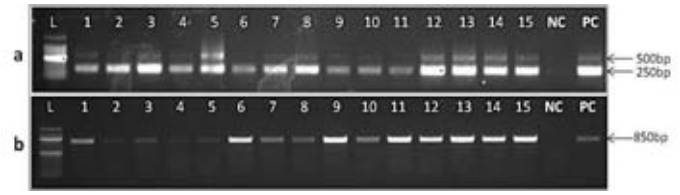

Figure 3: Molecular confirmations of putative transgenic plants.

a. Confirmation of 4XEn in lanes 1-15 plants with en F and R primer, b. Confirmation of bar gene in lanes 1-15 plants with bar F and Nos R primer, PC; Positive control (pDEB plasmid), NC; Negative control (untransformed BPT 5204).

Phenotypic evolution of mutated lines: Approximately 700 transformed tagged plants (T_1) were used to screen the phenotypic characteristics. The data was recorded systematically for all these T_1 transformed lines. These transformed lines were separated into different categories based on phenotypic observations like plant height, number of tillers, leaf length, leaf width, heading date and variations in root length. From each T_1 mutated tagged lines, 10 seeds were propagated for T_2 generation and selected for phenotypic similarities with previous generation. The selected similar T_1 and T_2 phenotypic mutants were further propagated to next generation.

Results

Generation of activation tagged lines

More than 3000 embryogenic calli were co-cultivated with *Agrobacterium (*strain, *EHA*105) containing DEB vector. After co-cultivation, all these calli were subjected to selection medium supplemented with 5 mg L^{-1} PPT. After repeated subculture, Approximately 700 plantlets (T_0) were produced after survived on plant selection medium supplemented with 5 mg L^{-1} PPT. Among them, only 165 plants had shown expected PCR amplification with gene specific primers of bar gene and enhancer element. From the selected PCR positive T_0 plants, 20 seeds (T_1) were subjected to selection medium containing 5 mg L^{-1} PPT (Table.1). Out of 165 lines only 105 lines shown Mendelian segregation ratio of 3:1. All survived plants were transfer to transgenic green house out of these only 66 lines shown the expected amplicon size. All the PCR positive transgenic plants were maintained in transgenic green house and phenotypic variations was recorded followed by seeds were collected from these positive transformants. 20 seeds from each 66 lines were subjected to selection medium containing 5 mg L^{-1} PPT and these plants were maintained in transgenic green house followed by observing phenotypic similarities with previous generations.

Table 1: Transformation efficiency based on PPT selection and PCR confirmation

Vector used for transformation	Generation	No. of calli co-cultivated	No. of explants survived on PPT selection media	No. of calli regenerated	No. of plants produced in regenerated media	No. of plants shows PCR +ve
pDEB	T_0	3,000 (calli)	700	490	680	165
	T_1	3,300 (seed)	480	-	480	66
	T_2	660 (seed)	220	-	220	38

Phenotypic evolution of mutated tagged population

The phenotypic evolutions of tagged lines were screened based on different morphological characteristics. For this study we used approximately 700 putative T_0 transformed plants and these plants were phenotypic screened for further two generations (T_1 and T_2). The frequency of the phenotype being carried to next generation (T_2) was very low in T_1 mutated lines. Mutated lines of STD3, STD8, STD17 and STD23 showed a dominant plant height ranging from 72.37-82.27 cms with productive tillers ranging from 13-17.67 cms compared to untransformed control plants (Table. 2). These mutated lines are valuable resources for functional genomics studies.

Table 2: Phenotypic data of mutated tagged lines

Tagged line	Plant height (cm)				No. of productive tillers			
	T_1		T_2		T_1		T_2	
	UC (mean±SE)	ML (mean±SE)	UC (mean±SE)	ML (mean±SE)	UC (mean±SE)	ML (mean±SE)	UC (mean±SE)	ML (mean±SE)
SDT 3	71.5±0.87	72.36±0.88	73.9±1.02	76.66±0.67	9.66±0.33	14.33±0.88	11±0.58	16.66±0.88
SDT 8	72.8±0.12	76.73±2.26	75.13±0.64	82.26±0.67	9.00±0.58	13.66±0.88	10±0.58	17±0.58
SDT 17	75.46±0.66	80.96±0.90	76±0.23	81.5±0.71	10.33±0.88	16.66±0.88	12±0.58	17.33±0.88
SDT 23	75.6±1.44	77.33±0.88	76.83±1.20	79.16±0.44	11±0.58	14±0.58	10±0.58	13±0.58

UC: untransformed control, ML: mutated line, T_1 and T_2: first and second generations. The data was considered statistically significant at $P< 0.05$ using one way ANOVA.

Discussion

The present study utilized an activation tagging vector pDEB in which a T-DNA carries a *Ds* element having a 4X *CaMV* enhancers, while the bar gene act as selectable marker. Even though large T-DNA insertion libraries have been generated in rice, effective T-DNA transformation system is present only for japonica subspecies (Hiei et. al., 1994). However difficulties are encountered during T-DNA transformation of the widely cultivated indica rice subspecies (Lin et. al., 2005). Nevertheless, transposon mutagenesis is a useful approach for plant functional genomics (Qu et. al., 2008). In T-DNA vector, *Ds* element carries four copies of *CaMV*35S enhancer elements. These tetrameric enhancers could mediate the activation of genes by interacting with their transcriptional factors. The activation of genes is based on the proximity of genes to transgene insertion site. Previous reports studied the activation of genes in different genetic distances (7 kb and 12.5 kb) from the insertion site (Hsing et. al., 2007, Wan et. al., 2009). The plants generated by using this vector were stable, because no transposition occurred in *Ds* element.

Some of these transformants showed phenotypic variations compared to the untransformed plants. These variations would be further confirmed for inheritance by studying these traits in further generations. We observe more number of phenotypic mutants in T_1 transformants like plant morphology, leaf morphology, root length difference, seed morphology, number of productive tillers and number of days to flowering. Among these phenotypic mutants plant height and number of productive tiller mutants were inherent to further generation. Some studies reported that phenotypic variation in activation tagged lines of javanica rice (Fladung et. al., 2012), japonica rice (Yang et al., 2013) and indica rice (Reddy et. al., 2018). Zhu and co-workers (2017) studied mutant phenotypes and identified a new uncharacterized gene involvement in spikelet heading date in rice. Dominant mutants were observed at a frequency of 0.3% (Tani et. al., 2004). Similar percentage of dominant mutations was observed in our study. Among all, four mutant lines showed dominant mutations in T_1 and T_2 generations. These mutant lines were confirmed by PPT selection and also positive results were obtained in PCR with transgene specific primers. These AT mutant lines developed in this study would be propagated further for screening of important traits. AT lines with enhancement in specific traits of importance could be a storehouse of vast and valuable informations which will be useful to the rice growers and researchers.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Effect of post- emergence herbicides after pre-emergence application of Pendimethalin in aerobic rice (*Oryza sativa* L) in north-western himalayas

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Abstract

Benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methxyphenyl)-5-fluoropyridine-2-carboxylate is a new arylpiconilate systemic broad spectrum post emergence herbicide with trade name Rinskor, is mainly absorbed by foliage, translocated through the xylem and phloem tissues to meristemetic regions where it exhibits unique molecular interaction with auxin receptors and thereby killing weeds. A field experiment was conducted at CSK HP Krishi Vishvavidyalaya Rice and Wheat Research Centre, Malan under All India Co-ordinated Rice Improvement Project during *kharif* 2016 to find out the bio-efficacy of post emergence herbicide Rinskor 2.5% EC after pre-emergence application of Pendimethalin in aerobic rice (sowing of seeds in well aerated soil at optimum moisture conditions and maintaining soil moisture near to field capacity). Treatments comprised of pre-emergence herbicide Pendimethalin 30% EC followed by (*fb*) post-emergence herbicide Rinskor 2.5% EC (750 *fb* 31.25 g a.i/ha), Pendimethalin 30% *fb* Rinskor 2.5% EC (750 *fb* 37.5 g a.i/ha), Pendimethalin 30% followed by Bispyribacsodium 10 SC (750 *fb* 30 g a.i./ha) and Pendimethalin 30% EC followed by Metsulfuronmethyl + Chlorimuronehtyl 20% WP (750 *fb* 4 g a.i./ha) along with weed free and un-weeded control. Rinskor was applied at 4-7 leaf stage of weeds & Bispyribacsodium & Metsulfuronmethyl+chlorimuronethyl at 3-4 leaf stage of weeds. Reduction in dry matter and density of weeds was observed by the herbicides applied. The density and dry matter of weeds in weed free condition was statistically at par with pre emergence application of Pendimethalin fb Rinskor @ 37.50 g or 31.25 g/ha. Rinskor is safe to the rice crop. Weedy check recorded significantly poor growth (plant height) yield attributes (panicles per unit area), panicle weight and thus, on an average 121.4 per cent increase in yield was recorded due to weed control treatments; highest being with Pendimethalin *fb* Rinskor 37.50 g/ha (139.2%). Post emergence application of Rinskor (3.66 Mg/ha) and Bispyribac sodium (3.56 Mg/ha) recorded statistically equal productivity and yield attributes. Thus, pre-emergence application of Pendimethalin 30% EC followed by Rinskor 2.5% EC @ 37.5 g/ha 4-7 leaf stage of weeds is efficient for control of mixed weed flora in aerobic rice.

Key words: Herbicides, weed management, aerobic rice, pre-emergence, post-emergence

Introduction

In the present scenario of increasing water scarcity, aerobic rice is the one of the contingent crop production systems. Weed menace is the major biological constraint in aerobic rice cultivation causing huge grain yield losses up to 50 to 90 per cent. Meager information is available on the weed management practices and weed-crop dynamics, which influence grain yield, energy use pattern in aerobic rice cultivation. Sharma *et al*. (2016) have reported huge losses in terms of productivity and nutrients. Rice is the second most important crop in Himachal Pradesh and is grown under various topo-sequences at various elevations (300 to 2200 m above mean sea level. Of late, the increased emphasis is being laid on the use of low dose high efficacy herbicides capable of controlling diverse weed flora (Shekhar *et al*., 2004). Continuous application of same herbicide leads to shift in weed flora and development of resistance to herbicides (Singh *et al*, 2013). Keeping these in view, the present investigation was undertaken to find the bio-efficacy of new herbicide in sequential application after Pendimathalin in aerobic rice. Benzyl 4-amino -3-chloro -6- (4-chloro-2-fluoro-3-methxyphenyl) -5-fluoropyridine -2-carboxylate is a new arylpiconilate systemic broad spectrum post emergence herbicide with trade name Rinskor. It is new arylpiconilate systemic broad spectrum post emergence herbicide mainly absorbed by foliage, translocated through the xylem and phloem tissues to meristemetic regions where it exhibits unique molecular interaction with auxin receptors and thereby is lethal to weeds.

Materials and Methods

A field experiment was conducted in *kharif* 2016 at CSK Himachal Pradesh Krishi Vishvavidyalaya Rice and Wheat Research Centre, Malan located at 76°2'E, 32°1' N and 950 m above mean sea level. Pre-emergence herbicide Pendimethalin 30% EC followed by (*fb*) post-emergence herbicide Rinskor 2.5% EC (750 *fb* 31.25 g a.i/ha), Pendimethalin 30% *fb* Rinskor 2.5% EC (750 fb 37.5 g a.i/ha) was evaluated against Pendimethalin 30% followed by Bispyribacsodium 10 SC (750 fb 30 g a.i./ha) and Pendimethalin 30% EC followed by Metsulfuronmethyl + Chlorimuronehtyl 20% WP (750 fb 4 g a.i./ha) treatments were tested along with weed free (three hand weedings at 20, 40 & 60 days after sowing- DAS), two hand weeding (20 & 40 DAS) and weedy check. The treatments were tested in randomized block design replicated thrice.

The soil of the experimental site was salty clay loam in texture, acidic (pH 5.7) in reaction and medium in available nitrogen (296 kg/ha), phosphorus (29 kg/ha), potash (246 kg/ha) and organic carbon. Rice cultivar 'HPR 1068' was sown in lines 20cm apart using 60 kg seed/ha, on June 21, 2016. Basal application of 45 kg N (through neem coated urea), 40 kg P_2O_5 (through 16%SSP) and 40 kg K_2O (through 60%MOP) was done and 45 kg N was top dressed in two equal splits at maximum tillering and panicle initiation stages. In 64 rainy days from June to October, a total of 1716.8 mm rainfall occurred; supplementary irrigations were given whenever required to keep the soil near full saturation. The monthly ambient temperature varied from 8.5 - 13.1 (minimum temp.) to 28.6-33.4 (maximum temp.) during June to October. The herbicides were applied with knapsack sprayer with flat fan nozzle using 750 L water / ha. The data on weed density and dry matter were recorded at flowering stage of crop. Weed index was calculated as percentage increase in yield over weedy check. Herbicide toxicity rating was done on 1-10 scale.

Results and Discussion

The major weed flora comprised of *Digitaria sanguinalis, Eleusine indica, Paspalum paspalodes, Setaria glauca, Aeschynomene indica, Echinochloa colona, E. crusgalli, Phyllanthus niruri, Commelina benghalensis, Monochoria vaginalis.* Weed density and biomass of grasses & broadleaved weeds were varied significantly by the weed control treatments (Table 1). Reduction in dry matter and density of grass and broad leaf weeds was observed by the herbicides applied (Table 1).The density and dry matter of weeds in weed free condition (weeding at 20, 40 & 60 DAS) was statistically at par with pre emergence application of Pendimethalin *fb* Rinskor @ 37.50 g or 31.25 g/ha 4-7 leaf stage of weeds. Pendimethalin 30% followed by Metasulfuronmethyl + Chlorimuronethyl -20%WP was observed to control more of broad leaves and recorded less dry weight of broadleaf weeds whereas this treatment controlled less of grasses. All the herbicides were observed to be safe to the crop (toxicity rating 1-10 scale).

