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DETECTION OF POLYMORPHISM IN RICE GERMPLASM USING RAPD MARKER

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Abstract

Preliminary studies on 34 rice accessions (aromatic and non-aromatic) originated from South and Southeast Asian countries were analyzed for genetic diversity applying random amplified polymorphic DNA (RAPD) technique. Amplification of genomic DNA from these accessions using 14 random primers produced a variety of RAPD patterns. UPGMA based cluster analysis resolved the accessions into three major clusters ranging from 47.83 to 97.52 similarity coefficients. The least similarity was shown by accession YAU-R2-1 which shared with none of the other clusters (a red pericarp accession belonging to China). The mutants in cluster A (Pakistani origin) showed more than 94% similarity among themselves whereas the accessions belonging to Philippines were grouped in two clusters. Cluster B consisted of 15 accessions (land races) with similarity coefficients ranging from 62.0 to 93.6% while 6 accessions were grouped in cluster C (mutants along with their parents) with similarity coefficients ranging from 72.9% and 87.1%. This might be an indicative of a relatively broader genetic base as compared to the germplasm belonging to Pakistan. This study demonstrated the ability of RAPD markers to reliably differentiate between different accessions of diverse origin and also represented an initial but important step in using RAPD markers as a tool for evaluating genetic diversity in rice germplasm.

Introduction

Rice (*Oryza sativa* L.) is life-blood of the Asia-Pacific Region where 56% of humanity lives, producing and consuming more than 90% of the world's rice. The demand for rice is expected to grow faster than the production in most of the countries. How the level of annual production of 524 million tonnes could be increased to 700 million tonnes by the year 2025 using less land, less water, less manpower and fewer agro-chemicals is a big question. Alternative ways to meet the challenge by horizontal and vertical growth have their own prospects and limitations. Based on this scenario, bridging of the yield gap for producing more rice appears to be promising. Improving the productivity of rice systems would contribute to hunger eradication, poverty alleviation, national food security and economic development. In Pakistan, rice occupies about 10% of the total cultivated area, accounts for 6.1% of value added in agriculture and 1.3% in gross domestic product (GDP). Production of rice during 2005–2006 was estimated at 5,547 thousand tonnes, 10.4% higher than last year with 6.1% increase in yield per hectare (Anon., 2006a). It is planted on an area of 2,621 thousand hectares with grain yield of 2,116 kg ha^{-1} (Anon., 2006b).

Over the last two decades, humanity has acquired biological knowledge that allows it to tamper with the very nature of creation. Biotechnological developments (James, 1997) are poised to complement and speed up the conventional rice improvement approaches in many areas (Khush, 1995), which could have immediate and long term impacts on breaking the yield ceiling, stabilizing the production and making rice nutritionally superior. In summary, the tools of genetic engineering will help to increase and stabilize

rice yields under varied situations of its growing, and thereby reducing the yield gap. These tools could be used to introduce superior kind of plants through wide hybridization, anther culture, marker aided selection, and transformation. These tools and tagging of quantitative trait loci would help enhance the yield potential. Rice transformation enables the introduction of single genes that can selectively perturb yield-determining factors. Approaches like differential regulation of a foreign gene in the new host for partitioning sucrose and starch in leaves, the antisense approach as used in potato, and transposable elements *Ac* and Ds from maize have opened up new vistas in breaking yield barriers (Bennett *et al.,* 1994).

For any breeding programme, it is essential to study genetic diversity in the germplasm it contains. Morphological features are indicatives of the genotype but are represented only by a few loci because there is not a large enough number of characters available. Moreover, they can be affected by environmental factors and growth practices. A large number of methodologies are available for the assessment of genetic variability, diversity and interrelationships in the germplasm, as well as for individualization through macromolecular fingerprinting. While the protein-based technologies are influenced by environment and more complex to analyze, the DNA-based ones have provided reliable tools, enabling not only the assessment of genetic variability but also a high-through-put individual DNA typing (Bligh, 2000). The genetic potential of germplasm cannot be assessed on phenotype alone and that marker methods will assist evaluation (Garris *et al*., 2004; Rajapakse, 2003). Molecular markers can also be used to detect relationships between the various accessions of an individual taxon (Dehmer, 2003). These molecular markers have been developed based on the more detailed knowledge of genome structure and considerable emphasis has been laid on the use of molecular markers in practical breeding and genotype identification (Ovesna *et al*., 2002). To have an accurate and reliable estimate of genetic relationships/diversity, the random amplified polymorphic DNA (RAPD) technique of William *et al*., (1990) provides unlimited number of markers which can be used for various purposes. In addition to technical simplicity and speed of RAPD methodology (Gepts, 1993), its level of genetic resolution is equivalent to restriction length polymorphism for determining genetic relationships (Dos Santos *et al*., 1994; Hallden *et al.,* 1994). RAPD markers have been successfully used for the estimation of genetic similarities and cultivars analyses of various plant species including rice (Yu & Nguyen, 1994; Mackill, 1995).

