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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.

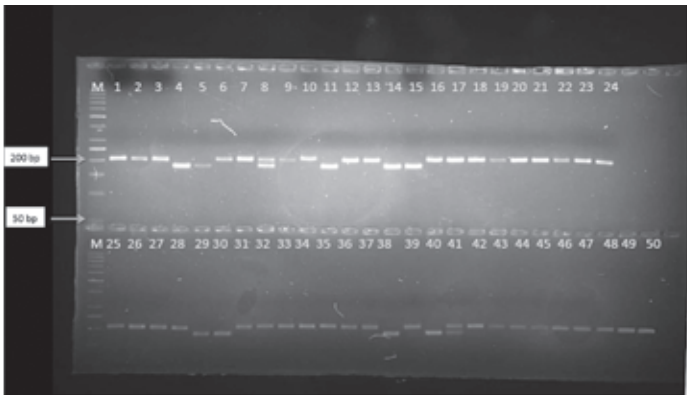

Fig.2: Amplification profile of the DNA of 50 rice genotypes using the primer RM 218

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

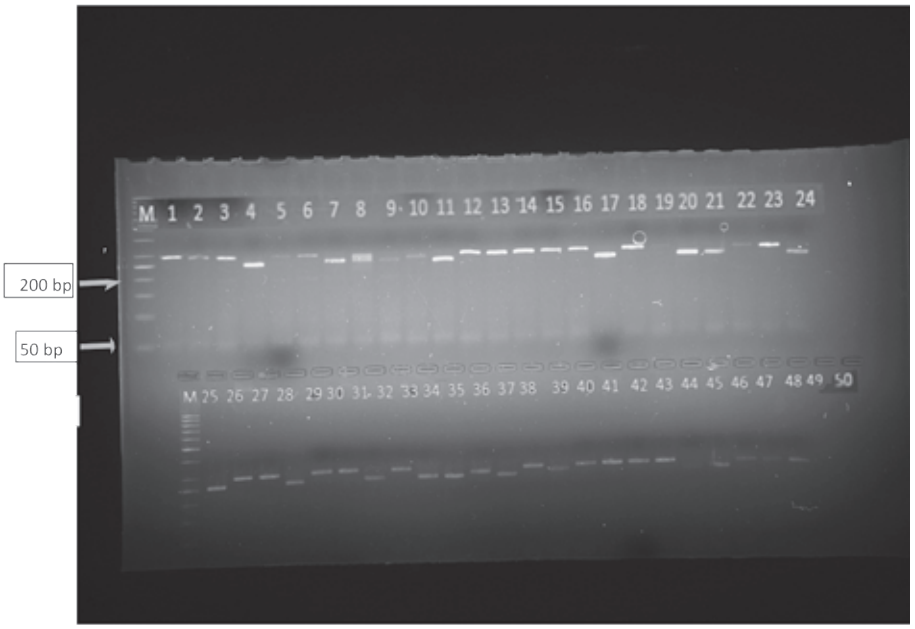

Fig.3: Amplification profile of the DNA of 50 rice genotypes using the primer RM 480

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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