Different weed control treatments had significant effect on weeds and the same was reflected in the growth of crop (plant height), panicle length, number of panicles per unit area, panicle weight (Table 2). Significantly lower values of these parameters were observed in weedy , on an average 121.4 per cent increase in yield was recorded due to weed control treatments. Plants were observed to be taller with pre-emergence application of Pendimethalin 30% followed by Metsulfuronmethyl + Chlorimuronethyl -20%WP, followed by the treatment Pendimethalin 30% *fb* Rinskor 2.5% EC (750 *fb* 37.5 or 31.25 g.a.i/ha). All the four herbicide treatments recorded number of panicles, panicle weight at par with weed free treatment. After pre emergence application of Pendimethalin, post emergence application of Rinskor or Bispyribac sodium recorded grain productivity at par with weed free condition. Significant variation in 1000 seed weight of the crop was not observed by weed control treatments. Weed index i.e. increase in yield over the weedy check by different treatments varied from 94.8 to 139.2 per cent; highest being with Pendimethalin *fb* Rinskor 37.50 g/ha. Similar results have been reported by Anonymous (2016).

Conclusion

Pre-emergence application of Pendimethalin 30% 750 g a.i./ ha followed by Benzyl 4-amino-3-chloro-6-(4-chloro-2-fluoro-3-methxyphenyl)-5-fluoropyridine-2-carboxylate with trade name Rinskor 2.5% EC @ 37.5 g/ha 4-7 leaf stage of weeds provides efficient control of mixed weed flora in aerobic rice in mid hill conditions of Himachal Pradesh.

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Table 1. Effect of different treatments on weed density and dry matter in aerobic rice

Treatment	Time of application	Weed density (No./m²)		Weed dry matter at flowering (g/m²)		Herbicide toxicity
		Grass	BLW	Grass	BLW	
Pendimethalin 30% *fb* Rinskor 2.5% EC (750 *fb* 31.25 g.a.i/ha)	Within 2 days of sowing (DAS) *fb* 4-7 leaf stage of weeds	8.11 (65.33)	8.20 (68.00)	11.29	15.84	1
Pendimethalin 30% *fb* Rinskor 2.5% EC (750 *fb* 37.5 g.a.i/ha)	With in 2 DAS *fb* 4-7 leaf stage of weeds	8.39 (72.00)	7.67 (58.67)	10.34	15.14	1
Pendimethalin 30% *fb* Bispyribacsodium 10SC (700 *fb* 30)	Within 2 DAS *fb* 3-4 leaf stage of weeds	10.15 (102.67)	6.64 (44.00)	18.79	12.87	1
Pendimethalin 30% *fb* Metsulfuronmethyl + Chlorimuronethyl -20%WP (750 *fb* 30)	Within 2 DAS *fb* 3-4 leaf stage of weeds	8.91 (81.33)	8.56 (73.33)	17.03	14.39	1
Weed free Condition	Weedings at 20, 40, 60 DAS	6.80 (46.67)	4.90 (24.00)	8.8	13.79	
Hand weeding twice	Weedings at 20,40 DAS	10.85 (118.67)	8.96 (81.33)	12.68	23.55	
Weedy Check		12.43 (154.67)	9.80 (96.00)	26.87	35.72	
CD(0.05)		2.45	1.59	5.74	1.23	-

fb = followed by; Figures in parentheses are means of original values

Table 2. Effect of treatments on yield attributes and yield of aerobic rice

Treatment	Time of application	Plant height (cm)	Panicle (No./m²)	Panicle length (cm)	Panicle weight (g)	Test wt. (g)	Grain yield (Mg/ha)	Straw yield (Mg/ha)	Weed index
Pendimethalin 30% followed by (*fb*) Rinskor 2.5% EC (750 *fb* 31.25 g.a.i/ha)	Within 2 days of sowing (DAS) *fb* 4-7 leaf stage of weeds	72.3	343	18.7	1.92	28.07	3.48	4.03	127.4
Pendimethalin 30% *fb* Rinskor 2.5% EC (750 *fb* 37.5 g.a.i/ha)	Within 2 DAS *fb* 4-7 leaf stage of weeds	72.6	342	18.4	1.86	28.38	3.66	4.23	139.2
Pendimethalin 30% *fb* Bispyribacsodium 10SC (750 *fb* 30)	Within 2 DAS *fb* 3-4 leaf stage of weeds	74.7	331	18.6	1.82	28.71	3.56	4.22	132.7
Pendimethalin 30% *fb* Metasulfuronmethyl + Chlorimuronethyl -20%WP (750 *fb* 30)	Within 2 DAS *fb* 3-4 leaf stage of weeds	71.3	367	17.4	1.79	28.57	3.29	3.96	115.3
Weed free Condition	Weedings at 20, 40, 60 DAS	71.3	333	17.8	1.83	28.8	3.35	3.95	118.9
Hand weeding twice	Weedings at 20,40 DAS	71.2	287	18.0	1.81	28.76	2.98	3.45	94.8
Weedy Check		70.5	217	16.8	1.45	27.78	1.53	2.45	-
CD(0.05)		0.7	66.9	0.9	0.16	NS	0.35	0.47	

fb = followed by

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Evaluation of antagonistic fluorescent pseudomonads for the suppression of bacterial blight of rice

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Abstract

Rice (*Oryza sativa*) is the most widely cultivated food crop in the world. India has the largest area under rice crop and ranks second in production after the China. In India, bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is considered as a serious production limiting factor especially in irrigated and rainfed lowland ecosystem. In the absence of highly effective chemicals against BB, biological management provides an alternative approach. In the present study, we isolated 21 strains of antagonistic fluorescent pseudomonads (FP) from rice rhizospheric soils, farm yard manure and vermicompost. All the isolates were biochemically tested and identity confirmed. All the FP strains except FP-3, FP-13, FP-14 and FP-15 tested positive for HCN production. Among the positive isolates, FP-18, FP-19, FP-20 and FP-21 were comparatively strong producers of HCN. Volatile metabolites produced by different FP isolates strongly inhibited the growth of *Xoo*. The strains like FP-2, FP-4, FP-5 and FP-14 were most effective in inhibiting the growth of *Xoo*. *In vitro* antagonistic activity of FP strains showed positive zone of inhibition ranging from 1.47 cm with FP-14 to 6.80 cm with FP-8. The FP strains like FP-6, FP-8, FP-13, FP-17, FP-19 and FP-21 were highly promising with inhibition zone ranging from 3.83-6.8 cm. These selected five strains significantly suppressed the BB disease severity under glasshouse conditions. Among the strains, FP-13 was the most effective and reduced the BB lesion length by 57%.

Key words: Rice, bacterial blight, biological control and fluorescent pseudomonads

Introduction

Rice (*Oryza sativa*) is the most widely cultivated food crop in the world, feeding about half of humanity with a worldwide production of 470.63 million metric tons from an area of 157.46 million hectares (Annonymous, 2016). India has the largest area under rice crop and ranks second in production after China. Rice forms an important part of diet of Indian population. Rice contributes 43 percent of total food grain production and 46 per cent of total cereal production. Rice is highly vulnerable to different biotic stresses that affect its quality and yield. Among different biotic stresses, bacterial blight (BB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) is the most important one in different rice producing countries in Asia (Nayak *et al*., 2008; Sudir and Yuliani, 2016). BB is known to occur in epidemic proportions in many parts of the world, causing 6-81% yield loss in some rice varieties (Win *et al*., 2013; Yugander *et al*., 2017). In India, BB is considered a serious production limiting factor especially in irrigated and rainfed lowland ecosystem. Generally, the stage between maximum tillering and booting is highly sensitive to disease infection, as it significantly affects grain filling and total yield. BB management strategy, such as use of resistant cultivars, is the most economical strategy for disease management, although there has been only partial success because of an enormous diversity in the pathogen. In spite of extensive trials conducted, chemical management of the disease has not been very successful.

Bio-control assumes a special significance in being an ecologically conscious, environmentally safe and cost-effective alternative strategy for management of plant diseases, without the negative effect of synthetic chemicals that can cause environmental pollution and induce pathogen resistance in some cases. This approach can be integrated with other strategies to afford greater levels of protection and sustain rice yields. Fluorescent pseudomonads (FPs) are considered as potential biological control agents because of production of diverse secondary metabolites

and their ability to show 'induced systemic resistance (ISR) (Whipps *et al*., 2001; Velusamy *et al*., 2006). FPs exhibit diverse mechanisms of bio-control which include antibiosis, cyanide production, siderophore production, competition for space and nutrient and induced systemic resistance. Certain strains of FPs have been used as bio-control agents to suppress rice BB (Vasudevan *et al*., 2002). In the present study, we studied the efficacy of FPs isolated from rice rhizosphere and other sources against BB disease of rice.

Material and methods

The study was carried out at ICAR-Indian Institute of Rice Research (ICAR-IIRR), Hyderabad, India. Rice rhizosphere soil samples were collected from different rice growing fields from Telangana. In addition to rice rhizosphere, farm yard manure and vermicompost was also used for isolation of FPs. All samples were properly labeled after collection and stored at 4°C till bacterial strains were isolated.

Isolation of FPs and culture conditions: Isolation of FPs was carried out by serial dilution method. One gram of each sample was added in a 100 ml capacity conical flask containing 10 ml of sterile distilled water and constantly shaken in an orbital shaker. Each sample suspension was then serially diluted up to 10^{-4} and an aliquot of 100 µl of each sample of the highest dilution was spread on a King's B (KB) medium plate using a sterilized glass spreader and the plates were incubated at 28±2°C for 3-4 days. Well separated colonies of FPs showing typical yellowish green pigment around the colony were picked up and further purified and then sub-cultured on King's B agar slants. A total of 21 FPs colonies were picked up from different soil/FYM/vermicompost samples and were coded by putting a prefix FP-1 to FP-21 (Table-1). The isolates were maintained in 15% glycerol at -80°C for long term storage and in the King's B agar slants at 4°C for short term storage.

In-vitro screening of FPs for antagonistic activity: All the FP isolates were screened for their antagonistic activity against *Xoo* by double layer technique. Each FP isolate was spot inoculated in the centre of freshly prepared King's B agar plate and the plates were incubated at 28±2°C for 3 days. The bacteria were then killed by chloroform vapor (by adding 1 ml chloroform in the upper lid after inverting the plate) in a laminar air flow for 2 hours. The plates were then kept open in the laminar air flow for 10 minutes to allow the chloroform vapor to evaporate completely. A suspension (~10^6 cfu/ml) was made by mixing a 3-day old culture of *Xoo* (strain IX-133) with 5 ml sterile distilled water under a laminar air flow chamber and then this suspension was mixed with melted and cool soft modified Wakimoto's agar (MWA) medium (0.4% agar). This soft medium with *Xoo* culture was then gently poured on the King's B plate having the dead FPs colony. The plates were allowed to solidify under the laminar air flow chamber and then incubated at 28±2°C. After two days of incubation, zone of growth inhibition of *Xoo* around the FPs colony was measured. The data were analyzed statistically following Gomez and Gomez (1984).

Biochemical characterization of FPs: The FP isolates were characterized by performing standard biochemical tests following Schaad *et al*. (2003). Different biochemical testes viz., Gram test/KOH test, production of green fluorescent pigment on King's B medium (King *et al*.1954), gelatin liquefaction, arginine dihydrolase, oxidase, and growth at 45°C were conducted following Fahy and Persley (1983) and Schaad (2003).

Hydrogen Cyanide (HCN) production by FPs: HCN production by FP isolates was tested following the method described by Lorck (1948). FP isolates were inoculated by streaking on freshly prepared King's B agar medium plates supplemented with the glycine amino acid (4.4 g/liter of medium). Simultaneously, a strip of sterilized Whatman filter paper No.1 impregnated with alkaline Picric acid (yellow) solution [0.5% Picric acid (w/v) in 2.0% Sodium carbonate] was placed in the upper lid of the inoculated petri plates under aseptic condition. Petri plates were sealed with petri seal or parafilm and incubated at 28±2°C for four days. The un-inoculated plates served as control. A change in strip color from yellow to light brown (+), moderate brown (++) or strong reddish brown (+++) was taken as indication of HCN production.

Effect of volatiles produced by FPs on the growth of *Xoo:* The inhibitory effect of volatile compounds produced by FP isolates on the growth of *Xoo* was performed by the paired petri plate technique using two different culture media. Two culture media *viz*., King's B medium (with and without glycine) and MWA was separately poured in sterile petri plates in a laminar air flow chamber. When the culture media got solidified, the MWA plate was streak inoculated with *Xoo* (strain IX-133) and the King's B medium plates with respective FP isolates. Then bottom Petri plate half with *Xoo* (upper) was face to face paired with bottom Petri

plate half with FP isolate (lower) and then sealed with petri seal or parafilm to prevent the leakage of volatiles from the plates and incubated at 28 ± 2°C for four days. Observations were taken visually on the suppression of growth of *Xoo*.