The understanding and knowledge of genetic variation and genetic similarities present within individuals or populations are useful for the efficient use of genetic resources in a breeding program. The breeder can use the genetic similarity information to complement phenotypic information in the development of breeding populations (Smith *et al*., 1990; Nienhuis *et al*., 1993; Galvan *et al*., 2001; Pengelly & Liu, 2001; Greene *et al*., 2004). The objectives of this study were to estimate the genetic similarity/diversity in the rice germplasm belonging to South and Southeast Asian countries of diverse agro-climates.

Materials and Methods

Seeds of 34 accessions (aromatic and non-aromatic) originated in the South and Southeast Asian regions of diverse agro-climates were selected and grown in the glass house at the Philippine Rice Research Institute (PhilRice), the Philippines. The genomic DNA from the rice seedlings was extracted in the laboratory following mini-prep CTAB (cetyldimethyl triethylammonium bromide) method.

DNA extraction: After two weeks of germination, leaf samples weighing 1-2 gm of fresh weight each were collected and cut away both ends of the leaves leaving 3-5 cm long sample for DNA extraction. The samples were kept in a tube with water until before grinding. Each sample was ground to a very fine powder in liquid nitrogen and transferred to a chilled (−20ºC) 2 ml centrifuge tube. Then added 665 µl of extraction buffer without SDS and subjected the samples to Vortex to suspend avoid samples. 35 µl of 20% Sodium dodecyl sulphate (SDS) was added in each sample, incubated no longer than 10 minutes at 65ºC in water bath. Added 115µl of 5M NaCl, mixed well by gentle inversion until samples were evenly suspended, added 90 µl of 10% CTAB (10% CTAB in 0.7M NaCl) and incubated for 10 minutes at 65 ^{0}C. Added 900 µl Chloroform and mixed well, centrifuged at 12000 rpm (rotation per minute) for 2 minutes. Collected the supernatant (upper layer), transferred in a new tube, added 600 µl isopropanol, spun down of fish out the DNA when visible strands were formed. Rinsed with 70% ethanol and spun down at 12000 rmp for 2 min., and the DNA was air-dried. Added 50 µl of 1 X TE buffer, spun down at 12000 rmp for 1 min., added 3 µl RNase and kept in ice. 1µl sample was taken and 9 µl TE buffer was added to make 10 µl volume of the sample. After this treatment, in 1µl of each sample, 4 µl of Blue Juice were added for measuring the concentration of DNA using UV-visible spectrophotometer. DNA was diluted in sterile distilled water to a concentration of 12.5 ng/µl for PCR analysis.

PCR and primers: Random decamer primers (Invitrogen Tech-Line Inc.) were dissolved in sterilized distilled water at a concentration of 25 ng/µl. Fourteen primers belonging to Operon kits OPA (5 primers; OPA-09, OPA-10, OPA 11, OPA-13, OPA-20), OPB (3 primers; OPB-10 OPB-12 OPB-18), OPC-12, OPD-20, OPE (2 primers; OPE-01, OPE-17), OPF (4 primers; OPF-09, OPF-13, OPF-15, OPF-16) and OPJ (2 primers; OPJ-01, OPJ-15) were employed for PCR amplification.

Amplifications were carried out in a 25-µl reaction volume containing 1.50 µl of 10X PCR buffer [(100 mM Tris-HCl, Ph 8.3 at 25^{o}C), (500 mM KCl, 0.01% gelatin)], 0.90 µl of 25mM $MgCl_2$, 0.15 µl of 10mM dNTPs (10 mM each of dATP, dGTP, dCTP and dTTP), 0.05 µl of unit *Taq* DNA polymerase, 1.00 µl of 25 mM primer, 5.00 µl of 12.5ng/µl-template DNA and 6.40 µl of sdH_2O. The reaction mixture was overlaid with one drop of mineral oil to avoid evaporation. The amplifications were carried out in A Perkin Elmer Thermal Cycler 480, programmed for denaturation steps of 2 min., at 94^{o}C and of 1 min., at 94^{o}C, annealing for 1 min., at 35^{o}C and for 2 min., at 72^{o}C and then followed by 45 cycles for 1 min., up to 2nd step of denaturation, extension for 2 min., at 72^{o}C. After completion of all the steps, final extension step was kept at 72^{o}C for 5 min., and then held at 4^{o}C until the PCR products were separated on 1.5% agarose gel (1.5 g agarose/100 ml of 1X TE buffer, stained with 5 µl of Ethidium bromide) along with 1 kb ladder at 100 voltage.