Suppression of BB under glass house condition: Five selected strains of FP which showed maximum inhibition zone against *Xoo* under *in vitro* tests, were evaluated for their disease suppression ability under glasshouse condition. A combination of seed treatment and foliar spraying with respective FPs was done to study the disease suppressing ability of the FPs. Selected antagonistic FPs (FP # -8, 13, 17, 19 and 21) were mass multiplied in King's B broth at 28 ± 2°C for 48 hrs in an incubator shaker. In a sterilized glass beaker, 10 ml bacterial culture broth, 2 g commercial talcum powder, 0.5 g gum acacia and 0.5 g carboxy methyl cellulose (Sodium salt) were added and mixed thoroughly. About 15 g seeds of BB susceptible rice variety, TN1 was added to this mixture and seeds were thoroughly coated with bacterial culture. The bacterized seeds were then dried under shade for 12 hours and then used for sowing. TN1 seeds coated with selected FP isolates were directly sown in earthen pots filled with a mixture of field soil and farm yard manure (3:1). For each isolate, three replications were maintained. Standard agronomic practices were followed to raise the plants. The plants at 35 days old stage were sprayed with respective FP isolate (0.5×10^7 cfu/ml). *Xoo* (strain IX-133) was multiplied in MWA culture medium as described by Yugander *et al*. (2017). The plants were clip-inoculated with a freshly grown *Xoo* culture suspension (~10^8 cfu/ml), 3 days after antagonist spray. The plants were again sprayed with the respective FP isolate, 3 days after inoculation with BB pathogen. Observations were recorded by measuring the lesion length of bacterial blight disease after 15 days of inoculation.

Results and discussion

Twenty one FP strains were isolated from various sources. These included 12 isolates from farm yard manure, 6 isolates from rice rhizosphere and 3 isolates from vermicompost. The isolates were designated as FP # 1-21. All the FP isolates were gram negative, produced typical yellowish green fluorescent pigment on King's B agar medium and were positive for oxidase, arginine dihydrolase (except one strain) and gelatin liquefaction. None of the FP strains could grow at 45°C. The strain FP-3 showed negative reaction for gelatin liquefaction. Among the common plant associated FPs, *Pseudomonas fluorescens* is known to be positive for gelatin liquefaction while *Pseudomonas putida* shows negative for gelatin liquefaction (Schaad *et al*. 2003). Five FP strains (FP # 17-21) showed comparatively higher gelatin liquefaction ability. The results of various biochemical tests conducted confirmed the identity of these bacteria as fluorescent *Pseudomonas* spp. strains.

In vitro antagonistic activity of FP strains

All the FP strains produced typical inhibition zone in dual culture plate against *Xoo* under *in vitro* condition. The diameter of inhibition zone ranged from 1.47 cm with FP-14 to 6.80 cm with FP-8 (Table 1). *In vitro* growth inhibition (%) ranged from 2.82 % (FP-14) to 57.22 % (FP-8) (Table 1). The FP strains like FP-6, FP-8, FP-13, FP-17, FP-19 and FP-21 were highly promising (inhibition zone ranging from 3.83-6.8 cm) with percentage of growth inhibition ranging from 18.15% to 57.22% (Table 1). The study revealed that different FP isolates have different capacities as biological weapons in inhibiting the *Xoo* strains. Use of bacterial bio-control agents to suppress plant diseases is very common. Jambhulkar and Sharma (2014) evaluated potential of *P. fluorescens* isolate RRb-11 against BB pathogen *Xoo* under *in vitro* and *in vivo* conditions. Similarly, Velusamy *et al*. (2013) and Yasmin *et al.* (2016) evaluated different rice rhizosphere associated antagonistic bacteria for plant growth promotion and BB disease suppression. Salaheddin *et al.* (2010) evaluated several fluorescent *Pseudomonas* spp. against *Xanthomonas axonopodis* pv. *malvacearum* causing cotton leaf blight under *in vitro* condition.

Hydrogen Cyanide (HCN) production by FPs

All the FP strains except FP # -3, 13, 14 and 15 tested positive for HCN production. Among the positive isolates, FP-18, FP-19, FP-20 and FP-21 were comparatively strong producer of HCN as indicated by change in color of the strips from yellow to reddish brown. Production of HCN is an important mechanism of many antagonistic fluorescent *Pseudomonas* spp. (Kumar *et al.,* 2012). HCN producing *Pseudomonas* strains effectively reduce or kill the plant pathogenic microorganism. Microbial cyanogenesis has been demonstrated only in a few species of bacteria in the genera *Chromobacterium* and *Pseudomonas* (Patty, 1921; Michaels and Corpe, 1965).

Effect of volatiles produced by FPs on the growth of *Xoo*

Volatile metabolites produced by different FP isolates strongly inhibited the growth of *Xoo* (Table 1). *Xoo* growth suppression was more when the King's B medium

was supplemented with glycine (4.4 g/l) indicating the enhanced production of HCN had more detrimental effect on the growth of *Xoo*. The strains like FP # 1, 6, 8, 13, 16, 17, 18, 19 and 20 showed stronger growth suppression when the King's B medium was supplemented with glycine (Table 1). On the other hand, strains like FP # 5 and 14 showed stronger growth suppression when cultured in King's B medium without glycine. Some of the strains like FP # 13, 14 and 15 which did not show the production of HCN, suppressed the *Xoo* growth to some extent indicating production of volatiles other than HCN (Table 1). The antagonistic activity of the selected bacterial isolates might be due to the production of HCN or synergistic interaction with other metabolites and it has been documented earlier that microorganisms showing the ability to produce HCN can be used as bio-control agents for the suppression of plant pathogens (Ramette *et al.*, 2003). Sarangi *et al.* (2010) reported production of different volatile compounds like nonanal, benzothiazole and 2-ethyl-1-hexanol as the primary mechanism of bio-control of *Sclerotinia sclerotiorum* in canola by *Pseudomonas chlororaphis* strain PA23.

Table 1: *In vitro* antagonistic effect of fluorescent Pseudomonad strains against *Xanthomonas oryzae* pv. *oryzae*

FP Isolates	Source	Mean Inhibition zone (cm) ± SE	Growth inhibition (%)	HCN production	*Xoo* growth suppression by volatiles of FPS (w/o glycine)	*Xoo* growth suppression by volatiles of FPS (with glycine)
FP-1	Rice rhizosphere	3.17 ± 0.18^{gh}	12.46(20.60) ^{gh}	+	-	+++
FP-2	Rice rhizosphere	3.40 ± 0.10 ^{fg}	14.30 (22.19) ^{fg}	+	+++	++
FP-3	Rice rhizosphere	3.03 ± 0.03 ^{h}	11.36 (19.69)^{h}	-	-	-
FP-4	Rice rhizosphere	1.83 ± 0.09 ^{j}	4.17 (11.75) ^{k}	+	+++	++
FP-5	Rice rhizosphere	4.17 (11.75)	8.81 (17.23) ^{ij}	+	+++	+
FP-6	Rice rhizosphere	3.83 ± 0.07 ^{de}	18.15 (25.20) ^{de}	+	+	+++
FP-7	FYM	3.63 ± 0.07 ^{ef}	16.31 (23.80) ^{ef}	+	+	+
FP-8	FYM	6.80 ± 0.23^{a}	57.22 (49.16) ^{a}	+	-	+++
FP-9	FYM	3.27 ± 0.03 ^{gh}	13.18 (21.27) ^{gh}	+	+	+
FP-10	Vermicompost	3.10 ± 0.12 ^{gh}	11.90 (20.14) ^{gh}	+	+	-
FP-11	Vermicompost	3.40 ± 0.06 ^{fg}	14.28 (22.19) ^{fg}	+	-	+
FP-12	Vermicompost	3.23 ± 0.03 ^{gh}	12.91 (21.05) ^{gh}	+	-	-
FP-13	FYM	4.03 ± 0.03 ^{D}	20.09 (26.62) ^{d}	-	+	++
FP-14	FYM	1.47 ± 0.26 ^{K}	2.82 (9.38) ^{k}	-	+++	-
FP-15	FYM	2.63 ± 0.07 ^{I}	8.57 (17.01) ^{j}	-	+	-
FP-16	FYM	3.00 ± 0.12 ^{h}	11.14 (19.47) ^{hi}	+	-	++
FP-17	FYM	4.47 ± 0.03 ^{C}	24.63 (29.74) ^{c}	++	+	+++
FP-18	FYM	2.67 ± 0.12 ^{i}	8.81 (17.23) ^{ij}	+++	-	+++
FP-19	FYM	3.83 ± 0.17 ^{de}	18.21 (25.21) ^{de}	+++	-	++
FP-20	FYM	2.47 ± 0.03 ^{I}	7.51 (15.90) ^{j}	+++	-	+++
FP-21	FYM	5.03 ± 0.03 ^{b}	31.28 (33.99) ^{b}	+++	-	+
CV (%)		6.60	5.89			
LSD (*P=0.05*)		2.43	0.33			

Data in parentheses are arc sine transformed values; means followed by a common letter in a column are not significantly different at 5% level by DMRT.

Suppression of BB under glasshouse condition

Based on the consistent performance in the laboratory tests, five FP isolates were selected for their efficacy to reduce BB disease severity under glasshouse condition. The results indicated that combined application of seed treatment as well as foliar spray with respective FP isolates significantly reduced the BB lesion length (5.33-7.00 cm) when compared to untreated control (12.33 cm) (Table 2).

Table 2: Efficacy of selected fluorescent pseudomonad strains against bacterial blight of rice under glasshouse condition

FP isolate	Mean lesion length (cm) ± SE
FP-13	5.33 ± 0.33
FP-8	7.00 ± 0.58
FP-21	5.67 ± 0.33
FP-17	6.33 ± 0.88
FP-19	5.67 ± 0.33
Un-inoculated control	12.33 ± 1.45
CV (%)	19.99
LSD (*P=0.05*)	2.57

Among the strains, FP-13 was most effective and reduced the lesion length by 57%. Seed treatment with some of these FP isolates, showed significant improvement in different plant growth parameters like germination (%), shoot length, root length and fresh plant weight when compared with the control treatment. Some of the promising FP strains which significantly improved different plant growth parameters were FP-8, FP-13, FP-17, FP-19 and FP-21. Management of plant diseases using antagonistic micro-organisms offers the best alternative to chemical control. Timely and augmented application of bio-agent is required for proper establishment on the plant surface. The combination of different application methods and different bio-agents has been advocated for better bio-control of plant diseases (Van Loon, 1998). As chemical control is not successful in managing bacterial blight of rice, bio-management of the disease offers an alternative approach. Many workers have used different bio-control agents for management of BB in rice. Vidhyasekaran *et al*. (2001) reported the effectiveness of selected *P. fluorescens* in the management of BB in rice. Kaur and Thind (2002) reported that seed bacteriazation followed by two foliar applications of antagonistic strains of *Pseudomonas fluorescens* can significantly reduce BB severity in both glasshouse and fields. Reduction of BB severity through seed bacterization and foliar spray has been reported by several workers (Shivalingaiah and Sateesh, 2012; Singh and Sinha, 2005; Sivamani *et al*., 1987). It can be concluded from this study that combined application of seed treatment and foliar application of selected fluorescent pseudomonads can significantly reduce the bacterial blight severity.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Characterization of native rice specific isolates of *Trichoderma* and evaluation of its effect on sheath blight pathogen *Rhizoctonia solani*

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Abstract

Trichoderma species is one of the most important antagonistic fungi commonly found in soil and is effective against many soil borne plant pathogens. It has been reported to improve plant growth and development in addition to its biocontrol effects on the pathogens. Sheath blight is one of the most economic important disease of rice causing significant yield loss. Biocontrol using *Trichoderma* spp. is reported to be a safe and effective alternate strategy to the use of fungicides which are harmful to the environment and consumers alike. About 4 native isolates of *Trichoderma* spp. were isolated from rhizospheric soil collected from different rice growing areas in India including the states of India viz., Telangana, Karnataka, Chhattisgarh, Jharkhand and Madhya Pradesh. The isolates were characterized both morphologically and by molecular means using ITS sequencing of *Trichoderma* specific 18s rRNAs.(NCBI ID2146474-IIRRCK1 to IIRRCK4) Accordingly the isolates were identified and the most potential isolate identified from the results was found to be *Trichoderma asperellum* based on the query score and similarity percentage. Results from the studies on the bio-efficacy of the isolates indicated that *T. asperellum* isolate IIRRCK1 was found to be the most effective in improving plant growth and high inhibition of *R.solani* (51.8%) under *in vitro* conditions.

Key words: *Trichoderma sp., Rice, Sheath blight, Rhizoctonia solani*

Introduction

Trichoderma is a genus of asexually reproducing fungi that are often the most frequently isolated soil fungi, from all temperate and tropical soils. *Trichoderma* strains may have one or all mechanisms of action according to species and strain. *Trichoderma* can offer several advantages over synthetic chemicals i.e., they are environment friendly, do not leave toxic residues, do not harm non-target friendly micro/macro flora and fauna and finally promote natural defense, growth and health of the host plants in addition to their biocontrol ability (Tahia et al., 2004).The mode of action of *Trichoderma* spp. include antibiosis, hyper-parasitic activity against pathogens and induction of systemic resistance in the host plants. *Trichoderma* strains produce a great variety of lytic enzymes most of which play a great role in their biocontrol activity against a broad spectrum of fungal pathogens *viz.,* species of *Rhizoctonia*, *Fusarium*, *Alternaria*, *Ustilago*, *Venturia* and *Colletotrichum* and the Oomycetes like *Pythium* and *Phytophthora* which lack chitin in their cell walls (Sriram et al., 2004). *Trichoderma* produces secondary metabolites and some of them are involved in the antibiosis activities against the pathogens. The secondary metabolites are classified into i) volatile antibiotics ii) water-soluble compounds, i.e. heptelidic acid or koningic acid and iii) peptaibols (Susanne et al., 2006). For successful; deployment, *Trichoderma* strains should be carefully chosen according to their mechanism of action and adaptation to the specific environment.

Rice (*Oryza sativa*) is India's most important cereal crops and is the staple food for more than 60 % of the world's population. Intense cultivation and adoption of semi-dwarf, early maturing and fertilizer responsive high-yielding rice varieties, coupled with increased fertilizer application have brought in the risk of increased incidence of pest and disease. Sheath blight disease caused by the fungus *Rhizoctonia solani* Khun, a soil borne saprophytic fungi, is one of the most destructive diseases of rice next to blast (Singh *et al*., 2003). The disease was first reported in Japan in 1910, later from all over the world (Prasad and Eizenga, 2008). The disease is severely endemic in areas where temperature and relative humidity are high and cultivation is intensive. The disease can cause yield losses up to 50

per cent in advanced stages and even adversely affects the quality of straw thereby limiting its value as fodder (Rajan, 1987). Results from several investigations have indicated that *Trichoderma* spp. are effective for control of sheath blight disease either single or in combination (Jasmine et al., 2005).

In the present investigation *Trichoderma* spp. isolated from rhizosphere soils of rice were characterized and evaluated for their efficacy on rice growth promotion and against *R. solani* in vitro and in vivo conditions.

Material and Methods:

Materials: *Trichoderma* isolates were collected from rhizosphere soils of farmer's rice fields during the monitoring trials and in the nearby fields around Hyderabad. The dilution-plate method was used to isolate *Trichoderma* from soil as described by Moubasher and Abdel-Hafez (1978) using *Trichoderma* medium E (Papavizas *et al.*, 1982). The cultures were maintained in PDA slants under refrigerated conditions. Morphological characterization was done as per the key characters of *Trichoderma* as described by Rifai (1969) and Lieckfeldt et al., (1999).

DNA extraction ITS analysis: The DNA extraction was done as per the standard cTAB (cetyl trimethyl ammonium bromide) protocol (Moeller et al., 1992: White at al., 1990). Mycelium disc was collected individually from the isolates and ground to a fine powder using liquid nitrogen. The powdered mycelium was mixed with 2x (CTAB) buffer and incubated at 65°C for 30 min, followed by extraction with chloroform phenol and iso-amylalochol (25:24:1) micture. The tubes were centrifuged and resulting pellets were washed with 0.1 ml of 70% ethanol, followed by air drying of the pellet. A region of nuclear rDNA, containing 18S ribosomal RNA gene (partial sequence); internal transcribed spacer 1, 5.8S ribosomal RNA gene, and internal transcribed spacer 2 (complete sequence); and 28S ribosomal RNA gene (partial sequence) was amplified by PCR using the primer combinations ITS1 (5'TCCGTAGGTGAACCTGCGG-3') and LR3R (5'-GGTCCGTGTTTCAAGAC-3') in 25 µl volume, in an automated temperature-cycling device (Eppendroff) was adopted (White *et al.*, 1990). PCR conditions was 1 min denaturation at 94°C: 30 cycles of 1 min denaturation at 94°C: 1 min primer annealing at 50°C: 90 s extension at 74°C: and final extension period of 7 min at 74°C. The desired amplified product was around 1200bp . The PCR product was purified using Wizard® SV Gel and PCR Clean-Up System (Promega, India.) DNA sequences were aligned with Clustal W and NCBI Blast N was used for identification of the 4 isolates that were isolated from the soil samples.

Studies on the efficacy of *T. asperellum* on *R. solani:* The antagonistic potential of the different isolates of *T. asperellum* on *R. solani* was tested under in vitro conditions. Petri plates containing PDA was inoculated at the with 5mm mycelial discs of *T. asperellum* was inoculated at 1cm away from the edge of the plate opposite to each other. After two days, a disc of Petri plates inoculated with pathogen at one end alone served as control. The Petri plates were incubated at room temperature for 3-4 days and radial growth afterwards were measured and recorded. Three replications were maintained per treatment. The antagonists found effective against these pathogens under *in vitro* conditions were further evaluated in the glass house on susceptible rice cultivar, TN-1. The plants were raised in plastic pots of 15 cm diameter and about 4 hills per pot were maintained in the glass house at a temperature of 27 ± 2^0 C and RH of 75-90%. Three different application methodologies, i.e., soil application, seed treatment and seed treatment followed by soil application were used to assess the biocontrol potential of the antagonist in suppressing rice sheath blight disease caused by *R. solani*. The antagonist, *T. asperellum* IIRRCK1 was applied in different ways i.e., as seed treatment followed by inoculation of *R. solani* on 30DAS, applications of T. *asperellum* IIRRCK1 as seed treatment followed by soil application on 25 DAS and followed by inoculation of *R. solani* on 30 DAS. *R. solani* was inoculated by placing the colonized shoot pieces of *T. angustata* between the tillers in the central region of the rice hills, 5-10 cm above the water line.

The severity of sheath blight disease was assessed 20 days after pathogen inoculation according to relative lesion height (RLH) method as suggested by Sharma *et al*. (1990) and the disease incidence was recorded using 0-9 grade scale. The disease progression in terms of vertical disease spread (cm) was measured with the help of scale from 3rd day to 20th day after inoculation of *R. solani*. The observations were taken on 3rd, 5th, 10th, 15th and 20th day to calculate PDI on respective days and to analyze the disease progression in the presence of the respective antagonists to know their potency to check the disease.

Results and Discussion:

Characterization of *Trichoderma* isolates

Four different isolates of *Trichoderma* spp. were isolated from the soils collected from different regions as given in the table (Table 1).

Table 1: Geographical location colony characters, conidial initiation growth and percent inhibition of *R. solani* over control of the isolates of *Trichoderma* spp. collected from the rice-rhizosphere soils of different regions in India.

Isolate No	Isolates	Location	Colour of the colony	Conidiation Initiation	Percent inhibition of R. solani over control in vitro in 5 days
1	*T. asperellum*	Hyderabad	Light green	46 hrs	**51.8 (46.03)**
2	*T. asperellum*	Hazribagh	Dark green	36 hrs	38.2 (38.04)
3	*T. asperellum*	Raipur	Light green	7 days	37.0 (36.54)
4	*T. asperellum*	Rewa	Dark green	72 hrs	35.2 (33.04)
CD					0.652
SE(±m)					0.274
CV					0.871

The isolates were characterized based on the colour of the colony, initiation of conidia in the media. The isolates varied in the initiation of conidiation ranging from about 36 hours (*T. asperellum* HZB) to 7 days (*T. asperellum* RPR). Conidial colour change was observed from white to varying shades of green (Table 1). In the microscopic studies of *T. viride* and *T. asperellum* similar type of arrangement of conidiophores and phialides were observed in all the 6 isolates. The isolates were characterized by highly branched divergent and dendritic conidiophores. Divergent phialides were typically arranged in whorls of 3-5 and held at 90° with respect to the hyphae from which they arose, or solitary. Those in whorls were typically flask-shaped, enlarged in the middle, sharply constricted below the tip to form a narrow neck and slightly constricted at the base. Terminal phialides were arranged in a whorl or solitary, were typically cylindrical or at least not conspicuously swollen in the middle and longer than the sub-terminal phialides. Majority of these above characteristics resembled with *T. asperellum* and *T. viride.*

In order to arrive at a conclusion on the identity of the species, 18sRNA sequencing and subsequent blast with the NCBI repository was carried out (Table 2). Several reports have been published on isolation and identification of *Trichoderma* in Saudi Arabia (Molan, 2009; Hussein and Yousef, 2011).

Table 2: Molecular characterization of *Trichoderma* strains using ITS regions sequencing

Isolate Code	Accession Number	Similarity %	Given (anamorph) name
Isolate-1	KF723005.1	99%	*Trichoderma. asperellum*
Isolate-2	KF723005.1	97%	*Trichoderma. asperellum*
Isolate-3	KF723005.1	100%	*Trichoderma. asperellum*
Isolate-4	KF723005.1	93%	*Trichoderma. asperellum*

Based on the query score and similarity percentage, the isolates were identified the isolates were identified as *T. asperellum*. Four isolates were sequenced; the length of the amplified fragments was 1200 bp (Figure- X). The sequencing results have been blasted against gene bank. Four isolates showed similarity ranging from 100% to 93% with the sequence results of *Trichoderma asperellum* and *Trichoderma viride*. This complexity comes from the fact that many of these species types are overlapping and therefore, two closely related organisms become attributed to different species recognized based on incomparable criteria. The isolates 1, 2, 3 and 4 showed similarity percentages of 99%, 97%, 100% and 93% respectively with *T.asperellum (*Fig. 1). The ITS sequences of all the four isolates of T. asperellum IIRRCK1 to IIRRCK4 were submitted to NCBI with the reference ID 2146474 .

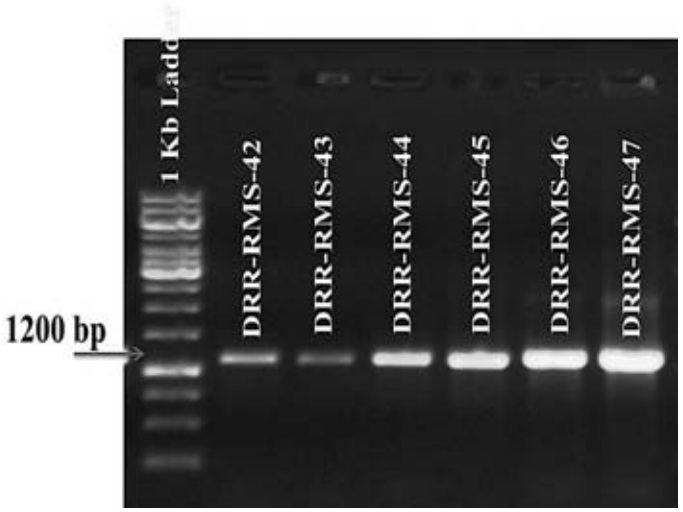

Figure 1: Gel picture representing the molecular characterization of Trichoderma isolates using ITS regions

Lanes: DRR-RMS-42- Isolate-1, DRR-RMS-43- Isolate -2, DRR-RMS-44- Isolate -3, DRR-RMS-45- Isolate -4

Biocontrol efficacy of *T. asperellum* IIRRCK1 against *R.solani*

All the four isolates of *T. asperellum* inhibited the radial growth of *R. solani* when inoculated in the plates; three days post inoculation of *R. solani* (Table 1, Fig. 2). Maximum growth suppression was recorded by the isolate 1 named as *T. asperellum* IIRRCK1 (51.8% over control), followed by the other three isolates. Due to its maximum antagonistic potential, the isolate *T. asperellum* IIRRCK1 was used in the study to estimate the progress of sheath blight disease in potted rice plants.

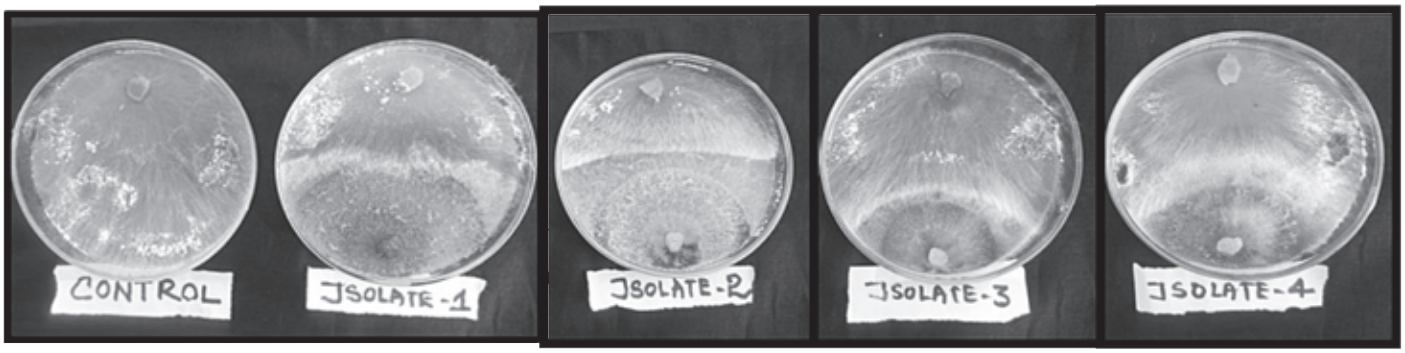

Figure 2: Effect of different isolates of T. asperellum on R. solani under in vitro conditions.

Progress of sheath blight disease in the presence of *T. asperellum* IIRRCK1

Results from the pot culture studies on the biocontrol efficiency of *T. asperellum* IIRRCK1 on sheath blight disease in rice (Table 3) indicated that the biocontrol agent was able to suppress the rate of development of sheath blight disease during the entire period of observation. Seed treatment with *T. asperellum* IIRRCK1 was more effective in suppressing the development of the disease (63.97 and 58.27 % over control) when compared to soil application alone (33.69 and 27.94 % over control). This indicated that *T. asperellum* IIRRCK1 could establish in the plants more effectively when the seeds are treated with the fungus. This may be due to the endophytic nature of this particular isolate of *T. asperellum* which shall be investigated. It has been reported by several workers that the endophytic *Trichoderma* strains have the ability to induce disease resistance and also improve the overall development of the host plants ((Bae et al., 2011: Chen et al., 2016: Elad et al., 1983). Further analysis of the data (Table 3) indicated that with progress of time, the biocontrol effect of *T. asperellum* IIRRCK1 got reduced suggesting that the antagonistic fungus population needs to be augmented with the second dose of soil application.

Table 3: Effect of indigenous isolate *T. asperellum* IIRR1 *in the* progress of Sheath blight disease of rice under pot culture conditions in the rice variety TN1.