Analysis of amplified profiles: Amplified profiles of 34 rice accessions were compared with each other and bands of DNA fragments were scored as present (1) or absent (0). The data obtained for all the 14 primers was used to estimate the similarity on the basis of the shared amplified products following Dice's coefficient of similarity. A dendrogram based on similarity coefficients was generated using the unweighted pair group method of arithmetic means (UPGMA) by NTSYS software.

Results and Discussion

The amplification profiles of 34 rice accessions with 14 primers were polymorphic and can be used for varietal identification. The maximum polymorphism was shown by the primer OPJ-01 i.e., for 13 accessions. DNA of individual seedlings/young plants were isolated for each accession and used for PCR amplification. The 14 primers studied amplified a total of 108 DNA fragments. Of these, 36 (33.3%) were not polymorphic. Rests of the 66.7% bands were polymorphic in one or other of the 34 accessions. The amplitude of polymorphism was almost intermediate (66.7% bands were polymorphic and 100% of the primers produced polymorphic profiles). There was not a single primer (out of 14 primers studied) that could differentiate clearly among all the accessions. This might be indicative of a narrow genetic base for most of the rice accessions studied.

The levels of polymorphism were different with different primers among different accessions (Fig. 1a, b and c). The accession YAU-R2-1 can be clearly distinguished from other accessions. Only 44 fragments were amplified from YAU-R2-1 genomic DNA with 14 primers as compared to the total of 108 amplified fragments. The lowest number of amplified fragments was 44 from YAU-R2-1. The number of amplified fragments from rest of the accessions ranged from 46 to 72 with the maximum of 72 from accession Sinampaguita. The number and size of amplified fragments also varied with different primers. A maximum number of 16 fragments were amplified with primer OPE-17 while the primer OPA-13 produced only one fragment. The main objective of this study was to estimate the genetic similarity/diversity in rice germplasm of diverse origin. The similarity matrix obtained after multivariant analysis using Dice's coefficient of similarity is shown in Table 1. Similarity matrix data revealed that similarity ranged between 47.83% and 97.52% among all the accessions. From the similarity matrix, the least similarity was shown by accession YAU-R2-1 (a red pericarp accession belonging to China). Its similarity ranged from 47.83% with Basmati 385 to 73.27% with IR 8. The lowest similarity of this accession with other accessions might be due to the fact that it belongs to old world whereas rests of the accessions belong to South and Southeast Asia. In the dendrogram, YAU-R2-1 did not cluster with any of the other accessions tested and was easily distinguishable. Japayuki (a traditional aromatic accession belonging to Philippine) was also distinct and its similarity varied from 57.50% with YAU-R2-1to 79.61% with Magueg (a traditional aromatic accession belonging to Philippine). Although the number of fragments amplified from YAU-R2-1 and Japayuki were 44 and 46 respectively, these accessions were only 57.50% similar with each other. It means that more than 50% of the amplified fragments were polymorphic. These results are indications of reliability of the RAPD technique for the evaluation of genetic similarities. These similarity coefficients were used to generate dendrogram (Fig. 2) following unweighted pair group method of arithmetic means (UPGMA) method in order to determine the grouping of different accessions. In the dendrogram, all the accessions were grouped in three main clusters except accession YAU-R2-1 that belonged to China. The accessions belonging to Pakistan were grouped in cluster A (12 accessions). In this cluster (Table 2), similarity coefficients ranged from 68.33 to 97.52%. The mutants Kashmir Basmati and DM 25 showed similarities of 97.39% and 94.49% respectively with Basmati 370 (parent). Both these mutants were 97.25% similar with each other. Mutant EL 30-2-1 was 95.00% similar with its parent Basmati Pak. Mutant DM 25 and P 52-9-2 were 97.52%, similar while Kashmir Basmati was 97.39% and 94.74% similar with P 52-9-2 and EL 30-2-1 respectively. All these mutants/lines had been developed at the same breeding center. Based on the analysis, their genetic base looked very narrow.