Treatments	No. of days after pathogen inoculation									
	3		5		10		15		20	
	Mean	Percent reduction over control	Mean	Percent reduction over control	Mean	Percent reduction over control	Mean	Percent reduction over control	Mean	Percent reduction over control
Ta St *fb Rs* on 30DAS	7.4 (15.66)	63.97	13 (21.18)	51.43	20.52 (26.92)	34.75	22.95 (28.57)	33.95	22.95 (28.60)	46.25
Ta ST *fb Ta* SA on 25 DAS 25DAS *fb Rs* on 30 DAS	8.57 (17.01)	58.27	14.77 (22.57)	44.82	20.9 (27.19)	33.54	21.1 (27.43)	39.28	21.1 (27.36)	50.58
Ta SA on 25DAS *fb Rs* on 30DAS	13.65 (21.67)	33.69	21.07 (28.49)	21.29	23.22 (28.81)	26.16	28.67 (32.36)	17.49	35.05 (36.25)	17.91
Rs on 30DAS *fb Ta* SA on 35 DAS	14.8 (22.63)	27.94	16.92 (24.28)	36.79	22.25 (28.11)	28.61	24.32 (29.53)	30.01	27.47 (29.65)	35.66
Rs on 30DAS (Control)	20.54 (26.96)	0.00	26.77 (31.14)	0.00	31.45 (34.06)	0.00	34.75 (36.09)	0.00	42.7 (40.78)	0.00
CD	*0.20*		*0.12*		*1.05*		*1.15*		*0.05*	
CV	*0.77*		*0.40*		*2.90*		*2.99*		*0.12*	
SE(m)	*0.06*		*0.04*		*0.35*		*0.38*		*0.01*	

However this needs to be standardized. Similar observations in the need for augmentation of the biocontrol inocula were reported by several workers (Kumari et al., 2016: Lenka *et al*. 2012: Prasad and Reddykumar, 2011).

Conclusions

The isolate of *T. asperellum* IIRRCK1 can significantly inhibit *R. solani* and suppress the sheath blight disease severity in rice. Further studies on the standardiszation of the dose and time of application and the suitable formulation for precise and sustained release of *T. asperellum* IIRRCK1 is under progress in this laboratory.

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Looking at rice adoption behaviour beyond Yield Criteria: How Farmers Adopt new rice varieties?

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Abstract

A study was conducted in Yadadri district of Telangana to understand the adoption behaviour among 80 rice farmers. The data were collected using personal interview method. An analysis of farmers' preferences and perceptions showed that majority of farmers expressed BPT 5204 variety has a better grain quality compared to other varieties grown. Head rice recovery of RNR 15048 harvested during *Kharif* season was better compared to that of Rabi. Farmers preferred the varieties which fetched the highest net income, having desired duration and having multiple stress resistance. Based on availability of other suitable varieties in this region, some of the predominant/existing varieties are likely to be discontinued. The continued adoption and discontinuation are influenced by several factors that go beyond yield criterion. The paper describes such factors.

Key words: Rice, Adoption behaviour, Farmers, Post adoption analysis

Introduction

Rice is one of the important cereal crops in India. Rice is not only the principal food crop of India, but it also occupies the largest area under cultivation. It is the major staple food of more than two billion peoples in Asia and more than 80 per cent of world's population. Presently rice caters to about 42 per cent of the total food need of the world?. Rice is main source of income for millions of people in the world and it is grown in all continents of the world. The world production of rice in the year 2016-17 was 758.9 million tonnes. (FAO 2017).

India is an important centre of rice cultivation and is cultivated in about 42.5 million hectares in India. Developing countries account for 95 per cent of the total production, with China and India alone responsible for nearly half of the world output. Rice is rich in genetic bio-diversity, with thousands of varieties grown throughout the world. It is life for thousands of millions of people in the globe. It is deeply embedded in the cultural heritage of their societies. About 4/5 of the world's rice is produced by small-scale farmers and is consumed locally.

With advent of novel trends in rice breeding varieties like transgenic breeding for improving yield, resistance/ tolerance against biotic/abiotic stresses and grain quality to cater to national and international markets and development of herbicide-tolerant transgenic rice, development of transgenic rice with nutrient acquisition, we are surplus in rice production. Even though several technological advancements in rice breeding have progressed they are becoming obsolete and are not catering to the complete needs of the rice consumers since there is a wide disparity in the consumption preferences of both rural and urban population. The most suitable variety is the one best meeting farmers' and consumers' needs.

The scientists' selection criteria will not always correlate with that of farmers. For example, apart from yield and panicle height, there was no significant correlation between breeders and the farmers' selection scores for quinoa variety selection in Equador (McElhinny *et al.* 2007). Due to the absence of varieties meeting their criteria, farmers will continue using the landraces or old varieties. In a participatory plant breeding (PPB) study in Ghana, Manu Aduening *et al.* (2006) reported that most farmers utilized the cassava landraces despite availability of modern varieties. In Zimbabwe, farmers were also growing old maize hybrids released in the 1970s and their own landraces in spite of availability of new and high-yielding hybrids (Derera *et al*. 2006).

Many such researches revealed that farmers may not always prefer the varieties with the highest yield potential and disease resistant traits, they also give preference to features such as good taste, high milling yield, whether the rice will be sold or consumed at their home. Hence, there is a need to lay emphasis on these factors; accordingly, more micro level studies should be planned. Based on the perception of the farmers, post adoption behaviour can also be guestimated. Keeping these points in view the following study was undertaken to assess differential perception of farmers about different rice varieties, factors associated with adoption or discontinuation of rice varieties.

An attempt is also made to understand the socio-economic and other factors associated with the adoption and likely discontinuation of varieties in farmers' fields.

Material and Methods

The study was carried out in nearby villages of Bhongir mandal in Yadadri district in Telangana. About 80 farmers were selected for studying their perception on different rice that are in vogue. The selection of farmers was based on the condition that each respondent has experience in cultivating all the varieties viz., BPT 5204, RNR 15048, JGL 18047 and IR 64. The perception variable encompasses two factors; the first consists of quality factors such as taste, smell, cooking quality and HRR. The second consists of production factors such as quantity of seed and fertilizer, Man days required etc. The factors that influence the adoption of varieties were also studied. The statistical tools such as frequency, percentage and ranking were employed to draw meaningful conclusions.

Varieties considered in the present study: Farmers' perception about production and quality factors was elicited for three newly released rice varieties which are being compared to the mega variety BPT 5204(Samba Mahsuri). Among the new varieties of rice considered in the study, super-fine Telangana Sona (RNR 15048), a comparable variety of the existing BPT 5204 (Samba Mahsuri) is a 120-day crop released for the states of Telangana, Andhra Pradesh, Karnataka, Tamil Nadu and Chhattisgarh. Bathukamma (JGL 18047) is also being considered a replacement to the existing BPT 5204. It is a 120-day crop and gives average productivity of 3 t/ acre. Another new variety, Kunaram Sannalu (KNM118) is said to be equally good and provides almost the same yield. BPT 5204 (Samba Mahsuri) was the most consumed variety of rice in Southern India, which was originally released in 1986.

Results and Discussion

Varietal preference is a carefully weighed balance between consumption and production characteristics. Farmers would not only select the high yielding varieties but prefer varieties because of their taste, nutritional value, duration, the ability to grow with fewer inputs and in different abiotic stress conditions. The conventional approaches always focus on linear model of demonstrations where no options are provided to farmers to pick from. Choice of a rice variety is determined by certain plant characteristics and the local natural environment.

An attempt is made here to understand the socio-economic and other factors associated with the adoption and likely discontinuation of varieties in farmers' fields.

Table I. Profile characteristics of the respondents (n=80)

S. No.	Independent variables	Category	Respondents	
			Frequency	Percentage
1	Age	Young age (< 35 years)	30	37.5
		Middle age (36-54 years)	38	47.5
		Old age (> 55 years)	12	15
2	Education	Illiterate	8	10
		Primary school	14	17.5
		Inter/Diploma	24	30
		Graduation	34	42.5
3	Farming experience	Below 10 years	16	20
		11 to 20 years	40	50
		21 to 30 years	24	30
4	Family size	Upto 5	64	80
		More than 5	16	20
5	Annual Income	Upto 2 lakh	32	40
		More than 3 lakh	48	60
6	Land Holding	1 to 5 acres	32	40
		6 to 10 acres	20	25
		11 to 15 acres	24	30
		More than 15 acres	4	5
7	Occupation	Farming	52	65
		Farming+ cast occupation	12	15
		Farming+ labour	16	20
8	Source of information	Friend and neighbours	22	27.5
		TV radio and mass media	20	25
		AEO+AO	20	25
		Scientists	18	22.5
9	Extension contact	Regularly	44	55
		Sometimes	24	30
		Rarely	12	15

Among the respondent farmers a considerable percentage was middle aged followed by young age. The plausible reason for the above trend might be the young and middle aged farmers are enthusiastic in making the choice of varieties for adoption. A glance of the above table revealed that 42 per cent of the farmers are graduates and 30 per cent of them had intermediate/diploma education followed by the rest belonging to primary school (17.5%) and illiterate category (10%). The above table illustrated that exactly half (50%) of the respondents had 11 to 20 years of farming experience followed by 30 per cent having 21 to 30 years of farming experience and a small portion of them had below 10 years of farming experience. This might be due to majority of the respondents belong to middle age (36-54) hence they had 11 years of farming experience.

A close investigation of the Table 1 reveals that three fourth of the farmers had up to five members in a family and only 25 per cent of the respondents had family size more than 5. It can be concluded from the above table that 60 per cent of the respondents are having annual income more than 3 lakhs followed by 40 per cent of them having below 2 lakhs of annual income. This is attributed to the family background of the respondents.

The Table 1 clearly shows that majority (40%) of farmers were owning 1 to 5 acres of land followed by 30 per cent of them having 11 to 15 acres of land, 25 percent had 6 to 10 acres land and only five percent of farmers possessed more than 15 acres of land. This reflects the representativeness of the sample selected for the study in terms of landholding.

It was evident from the table that about 65 per cent of the farmer's sole occupation is farming it is quite interesting to note that the farmers in the sample area mostly depend on farming for their livelihood followed by 20 per cent whose occupation is both farming and casual labour and about 15 % of them are relying on caste occupation along with farming.

Regarding the utilisation of sources of information by the farmers, 27.5 per cent of them obtain information from their friends and neighbours followed by 25 per cent of them depend on Agricultural extension officer (AEO) and Agricultural Officer (AO) for their information needs. An equal percentage of the respondents utilized mass media sources viz. Television, radio and newspapers for their information needs and 22 percent of them interacted with scientists for fulfilling their information needs.

With regard to extension contact, 55 per cent of the farmers expressed that they had regular extension contact and 30 per cent reported that they meet the extension personnel sometimes and 15 per cent of them had rarely contacted the extension agencies such as State Agriculture Department or University Scientists.

Table 2 portrays perception of farmers on different rice varieties with regard to some key production factors. Regarding the availability of the quality seeds all the sample farmers stated that quality seeds are available for the varieties such as RNR-15048 and JGL 18047 on the other hand some of the farmers expressed that the quality seeds are not available for BPT 5204 and IR 64. Farmers usually purchase seeds from the market yards and few of them purchase from local dealers. According to farmers, higher seed rate was used (30 kgs per acre or 75 kg/ ha) for varieties like BPT 5204, JGL 18047 and IR 64 whereas RNR-15048 variety required only 20 kg/ acre which is equivalent to 50 kg/ha. The cost of seed for JGL 18047 and IR 64 per one bag of 25 kg was Rs. 950 and for RNR-15048 and BPT 5204 varieties were Rs.800 and Rs.1200 per bag (of 25 kg) respectively.

Table 2: Perception of farmers about rice varieties – production factors (figures in parenthesis represent %)

S. No		Category	BPT 5204	RNR-15048 *Kharif*	JGL 18047	IR 64	RNR-15048 *Rabi*
1	Availability of quality seeds	Available	74 (92.5)	80 (100)	80 (100)	76 (95)	80 (100)
		Not available	6 (7.5)	0 (0)	0 (0)	4 (5)	0
2	Quantity of seed sown (kg/ha)		75	50	75	75	50
3	Cost of the seed (in Rs/bag)		1200	800	950	950	800
4	Quantity of fertilizer applied (In bags/ acre)	Urea	3	1	2	2	1
		DAP	2.5	1.5	2	2	1.5
5	Man days required (no of men employed X days)	Less	0 (0)	68 (85)	24 (30)	0 (0)	68 (85)
		Medium	20 (25)	12 (15)	56 (70)	4 (5)	12 (15)
		high	60 (75)	0 (0)	0 (0)	76 (95)	0 (0)
6	Pests and diseases incidence (less/more)	Less	0	72 (90)	22 (27.5)	4 (5)	72(90)
		Medium	24 (30)	8 (10)	48 (60)	24 (30)	8 (10)
		high	56 (70)	0	10 (12.5)	52 (65)	0
7	Intensity of weed (less/more)	Less	56 (70)	6 (7.5)	56 (70)	0	6 (7.5)
		Medium	24 (30)	10 (12.5)	24 (30)	8 (10)	10 (12.5)
		high	0	64 (80)	0	72 (90)	64 (80)
8	Harvesting (in days after planting)		120	95-105	105	110	100
9	Yield (t/acre)		2.25	2.47	2.30	2.25	3.0
10	Gross Income (Rs/acre)		41625	44460	35420	34875	45000
11	Tillering Capacity	Better	20 (25)	25 (31.25)	18 (22.50)	18 (22.50)	22 (27.50)
		Medium	40 (50)	42 (52.50)	35 (43.50)	32 (40)	39 (48.75)
		Shy	20 (25)	13 (16.25)	27 (33.75)	30 (33.75)	19 (23.75)

The fertilizer requirement of JGL 18047 and IR -64 varieties was identical, about 2 bags of urea and 2 bags of DAP are applied in three doses. While RNR-15048 required 1 bag urea and 1.5 bags DAP. However, according to the respondents, fertilizer requirement for BPT 5204 variety was more i.e. 3 bags of urea and 2.5 bags of DAP. Regarding the number of labour employed during the season - the RNR-15048 variety required extremely less number of workers, 70 per cent conveyed that moderate number of workers required in case of JGL 18047. While, 75 per cent opined that BPT variety required more man power or more labour days. The duration of variety, transplanting, weed population and pest incidence are the factors that influence number of workers to be employed during the crop season.

The pest and disease incidence was less in case of RNR-15048 variety in *kharif* and *Rabi* seasons as 90 per cent of the farmers inferred it as low. On the other hand, 60 per cent expressed that JGL 18047 has medium pest incidence and however IR64 variety and BPT 5204 have recorded high pest incidence rate (perceived by 65 per cent and 70 per cent of farmers respectively). In addition to this, severe rodent damage was also observed in BPT 5204 variety.

The weed intensity was very high in RNR-15048 and IR 64 varieties and low in case of BPT and Batukamma varieties. The plausible reason could be that spacing in earlier stated varieties is more; the thin spacing will aggravate the weed population. The harvesting was done after 120 days after transplanting in case of BPT 5204, JGL 18047 and IR-64 varieties whereas RNR-15048 variety is harvested in 95 to 105 days after transplanting. Farmers reported that RNR 15048, when transplanted before July 1st week, resulted in longer duration and when transplanted after July 1st week, resulted in most appropriate duration for Telangana state. In case of Rabi, the duration remained same in early and late planted condition.

Table 3: Perception of farmers about rice varieties – quality factors (figures in parenthesis represent %)

S.No		Category	BPT 5204	RNR-15048 Kharif	JGL 18047	IR 64	RNR-15048 Rabi
1	Taste	V. good	32 (40)	76(95)	0	0	16 (20)
		Good	48 (60)	4(5)	28 (35)	48(60)	40 (50)
		Average	0	0	52 (65)	32 (40)	24 (30)
2	Smell	V.good	72 (90)	36(45)	14 (17.5)	10 (12.5)	36 (45)
		Good	8 (10)	12 (15)	8 (10)	32 (40)	12 (15)
		Average	0	16(40)	58 (72.5)	38 (47.5)	32 (40)
3	Cooking quality	V.good	70 (87.5)	76 (95)	0	0	16 (20)
		Good	10 (12.5)	4 (5)	20 (25)	24 (30)	48 (60)
		Average	0	0	60 (75)	56 (70)	16 (20)
4	Head rice recovery (HRR) (%) Cumulative average		73	73	70	70	69

About 60 per cent of the respondents expressed that taste of BPT was good and 40 per cent stated it has a very good taste, 60 percent stated that IR64 has good taste and 65 per cent expressed JGL 18047 variety has average taste. It is quite interesting to note that the RNR variety in *Kharif* was having a very good taste compared to the RNR variety in the *Rabi* only 50 per cent opined that it has good taste. The differential taste perception between *Kharif* produce and *Rabi* produce is evident from the qualitative judgement of farmers. The varietal adoption may differ between *Rabi* and *Kharif*.

Among all the varieties BPT variety has very favourable smell as expressed by 90 per cent of the respondents; it has characteristic aroma and fine grain quality. Hence it was still the ruling variety and remaining varieties fall under average category with respect to the smell.

The cooking quality of RNR variety during *Kharif* was perceived as very good (95%) and Good (5%) whereas the cooking quality of RNR variety in Rabi was good perceived by 60 per cent of the respondents.

The head rice recovery (qualitative ratings by respondents) revealed that all the varieties have good head rice recovery. But the HRR of RNR 15048 harvested during *Rabi* (dry) season has recorded lowest HRR, where the same variety recorded highest HRR in *Kharif*.

Rating of factors influencing Adoption behaviour

Based on the analysis of above factors, an attempt was made to rank the factors that are likely to influence the adoption behaviour of rice farmers in respect of these varieties. The results are presented in the following Table 4.

Table 4: Perceived ranking of factors influencing adoption

S. No.	Perceived Factors	Number of favourable responses	Ranking
1	Availability of quality seeds	64	V
2	Lesser Seed rate – without Yield Penalty	48	XI
3	Reasonable Cost of the seed	52	IX
4	Fertilizer Responsiveness	60	VI
5	Lesser need for Labour Man days	56	VIII
6	Less Pests and diseases incidence	68	IV
7	Less Intensity of weed	60	VI
8	Favourable (Medium) duration	72	III
9	Better Yield	78	II
10	Market price (Procurement price)	72	III
11	Net Income per acre	80	I
12	Favourable Taste	56	VIII
13	Mild favourable Smell	50	X
14	Good Cooking quality	64	V
15	Head rice recovery (HRR) (%)	58	VII
16	Abiotic Stress Tolerance (floods, drought)	68	IV

Farmers opined that their varietal choice depended on several factors as indicated in the table 4. Apart from net income and yield criteria, duration and market prices influenced adoption of a particular variety. In Telangana due to assured procurement of both fine and bold grain type varieties through *Indira Kranthi Patham* (IKP) centres by providing minimum support price, very thin line is left out between varieties having fine grain and bold grains in terms of marketability.

The reasons from varietal choice shifted from mere yields to other factors such as favourable duration (borewell irrigated conditions), abiotic stress tolerance (due to aberrations in climate), availability of seeds (non availability of a particular seed leads to adoption of some other variety), cooking quality (for family consumption) etc.,

When asked about the post adoption behaviour, interesting results were obtained. Most of the farmers are thinking to discontinue adoption of these varieties except for RNR 15048 during *Kharif*. The continued adoption is observed in case of BPT 5204 and RNR 15048 (in Kharif) for home consumption purposes where as for marketing purposes the results are not that encouraging.

The discontinuation of adoption of these varieties will stem from either replacement or dissatisfaction. BPT 5204 is likely to be replaced by Improved Samba Mahsuri, Jai Sree Ram and HMT Sona. RNR 15048 during *kharif* was perceived as good when it is planted after July. Farmers who plant before July wanted replacement of RNR 15048 with Jai Sree Ram and HMT Sona. Interestingly, IR 64 may see replacement with new varieties like DRR Dhan 44, MTU 1153 and JGL 24423, RNR 15048 during *Rabi* may get discontinued due to several reasons that led to disenchantment. Reverse adoption is observed where farmers are returning to MTU 1010 after having adopted RNR 15048 during last two rabi seasons.

Table 5: Post adoption Indicators (figures in parenthesis represent %)

S. No	Post adoption Behaviour	Category	BPT 5204	RNR-15048 Kharif	JGL 18047	IR 64	RNR-15048 Rabi
1	Continued adoption	For marketing	12 (15)	28 (35)	12 (15)	8 (10)	12 (15)
		Home Consumption	5 (6.2)	4 (5)	0	0 (0)	0 (0)
2	Discontinuation	Replacement	49 (61.3)	40 (50)	36 (45)	48 (60)	48 (60)
		Disenchantment	14 (17.5)	8 (10)	32 (40)	24 (30)	20 (25)

Conclusion

In most parts of the country, farmers continue to adopt rice varieties developed several years back. Most of the farmers are thinking to discontinue adoption of these varieties except for RNR 15048 during *Kharif*. The continued adoption is observed in case of BPT 5204 and RNR 15048 (in *Kharif*) for home consumption purposes where as for marketing purposes the results are not that encouraging. The discontinuation of adoption of these varieties will stem from either replacement or dissatisfaction. BPT 5204 is likely to be replaced by Improved Samba Mahsuri, Jai Sree Ram and HMT Sona. RNR 15048 during Kharif was

perceived as good when it is planted after July. Farmers who plant before July wanted replacement of RNR 15048 with Jai Sree Ram and HMT Sona. Interestingly, IR 64 may see replacement with new varieties like DRR Dhan 44, MTU 1153 and JGL 24423. RNR 15048 during *Rabi* may get discontinued due to several reasons that led to disenchantment. Reverse adoption is observed where farmers are returning to MTU 1010 after having adopted RNR 15048 during last two rabi seasons.

The research and development organisations need to focus on multiple factors such as grain quality, favourable duration, procurement method, abiotic stress tolerance, etc., apart from the yield criteria. Only yield criterion would never lead to adoption of new varieties. Breeding programs need clear, formal product profiles to guide them. These profiles must specify: - Which variety currently grown by farmers will be replaced by the new product? - Which features of the current variety must be improved to drive adoption (eg, a specific quality parameter or disease resistance) - Which features must remain unchanged in the new product.

Another important dimension is availability of the quality seed at right time. The breeding objectives may be defined to suit to a particular regions/ state/ ecosystem/ consumption pattern, before a variety is developed. The varietal development and targeting should be felicitous to the target producers and consumers. After the release of varieties, the line departments should target the best suited areas for the cultivation of these varieties rather prioritizing the popular age old varieties. While targeting the varieties, we need to take into consideration the factors that affect the varietal choice among the farmers. This paper tried to highlight the way adoption studies need to be undertaken in describing the varietal adoption based on various qualitative parameters beyond 'yield alone' approach.

Acknowledgements

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ORIGINAL RESEARCH ARTICLE OPEN ACCESS

Economics of Paddy Cultivation in East Godavari district of Andhra Pradesh

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Abstract

This study examined the yield, input use, net returns, break-even output and resource use efficiency in paddy cultivation in East Godavari district of Andhra Pradesh. At district level, total variable cost per hectare was Rs.65160.22 whereas total cost of cultivation per hectare was Rs.97884.09. Total variable cost accounts for 66.57% to the total cost. Labour cost constitutes over 63 % of the total variable cost. At district level, total returns from paddy crop were Rs.79394.81 per hectare. Returns over variable cost were positive but returns over total cost were negative. It is observed that the actual yield was less than BEO. Cobb-Douglas production function estimation revealed that, except for manures and fertilisers, all other inputs were positively contributing to productivity. Among these, land and labour variables were significant. Keeping in view the recent proposal by the Government to fix MSP at 1.5 times of total expenses incurred by farmers, BEO simulations has been carried out under different scenarios. The results indicate that that proposed hike in MSP can improve viability of paddy cultivation provided the increase is based on cost of cultivation in the region and there is effective enforcement of MSP.

Key words: BEO, MSP, Rice, Paddy, East Godavari, Crop-holiday

Introduction

Reiterating the current government's commitment to the goal of doubling farmers' income by 2022, in the Union Budget 2018, the government has announced its decision to offer a Minimum Support Price (MSP) of at least 1.5 times the expenses borne by farmers for all crops. In this backdrop, in the present study an attempt has been made to evaluate economics of paddy cultivation in East Godavari district of Andhra Pradesh.

In A.P, within agricultural crops sector, major share of Gross Value Added (GVA) was contributed by paddy, but its share declined to 9 % in 2015-16 compared to 11% in 2014-15. East Godavari contributed 9% of Gross sown area (GSA) and 17.67 % of Paddy area in the state in 2015-16. In East Godavari, Paddy area constituted 56.22 % of GSA in 2015-16. East Godavari offers an important case study not only because of its importance in contribution to rice production in the state but also because of crop holiday observed in the district in 2011-12, reflecting farmer's unhappiness with returns from paddy cultivation.

Data and Methodology

Sampling framework

Keeping in view the objective of the study, a multistage sampling procedure was adopted in getting primary data from farmers. In the first stage, East Godavari district of Andhra Pradesh was purposively selected. In the second stage, four mandals namely Samalkota, Jaggampeta, Rajavommangi, Amalapuram were selected representing mandals with different pattern and levels of crop-diversification. Samalkota was low diversified mandal, Jaggampeta and Rajavommangi were highly diversified mandals and Amalapuram was medium diversified mandal. This type of mandal selection ensured capturing the contextual diversity in paddy cultivation. In the third stage, two villages from each mandal have been selected randomly. From these four mandals a total of 145 farmers were selected randomly for data collection as represented in Figure 1. Primary data was collected using specifically designed and pretested questionnaires for farmers. For further analysis paddy farmers were post stratified into marginal (<1 ha), Small (1-2 ha), semi medium (2-4 ha) and medium (4-10 ha) categories based on the size of their operational land holdings.