(a)

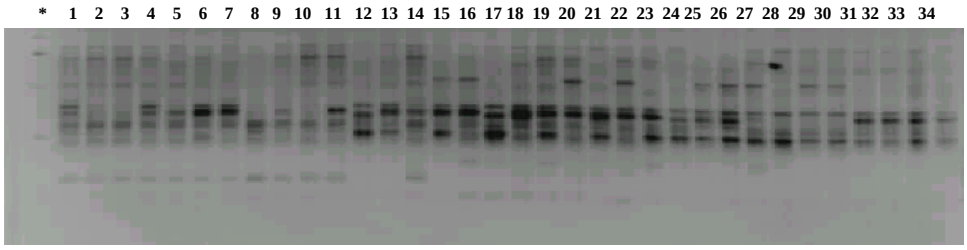

Fig. 1(a): Amplification of RAPD marker OPE-17 for rice accessions (1. EL 30-2-1,2. Basmati 370,3. Kashmir Basmati,4. Basmati Pak,5. Basmati 2000, 6. EF 2-3, 7. Basmati 385,8. P 38-6-1,9. Super Basmati,10. P 52-9-2,11. DM 25,12. IR 6,13. IR 8,14. Niab-Irri-9,15. IR 64,16. PERYA,17. PSC 23-3, 8. Magnolia,19. Magueg, 20. Kinabuong, 21. Sinampaguita, 22. Camoros, 23. Dinorado, 24. IR 58025 B, 25. YAU-R2-1, 26. PR 29164-R2-1-1-25Kr, 27. Japayuki, 28. PR30256-Jap-1-25Kr-195-1-1, 29. Matatag 1, 30. IR 69726-116-25kR-243-3-3, 31. Pulang Humot, 32. PR 30220-1-1-25kR-300-1-1, 33. Sigadis Milagrosa, 34. PSB RC 78)

(b)

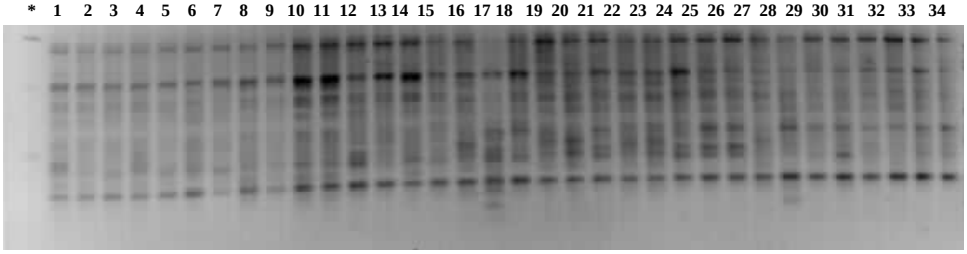

Fig. 1(b): Amplification of RAPD marker OPD-20 for rice accessions (1. EL 30-2-1,2. Basmati 370,3. Kashmir Basmati,4. Basmati Pak,5. Basmati 2000, 6. EF 2-3, 7. Basmati 385,8. P 38-6-1,9. Super Basmati,10. P 52-9-2,11. DM 25,12. IR 6,13. IR 8,14. Niab-Irri-9,15. IR 64,16. PERYA,17. PSC 23-3, 18. Magnolia,19. Magueg, 20. Kinabuong, 21. Sinampaguita, 22. Camoros, 23. Dinorado, 24. IR 58025 B, 25. YAU-R2-1, 26. PR 29164-R2-1-1-25Kr, 27. Japayuki, 28. PR30256-Jap-1-25Kr-195-1-1, 29. Matatag 1, 30. IR 69726-116-25kR-243-3-3, 31. Pulang Humot, 32. PR 30220-1-1-25kR-300-1-1, 33. Sigadis Milagrosa, 34. PSB RC 78).

(c)

Fig. 1(c): Amplification of RAPD marker OPA-10 for rice accessions (1. EL 30-2-1,2. Basmati 370,3. Kashmir Basmati,4. Basmati Pak,5. Basmati 2000,6. EF 2-3, 7. Basmati 385,8. P 38-6-1,9. Super Basmati,10. P 52-9-2,11. DM 25,12. IR 6,13. IR 8,14. Niab-Irri-9,15. IR 64,16. PERYA,17. PSC 23-3,18. Magnolia,19. Magueg, 20. Kinabuong, 21. Sinampaguita, 22. Camoros, 23. Dinorado, 24. IR 58025 B, 25. YAU-R2-1, 26. PR 29164-R2-1-1-25Kr, 27. Japayuki, 28. PR30256-Jap-1-25Kr-195-1-1, 29. Matatag 1, 30. IR 69726-116-25kR-243-3-3, 31. Pulang Humot, 32. PR 30220-1-1-25kR-300-1-1, 33. Sigadis Milagrosa, 34. PSB RC 78).

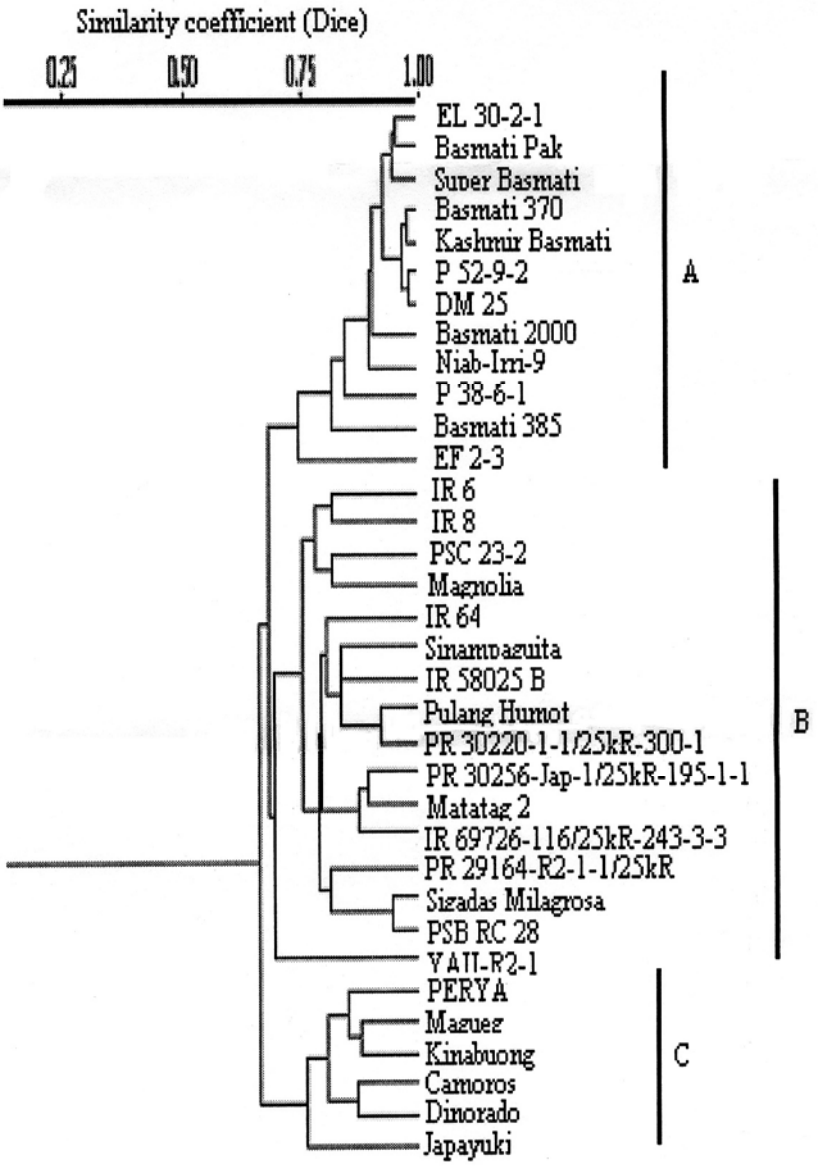

Fig. 2. Dendrogram showing genetic relationships among the 34 rice accessions analyzed by RAPD. Clustering was based on the Dice similarity coefficients between accessions.

Sun *et al*., (2001) comparing microsatellite and RAPD polymorphism in corn hybrids reported that most of the hybrids from the same company were closely related to each other. Iqbal *et al*., (1997) reported in cotton that the intervarietal genetic relationships of several varieties related to the center of origin. Nagaraju *et al*., (2002) also observed lowest genetic diversity in the traditional basmati (TB) rice varieties as compared to evolved basmati (EB) and non-basmati (NB) rice varieties.

Table 1. Similarity matrix for 34 rice accessions originated from divers origin.