Few errors were noticed in this article published in Vol. 10, Issue No. 2 of the Journal. Hence corrected version of the article is republished in this issue

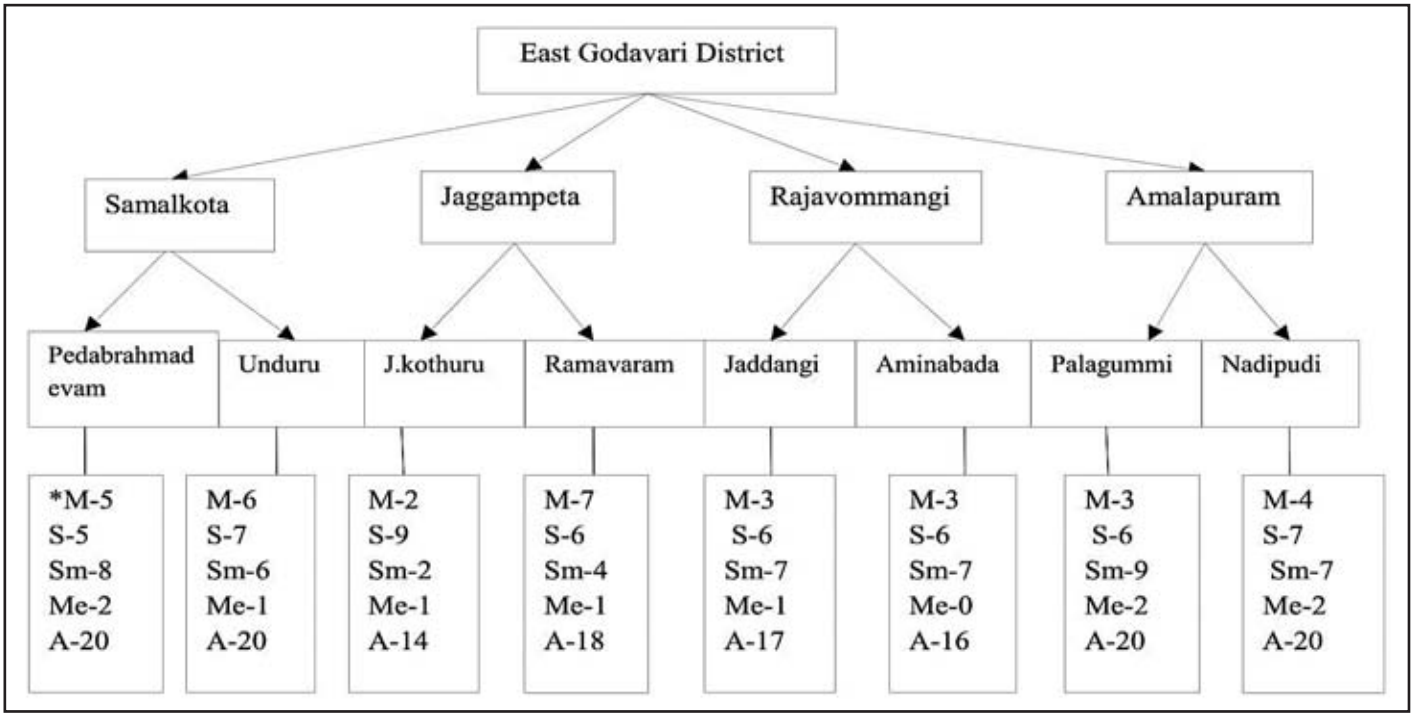
Analytical Framework

From the selected farmers, data regarding expenses incurred in cultivation of paddy for the *kharif* season of the year 2015-16 was collected. Using this data, computation of cost of cultivation of paddy was carried out on hectare basis in two parts namely, variable cost and fixed cost. Variable cost includes cost of human labour, machine/ bullock labour, seed, irrigation, manures, fertilizers, pesticides, and interest on working capital. The prevailing bank rate of interest (7%) was taken to work out the interest on working capital for the duration of the crop (150 days). Items included under the category of fixed costs are land revenue, rental value of land, interest on fixed capital. Interest on fixed capital was calculated in the same way as in case of interest on working capital at bank interest rate of 10%. Returns in paddy cultivation were assessed by computing returns over variable cost and returns over total cost. Cost of production was worked out as cost per unit of output i.e. per quintal of paddy and compared with output price realized by farmers.

For assessing viability of paddy cultivation, Break even analysis was carried out. Break even output (BEO) is the output level at which the total revenue received by a farmer just matches the total cost incurred. It is computed at hectare level using the formula

$$\text{Break even output (units)} = \frac{\text{Fixed cost}}{\text{Price per unit-Variable cost per unit}}$$

BEO was compared with actual yield realised.



*M – Marginal farmers; S - Small farmers; Sm –Semi medium farmers; Me – Medium farmers; A – All size categories

Figures adjacent to different categories of farmer indicate total number of farmers in that category.

Figure 1: Farmers sampling plan

Cobb-Douglas production function was estimated to find out whether farmers used various inputs in crop production efficiently. Cobb-Douglas production function in linear form was specified as

$$\text{Log } Y = \log A + b_1 \log X_1 + b_2 \log X_2 + b_3 \log X_3 + b_4 \log X_4 + b5 \log X_5 + b_6 \log X_6 + b_7 X_7$$

Where, Y = Rice yield (quintals/farm)

A – Constant (intercept)

X_1- Land in acres

X_2- No. of human labour days

X_3- Seed cost (Rs.)

X_4- No. of tractor hours

X_5- Manures and Fertilizers (Rs.)

X_6- Other expenses (Rs.)

X_7- dummy 1 for leased in farmers, otherwise 0.

b_1, b_2, b_3, b_4, b_5, b_6, b_7 - elasticity coefficients

The elasticity coefficients obtained in estimation in turn have been used to calculate their marginal value product (MVP) at their geometric mean for an average farm.

$$MPP = E \times \frac{\overline{Y}}{\overline{X}}$$

Where, MPP = Marginal physical product

E = Elasticity's of production

$\overline{Y}$ = Geometric mean of yield

$\overline{X}$ = Geometric mean of a given factor

$$MVP = MPP \times Py$$

Where, Py = Price of the output

Then the marginal value product was compared with their Marginal Input Cost (MIC) for evaluating resource use efficiency.

Results and Discussion

Salient characteristics of sampled farmers are presented in Table 1. It could be seen from Table 1 that average farm size in East Godavari was 1.83 ha. In selected mandals average farm size ranged from 1.67 ha to 2.10 ha. Paddy area share in total operational holding area was 79.66 % in East Godavari. In Samalkota mandal paddy area share was 100% followed by Amalapuram (95.67), whereas in other two mandals it was 55%. Share of leased in farmers in East Godavari district was 57.24% and this ranged from 27.27% to 80% in selected mandals. Share of leased in farmers was highest in case of small farmers in all mandals except in samalkota mandal, wherein medium farmers constituted highest share of leased in farmers. At district level average paddy yield was 57.89q/ha. Across selected mandals, paddy yield ranged between 56.19 to 59.37 q/ha. Across different categories of farmers at district level, highest paddy yield was observed in the case of medium farmers (59.67q/ha) followed by marginal farmers (59.041 q/ha); semi medium farmers (57.420 q/ha) and small farmers (57.079 q/ha). These facts indicate diverse contexts of paddy cultivation in the district.

Table 1: Salient characteristics of sampled farmers

Mandal name	Farmer category	Average total farm size (ha)	Paddy area share in total operational holding area (%)	Paddy yield(in quintals/ha)	Share of of leased-in -in farmers
Samalkota	Marginal	0.61	100.00	58.38	45.45
	Small	1.38	100.00	54.94	58.33
	Semi medium	2.37	100.00	55.69	64.28
	Medium	4.05	100.00	58.05	66.66
	All	**1.71**	**100.00**	**56.19**	**57.5**
Jaggampeta	Marginal	0.65	75.81	59.45	55.55
	Small	1.44	57.80	57.83	66.67
	Semi medium	2.63	41.00	56.96	50
	Medium	5.06	59.98	59.28	50
	All	**1.67**	**55.23**	**58.18**	**59.37**
Rajavommangi	Marginal	0.68	80.20	57.89	33.33
	Small	1.35	64.98	57.21	50
	Semi medium	2.46	42.34	57.22	7.15
	Medium	4.05	100.00	59.28	0
	All	**1.78**	**55.17**	**57.54**	**27.27**
Amalapuram	Marginal	0.64	100.00	60.46	71.43
	Small	1.39	93.23	58.61	92.31
	Semi medium	2.81	95.50	58.90	75
	Medium	4.15	97.59	61.13	75
	All	**2.10**	**95.67**	**59.37**	**80**
East Godavari district	Marginal	0.638	89.449	59.041	51.52
	Small	1.392	77.897	57.097	67.31
	Semi medium	2.567	75.707	57.420	50
	Medium	4.291	89.627	59.670	60
	All	**1.83**	**79.66**	**57.89**	**57.24**

Details of Cost of cultivation of paddy per hectare are presented in Table 2. At district level, total variable cost per hectare was Rs.65160.22 which accounts for 66.57% of the total cost. Across the farmer categories variable cost was highest in case of marginal farmers (Rs.68731.95) and lowest in case of small farmers (Rs.63565.94). At aggregate level maximum variable cost was observed in case of weeding which accounted for 17.02% of variable cost, due to more human labour involvement. The second highest cost item under variable costs was post harvesting operations comprising threshing, bagging, transportation costs (14.82%) and was followed by transplanting cost (14.28%). Expenses incurred on weeding were maximum in the case of marginal, small, and semi medium categories but in case of medium farmers post harvesting cost was the highest variable cost. At aggregate level, among the components of variable cost, human labour cost constituted the highest share (63.87%) followed by manures (11.90%). Sita Devi and Ponnarasi (2009) and Archana (2013) also reported that human labour cost constituted highest share in

Table 2: Cost of cultivation of Paddy (Rs. /Hectare)

Particulars	Farm Size Category				
	Marginal	Small	Semi medium	Medium	All
Ploughing	4695.66 (6.83)	3654.93 (5.75)	5075.85 (7.77)	5850 (8.97)	4802.95 (7.37)
Sowing	2424.85 (3.53)	2109.29 (3.32)	2401.05 (3.67)	2433.60 (3.73)	2331.05 (3.58)
Manuring	8929.18 (12.99)	8254.60 (12.99)	8458.72 (12.94)	8546.20 (13.10)	8462.07 (12.99)
Transplanting	9619.72 (14.00)	8476.17 (13.33)	9739.00 (14.9)	9282.00 (14.23)	9307.14 (14.28)
Fertilizer application	7258.88 (10.56)	6107.56 (9.61)	6440.53 (9.85)	5957.12 (9.13)	6336.33 (9.72)
Weeding	12522.63 (18.22)	14477.02 (22.77)	10239.69 (15.66)	7572.50 (11.61)	11090.70 (17.02)
Irrigation	1667.25 (2.43)	1667.25 (2.62)	1667.25 (2.55)	1667.25 (2.56)	1667.25 (2.56)
Application of Plant protection chemicals	4116.67 (5.99)	2459.36 (3.87)	3526.03 (5.39)	4618.90 (7.08)	3492.71 (5.36)
Harvesting	6950.53 (10.11)	6907.49 (10.87)	6522.86 (9.98)	6266.00 (9.61)	6617.10 (10.16)
Post harvesting costs	9075.25 (13.20)	8091.53 (12.73)	9898.01 (15.14)	11635.00 (17.84)	9658.06 (14.82)
Interest on working capital	1471.33 (2.14)	1360.74 (2.14)	1399.32 (2.14)	1396.25 (2.14)	1394.87 (2.14)
Total variable cost	68731.95 (100)	63565.94 (100)	65368.31 (100)	65224.82 (100)	65160.22 (100)
Land revenue	370.50	367.84	370.50	370.5	369.79
Rental value of land	29427.53	31382.38	30895.58	31720	31045.13
Interest on fixed capital	1241.58	1322.93	1302.75	1337.10	1308.95
Total fixed cost	31039.61	33073.15	32568.84	33427.60	32723.87
Total cost	99771.56	96639.09	97937.14	98652.42	97884.09
Share of variable cost in total cost	68.88	65.77	66.74	66.12	66.57
Share of human labour cost in variable cost	65.82	67.08	62.80	61.06	63.87
Share of seed cost in variable cost	2.78	2.84	3.23	3.25	3.09
Share of manure and fertilizer cost in variable cost	18.71	19.54	19.87	18.74	19.47
Share of plant protection chemicals in variable cost	3.72	2.69	4.13	5.84	4.03

Figures in parenthesis indicate percentages to total variable cost

cost of paddy cultivation. Human labour cost share ranged from 61.06 to 67.08% of variable cost across different farm size categories. Cost of seeds constituted only a small (2.78 to 3.25%) share of variable cost because most of the farmers used local varieties of seeds.

It could be seen from Table 3 that total cost of production per quintal of paddy was Rs.1690.86 at district level and it ranged from Rs.1653.30 to Rs.1705.63 across farm size categories. As observed earlier in Table 2 weeding constituted highest variable costs of production. Total cost of cultivation (Table 2) was highest in case of marginal farmers followed by medium, semi medium and small categories, whereas cost of production (Table 3) was highest in case of semi medium farmers followed by small, marginal and medium categories .

Table 3: Cost of Production of Paddy (Rs. /Quintal)

Particulars	Farm Size Category				
	Marginal	Small	Semi medium	Medium	Combined
Ploughing	79.53	64.01	88.40	98.04	82.97
Sowing	41.07	36.94	41.82	40.78	40.27
Manuring	151.24	144.56	147.31	143.22	146.17
Transplanting	162.94	148.44	169.61	155.56	160.77
Fertilizer application	122.95	106.96	112.17	99.83	109.45
Weeding	212.10	253.54	178.33	126.91	191.58
Irrigation	28.24	29.20	29.04	27.94	28.80
Plant protection chemicals application	69.73	43.07	61.41	77.41	60.33
Harvesting	117.73	120.97	113.60	105.01	114.30
Post harvesting costs	153.71	141.71	172.38	194.99	166.83
Interest on working capital	24.92	23.83	24.37	23.40	24.10
Total variable cost	1164.16	1113.24	1138.42	1093.09	1125.59
Total fixed cost	525.74	579.21	567.20	560.21	565.28
Total cost of production	1689.90	1692.45	1705.63	1653.30	1690.86

A perusal of extent of mechanization (Table 4) revealed that machine labour constituted 10.35% of total labour cost. Across categories it was highest in case of medium farmers (12.81%) followed by semi medium, marginal and small farmers in that order. Average paddy yield was highest in case of medium farmers (Table 5). This coupled with highest machine labour utilization led to lowest cost of production on these farms.