Accession	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34
1	1.0000000																																	
2	0.9268293	1.0000000																																
3	0.9473684	0.9739130	1.0000000																															
4	0.9500000	0.9076923	0.9565217	1.0000000																														
5	0.9203540	0.8870968	0.9056604	0.9166667	1.0000000																													
6	0.7155963	0.7933884	0.7647059	0.7179487	0.7500000	1.0000000																												
7	0.8288288	0.8196721	0.8076923	0.8135593	0.8214286	0.7339450	1.0000000																											
8	0.8761905	0.8672566	0.8659794	0.8148148	0.7640759	0.7450980	0.7592593	1.0000000																										
9	0.9411765	0.8992248	0.9189189	0.9440000	0.9075630	0.7241379	0.8000000	0.8288288	1.0000000																									
10	0.9180328	0.9430894	0.9739130	0.9243697	0.8672566	0.7522936	0.8173913	0.8703704	0.9090909	1.0000000																								
11	0.9401709	0.9446819	0.9724771	0.9193548	0.8813559	0.7543860	0.8333333	0.8363636	0.8976378	0.9752066	1.0000000																							
12	0.6440678	0.6400000	0.6726972	0.6115702	0.6206897	0.7142857	0.6153846	0.6545455	0.6016260	0.6557377	0.6341463	1.0000000																						
13	0.6721311	0.7218045	0.7272727	0.6821705	0.6774194	0.7333333	0.6240000	0.6666667	0.6870229	0.7200000	0.7230769	0.8125000	1.0000000																					
14	0.9280000	0.8571429	0.8965517	0.8992248	0.8455285	0.6833333	0.7936508	0.8275862	0.9090909	0.8992248	0.8787879	0.6511628	0.6861314	1.0000000																				
15	0.6363636	0.6991870	0.6866667	0.6386555	0.6956522	0.7272727	0.6140351	0.5849057	0.6178862	0.6434783	0.6666667	0.7179487	0.7460317	0.6190476	1.0000000																			
16	0.6551724	0.7244094	0.7102804	0.6666667	0.6495726	0.6491228	0.6000000	0.6607143	0.6507937	0.7166667	0.7200000	0.5853659	0.7022901	0.6515152	0.7213115	1.0000000																		
17	0.6101695	0.6511628	0.6239532	0.6240000	0.6166667	0.7068966	0.6065574	0.6315789	0.5781250	0.6229508	0.6141732	0.7680000	0.7819549	0.6119403	0.8032787	0.6875000	1.0000000																	
18	0.6666667	0.6942149	0.6972477	0.6440678	0.6964286	0.7102804	0.6017699	0.6666667	0.6166667	0.6612903	0.6610169	0.7666667	0.7903226	0.6406250	0.7610619	0.6722689	0.8099174	1.0000000																
19	0.7377049	0.7518797	0.7678571	0.7286822	0.6829268	0.6833333	0.6240000	0.6837607	0.6870229	0.7360000	0.7575758	0.6406250	0.7205882	0.6861314	0.6507937	0.8244275	0.6917293	0.6935484	1.0000000														
20	0.6666667	0.7200000	0.7238095	0.6611570	0.6434783	0.6785714	0.5486726	0.6346154	0.6333333	0.7017544	0.7118644	0.5862069	0.7096774	0.6129032	0.6842105	0.8644068	0.6666667	0.6785714	0.8709677	1.0000000													
21	0.7360000	0.7737226	0.7610619	0.7067669	0.7244094	0.7419355	0.7286822	0.6666667	0.6814815	0.7266822	0.7407407	0.7121212	0.8142857	0.6950355	0.7812500	0.6666667	0.7591241	0.8188976	0.7000000	0.6718750	1.0000000												
22	0.6428571	0.7049180	0.6730769	0.6271186	0.5882857	0.6055046	0.6086957	0.6542056	0.6115702	0.7008547	0.7166667	0.5762712	0.6825397	0.6141732	0.6896552	0.8264463	0.6829268	0.6782609	0.7777778	0.8318584	0.6769231	1.0000000											
23	0.6095238	0.6491228	0.6464646	0.6000000	0.5576923	0.5940594	0.5882353	0.6736842	0.5871560	0.6476190	0.6542056	0.5904762	0.6194690	0.6194690	0.6274510	0.7663551	0.6238532	0.6407767	0.7964602	0.7809524	0.5811966	0.8627451	1.0000000										
24	0.6666667	0.7130435	0.7111111	0.6545455	0.6666667	0.6923077	0.6909091	0.5818182	0.6371681	0.7169811	0.7207207	0.7678571	0.8067227	0.6779661	0.7962963	0.6842105	0.7586207	0.7619048	0.6554622	0.6415094	0.8320000	0.6422018	0.5567010	1.0000000									
25	0.5252525	0.5544554	0.5454545	0.4948454	0.4635165	0.5617978	0.4782609	0.5833333	0.5154639	0.5714286	0.5376344	0.6938776	0.7326733	0.5686275	0.6067416	0.5894737	0.6597938	0.6530612	0.5940594	0.5652174	0.6603774	0.5054945	0.4941176	0.7096774	1.0000000								
26	0.7083333	0.7358491	0.7674419	0.7254902	0.7070707	0.7346939	0.6930693	0.6600000	0.6666667	0.7234043	0.7272727	0.7378641	0.7850467	0.6666667	0.7474747	0.7184466	0.7663551	0.7083333	0.7522936	0.7000000	0.7964602	0.6464646	0.5652174	0.7572816	0.7692308	1.0000000							
27	0.7047619	0.6666667	0.6597938	0.6601942	0.6185567	0.6153846	0.6170213	0.6818182	0.6470588	0.6923077	0.7142857	0.6597938	0.6407767	0.7037037	0.6956522	0.7628866	0.6734694	0.6534653	0.7961165	0.7291667	0.6037736	0.7526882	0.7356322	0.6588235	0.5750000	0.6829268	1.0000000						
28	0.6611570	0.6721311	0.6851852	0.6218487	0.6546673	0.7037037	0.6260870	0.6250000	0.6065574	0.6666667	0.6611570	0.7540984	0.7777778	0.6769231	0.7826087	0.6280992	0.7967480	0.7419355	0.6346206	0.6194690	0.7906977	0.5982906	0.5384615	0.7522936	0.6930693	0.7422680	0.6019417	1.0000000					
29	0.6833333	0.7272727	0.7358491	0.6718750	0.7049180	0.7563025	0.6666667	0.6333333	0.6666667	0.6984127	0.7164179	0.6976744	0.7591241	0.6956522	0.7812500	0.6515152	0.7014925	0.6774194	0.6715328	0.6666667	0.8065106	0.6141732	0.5535714	0.7377049	0.6930693	0.7777778	0.5882353	0.8888889	1.0000000				
30	0.6666667	0.6964286	0.7010309	0.6238532	0.6078431	0.6800000	0.6139614	0.6734694	0.6238532	0.6846847	0.6478190	0.7339450	0.7256637	0.6956522	0.7551020	0.6415094	0.7289720	0.6972477	0.6666667	0.6666667	0.7586207	0.6213592	0.5833333	0.7157895	0.7472527	0.7272727	0.6086957	0.8468468	0.8828829	1.0000000			
31	0.7288136	0.7384615	0.7500000	0.6825397	0.6942149	0.7268136	0.6829268	0.6606996	0.6666667	0.6991870	0.7343750	0.7460317	0.8358209	0.6814815	0.8064516	0.6923077	0.7692308	0.7438017	0.7164179	0.7213115	0.8115942	0.6666667	0.6181818	0.8135593	0.7010309	0.7547170	0.6862745	0.8455285	0.8444444	0.8224299	1.0000000		
32	0.7422680	0.7547170	0.7654321	0.6923077	0.6930693	0.7291667	0.7474747	0.7096774	0.6990291	0.7368421	0.7600000	0.6666667	0.7924528	0.7299720	0.8163265	0.6930693	0.7307692	0.7157895	0.7358491	0.7070707	0.8440367	0.6875000	0.6292135	0.8453608	0.6585366	0.7524752	0.6896552	0.8085106	0.8269231	0.8235294	0.9158879	1.0000000	
33	0.7378641	0.8034188	0.8000000	0.7500000	0.7407407	0.7735849	0.6846847	0.6666667	0.7179487	0.7407407	0.7610619	0.7272727	0.8166667	0.7058824	0.8034188	0.7586207	0.7719298	0.7924528	0.7226891	0.7663551	0.8225806	0.6788991	0.6391753	0.7857143	0.6956522	0.8367347	0.8590909	0.8181818	0.8387097	0.8260870	0.8500000	0.8333333	1.0000000
34	0.7111111	0.7500000	0.7857143	0.7474747	0.7021277	0.6947368	0.6530612	0.6000000	0.6923077	0.7157895	0.7326733	0.6200000	0.7339450	0.6603774	0.7184466	0.7169811	0.7047619	0.7083333	0.7339450	0.7551020	0.7636364	0.6464646	0.6136364	0.7326733	0.6823529	0.7764706	0.6315789	0.7216495	0.7927928	0.8095238	0.8148148	0.7951807	0.9357798	1.0000000