Table 4: Cost incurred on manual and machine labour in paddy cultivation

Particulars	Human labour cost Rs./ha	Machine labour cost Rs./ha	Share of machine labour in total labour cost
Marginal	45237.52	4695.66	9.40
Small	42641.12	3654.93	7.89
Semi medium	41053.46	5075.85	11.00
Medium	39823.55	5850.00	12.81
All	41620.22	4802.95	10.35

Table 5 Returns and Break even output in paddy cultivation

Particulars	Farm Size Category				
	Marginal	Small	Semi medium	Medium	All
Total variable cost (Rs./ha)	68731.95	63565.94	65368.31	65224.82	65160.22
Total fixed cost (Rs/ha.)	31039.61	33073.15	32568.84	33427.60	32723.87
Yield in quintals/ha	59.04	57.10	57.42	59.67	57.89
Returns from main product (Rs/ha.)	79178.11	76129.53	78082.88	81172.00	78221.49
Returns from by product (Rs/ha.)	1187.67	1141.94	1171.24	1217.58	1173.32
Total Returns (Rs/ha.)	80365.78	77271.47	79254.12	82389.58	79394.81
Returns over variable cost (Rs.)	11633.83	13705.53	13885.81	17164.76	14234.59
Returns over total cost (Rs.)	-19405.78	-19367.62	-18683.03	-16262.84	-18489.28
Output Price per unit (Rs/quintal.)	1361.21	1353.27	1380.25	1380.75	1371.48
Break even output (BEO in quintals)	157.52	137.79	134.68	116.20	133.08
BEO to Yield ratio	2.67	2.41	2.35	1.95	2.30
Required variable cost per unit (Rs.) where current yield become BEO at ceteris paribus	835.47	774.05	813.05	820.55	806.20
Required price per unit (Rs.) where current yield become BEO at ceteris paribus	1689.90	1692.54	1705.63	1653.30	1690.86

Returns and Break Even Analysis

At district level, total returns from paddy crop were Rs.79394.81 per hectare (Table 5). Returns over variable cost were positive, but returns over total cost were negative in all farm size categories. From the results of Break Even Output (BEO) analysis in paddy cultivation it is evident that, the average yield obtained on different farm size groups was lesser than the break-even output. At district level BEO was 133.08 quintals indicating that a farmer should produce a minimum of 133.08q of paddy/ha so as to not incur any loss. Across categories break even output was higher in case of marginal farmers (157.52q) followed by small, semi medium and lower in medium farmers (116.20q). At aggregate level, the actual yield was 57.89 quintals. To make this yield (57.89q) as a break even output at given fixed cost, (i) variable cost per unit has to be reduced by 28.38% at actual price realized by farmers or (ii) price per unit has to be increased by 23.29% at actual variable cost incurred by the farmers.

In the context of proposed increase in M.S.P (at 1.5 times of expenses incurred by farmers) in recent union budget, different simulations of fixing support price was attempted and resultant price and BEO outcomes are depicted in Figure 2 and Figure 3.

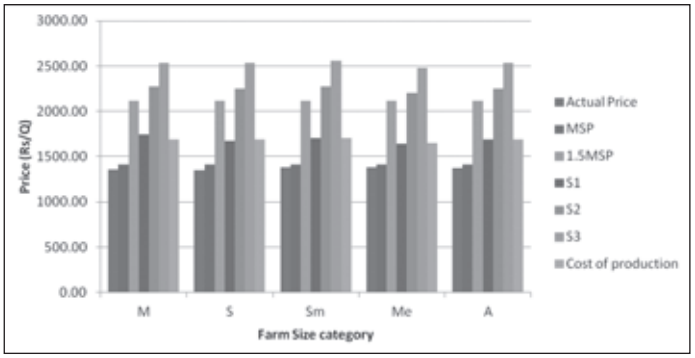


Figure 2: Paddy price under different scenarios

Under Scenario-1 (S1), total returns were computed as 1.5 times of Total Variable Cost (TVC). Under Scenario-2 (S2), total returns were computed as 1.5 times of TVC+ Actual Total Fixed Cost. In Scenario-3(S3), total returns were computed as 1.5 times of Total Cost (TC). Dividing these total returns under different scenarios for different size category farms, with respective paddy yield, prices were obtained. Using these prices BEO was calculated under different scenarios.

From the Figure 2, it is evident that at aggregate level both MSP and price under S1 were lower than cost of production. In rest of the scenarios price was higher than cost of production. From the Figure.3 it is clear that under S1 at aggregate level, BEO was almost equal to actual

yield resulting in zero safety margins. Under S2 safety margin was around 50% and it increased to 60% under S3. Thus across scenarios considered, highest safety margin was associated with S3

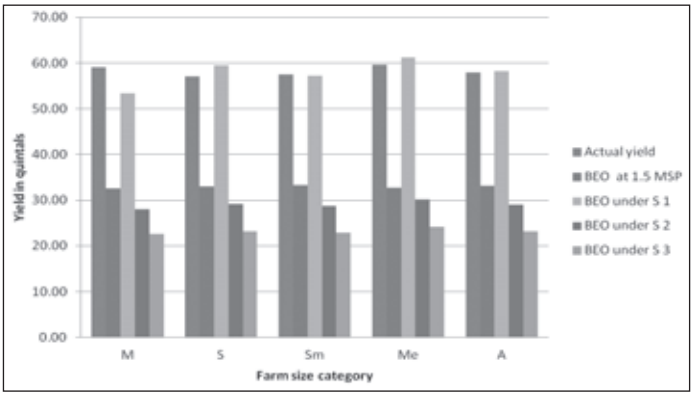


Figure 3: Analysis of Break Even Output under different price fixing Scenarios

M – Marginal S – Small Se – Semi medium
Me – Medium A - All

Aggregating cost of cultivation data of different states and fixing MSP at all India level is creating problem to some extent in ensuring reasonable returns to farmers. Further, scrapping the provision of bonus payment (over MSP) by states in recent years added to this the problem. It is observed that at all India level also; margin between MSP and different costs for paddy was low (less than 50%) in recent years (Himanshu, 2018). In the present study it is observed that MSP (2015-16) is lower than cost of production and, and at this price BEO was more than yield. At1.5 times MSP though price could cover cost of production; the safety margin was 43% percent only. Thus it is observed in the present study that safety margin is higher when price is fixed based on regional cost of cultivation compared to price fixed based on aggregate national level MSP. In 2014, in Karnataka, a state level advisory body viz Karnataka Agricultural Prices commission was constituted (KAPC, 2014). One duty of this commission is estimation of cost of cultivation of principal crops of the state including horticultural crops regularly and systematically using standard cost concepts reflecting the local conditions of demand and supply of inputs and outputs. Similar initiatives can be thought of in other states also to get help in arriving at regional level cost of cultivation estimates.

Under different scenarios returns over total costs were computed and presented in Table 6. It is observed that at aggregate level returns over total cost were negative in the case of Scenario1, and at MSP. In rest of the scenarios it was positive. Under Scenario3, returns over total costs were Rs 48942 per ha.

Table 6 Returns over total cost under different pricing Scenarios (Rs/ha)

Scenario		Farm Size Category				
		Marginal	Small	Semi medium	Medium	All
Actual		-19405.78	-19367.62	-18683.03	-16262.84	-18489.29
MSP		-16525.16	-16128.09	-16974.95	-14517.72	-16259.20
1.5*MSP		25098.04	24127.41	23506.15	27549.63	24553.25
1.5TVC	S1	3326.36	-1290.18	115.32	-815.19	-143.76
1.5*TVC+TFC	S2	34365.98	31782.97	32684.16	32612.41	32580.12
1.5*TC	S3	49885.78	48319.55	48968.58	49326.21	48942.05

BEO analysis is subject to assumption of constant rate of increase in variable cost. Keeping this limitation in view, to get further insights regarding resource use efficiency in paddy cultivation in East Godavari district, Cob-Douglas production function was estimated and the results are presented in Table 7.

It is evident from the Table 7 except manures and fertilizers, all other inputs were contributing positively to productivity. Among these inputs, land and labour were observed to be statistically significant. Though the expenditures on manures and fertilizers was with negative elasticity, it was statistically non significant. The variables considered in the model were able to explain 99.6% of variation in paddy production. While a study conducted by Jeena (2012) had reported diminishing returns to scale (0.69) in the context of Kerala, in the present study returns to scale is 1.00 indicating that cultivation of paddy in the study area is operating at constant returns to scale.

Results of resource use efficiency are furnished in Table 8. The ratio of marginal value product to marginal input cost in case of land was greater than unity, implying that production can be significantly increased by increasing

the area of land under paddy cultivation. Except land, other factors have ratio of less than unity means they are being overused. So use of these inputs needs to be reduced for optimization of resource use.

Table 7: Determinants of Production of paddy farmers

Particulars	Coefficients	Standard Error	P-value
Intercept	1.326	0.097	2.36257E-27
Paddy area (in acres)	0.957	0.034	1.29453E-58
labour days	0.048	0.025	0.053738441
Seed cost (Rs.)	0.019	0.014	0.177561088
No. of tractor hours	0.003	0.017	0.825755257
Manures and Fertilizers (Rs.)	-0.027	0.025	0.27947306
Other expenses (Rs.)	0.0008	0.018	0.965664386
LD (leased in dummy)	0.002	0.003	0.441039834
R Square	0.996		
Number of observations	145		

Table 8: Resource use efficiency in paddy cultivation

Particulars	Marginal value product (MVP)	Marginal Input cost (MIC)	MVP/ MIC
Paddy land (in acres)	30735.96	12568.88	2.45
labour days	38.02	308.67	0.12
Seed cost (Rs.)	0.82	1.00	0.82
No. of tractor hours	14.04	200.71	0.07
Manures and Fertilizers(Rs.)	-0.17	1.00	-0.17
Other expenses(Rs.)	0.02	1.00	0.02

Conclusion and Implications

This study examined the yield, input use, returns, break-even output and resource use efficiency in paddy cultivation in East Godavari district of Andhra Pradesh. At district level, total variable cost per hectare was Rs.65160.22 whereas total cost of cultivation per hectare was Rs.97884.09. Total variable cost accounts for 66.57% to the total cost. Labour cost constitutes around 63 % of the total variable cost. At district level, total returns from paddy crop were Rs.79394.81 per hectare. In present situation, it is observed that the actual yield was less than BEO. Cobb-Douglas production function analysis revealed that, land was the highest predictor of the productivity level which states its continued importance in agriculture. Resource use efficiency analysis indicated over use of inputs other than the land.

Developing suitable short duration rice varieties may help in reducing cost of cultivation. Efforts need to be taken to encourage farmers to carry out cultivation collectively so as to make paddy cultivation economically more remunerative. Farmers need to be educated regarding optimal resource use. The proposed increase in MSP can improve viability of paddy cultivation, provided it is fixed based on regional cost of cultivation / cost of production as it is evident from the present study.

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Notice for withdrawing of a published manuscript

Based on email request from the corresponding author, the manuscript entitled "Morphological and Physiological Studies in Rice Cultivars Reveal Critical Role of Root Length and Photosynthetic Rate in Adaptation to Aerobic Conditions" authored by Phule *et al.* in Vol. 10, Issue # 2 of the Journal stands withdrawn.

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Scope: Journal of Rice Research is a channel for publication of full length papers covering results of original research, invited critical reviews or interpretative articles related to all areas of rice science, rice based crop systems and rice crop management. The journal also publishes occasional short communications, book reviews and letters to the editor.

Articles reporting experimentation or research in any field involving rice or rice based cropping systems will be accepted as original articles while critical reviews are generally invited. Short articles concerned with experimental techniques or observation or observation of unique nature will be accepted as short communication. Letters to the editor concerning previous articles are welcome and are published subject to review and approval by the editorial board. The original authors will be invited to reply to the points raised in these letter for their response which are also published together.

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Submission to the journal must be reports of original research of at least two crop seasons and must not be previously published or simultaneously submitted to any other scientific or technical journal. At least one of the authors (in case of joint authorship) should be member of the Society for Advancement of Rice Research and not in arrears of subscription. Authors of invited articles are exempted from this.

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Manuscripts should be sent online to the Journal office to rms_28@rediffmail.com; rmsundaram34@gmail.com as an attachment. All the enclosed figures (as ppt files), graphs (as MS Excel worksheet with original data) and photographs (as jpg or ppt files with high resolution) may be submitted as separate files. Avoid using more than one font. The manuscript should be typed in double spaced with margins of at least 2.5 cm. On the first page give the title, a byline with the names of authors, their affiliation and corresponding author's e-mail ID. Abstract should be followed by a list of key words, and abbreviations used in the paper. The usual order of sections to be included after title and abstract pages are: Introduction which includes literature review; materials and methods; results and discussion; conclusion (optional), acknowledgements and references followed by figures and tables.

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Figures: Photographs and drawings for graphs and charts should be prepared with good contract of dark and light. Figure caption should be brief specifying the crop or soil, major variables presented and lace and year. Give careful attention to the width of lines and size, and clarity of type and symbols.

Tables: Tables are used for reporting extensive numerical data in an organized manner and statistically analyzed. They should be self explanatory. Prepare tables with the word-processing tables feature and tabs or graphics boxes should not be used. Table head should be brief but complete and self contained. Define all variables and spellout all the abbreviations. An exponenetial expression (eg. x 103) in the units line is often needed to keep length of the data reasonably short, and referenced with an explanatory note. Unless otherwise required, two decimal place values are suggested. Follow the articles published in recent journal for table format.

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