(1. EL 30-2-1,2. Basmati 370,3. Kashmir Basmati,4. Basmati Pak,5. Basmati 2000,6. EF 2-3,7. Basmati 385,8. P 38-6-1,9. Super Basmati,10. P 52-9-2,11. DM 25,12. IR 6,13. IR 8,14. Niab-Irri-9,15. IR 64, 16. PERYA, 17. PSC 23-3,18. Magnolia,19. Magueg, 20. Kinabuong, 21. Sinampaguita, 22. Camoros, 23. Dinorado, 24. IR 58025 B, 25. YAU-R2-1, 26. PR 29164-R2-1-1-25Kr, 27. Japayuki, 28. PR30256-Jap-1-25Kr-195-1-1, 29. Matatag 1, 30. IR 69726-116-25kR-243-3-3, 31. Pulang Humot, 32. PR 30220-1-1-25kR-300-1-1, 33. Sigadis Milagrosa, 34. PSB RC 78)

Table 2. Similarity matrix for 12 rice accessions originated in Pakistan.

Accession	EL 30-2-1	Basmati 370	Kashmir Basmati	Basmati Pak	Basmati 2000	EF 2-3	Basmati 385	P 38-6-1	Super Basmati	P 52-9-2	DM 25	Niab-Irri-9
EL 30-2-1	1.0000											
Basmati 370	0.9268	1.0000										
Kashmir Basmati	0.9474	0.9739	1.0000									
Basmati Pak	0.9500	0.9077	0.9565	1.0000								
Basmati 2000	0.9204	0.8871	0.9057	0.9167	1.0000							
EF 2-3	0.7156	0.7934	0.7647	0.7179	0.7500	1.0000						
Basmati 385	0.8288	0.8197	0.8077	0.8136	0.8214	0.7339	1.0000					
P 38-6-1	0.8762	0.8673	0.8660	0.8148	0.7647	0.7451	0.7593	1.0000				
Super Basmati	0.9412	0.8992	0.9189	0.9440	0.9076	0.7241	0.8000	0.8288	1.0000			
P 52-9-2	0.9180	0.9431	0.9739	0.9244	0.8673	0.7523	0.8174	0.8704	0.9091	1.0000		
DM 25	0.9402	0.9449	0.9725	0.9194	0.8814	0.7544	0.8333	0.8364	0.8976	0.9752	1.0000	
Niab-Irri-9	0.9280	0.8571	0.8966	0.8992	0.8455	0.6833	0.7937	0.8276	0.9091	0.8992	0.8788	1.0000

Table 3. Similarity matrix for 15 rice accessions originated in Philippine.

Accession	IR 6	IR 8	PSC 23-3	Magnolia	IR 64	Sinampaguita	IR 58025 B	Pulang Humot	PR 30220-1-1-25kR-300-1-1	PR30256-Jap-1-25Kr-195-1-1	Matatag 1	IR 69726-116-25kR-243-3-3	PR 29164-R2-1-1-25kR	Sigadis Milagrosa	PSB RC 78
IR 6	1.0000														
IR 8	0.8125	1.0000													
PSC 23-3	0.7680	0.7820	1.0000												
Magnolia	0.7667	0.7903	0.8099	1.0000											
IR 64	0.7179	0.7460	0.8033	0.7611	1.0000										
Sinampaguita	0.7121	0.8143	0.7591	0.6786	0.7813	1.0000									
IR 58025 B	0.7679	0.8067	0.7586	0.7619	0.7963	0.8320	1.0000								
Pulang Humot	0.7460	0.8358	0.7692	0.7438	0.8065	0.8116	0.8136	1.0000							
PR 30220-1-1-25kR-300-1-1	0.6869	0.7925	0.7308	0.7158	0.8163	0.8440	0.8454	0.9159	1.0000						
PR30256-Jap-1-25Kr-195-1-1	0.7541	0.7778	0.7967	0.7419	0.7826	0.7907	0.8523	0.8455	0.8085	1.000					
Matatag 1	0.6977	0.7591	0.7015	0.6774	0.7813	0.8085	0.7377	0.8444	0.8269	0.8889	1.0000				
IR 69726-116-25kR-243-3-3	0.7339	0.7257	0.7290	0.6972	0.7551	0.7586	0.7158	0.8224	0.8829	0.8468	0.8829	1.0000			
PR 29164-R2-1-1-25kR	0.7379	0.7850	0.7664	0.7083	0.7475	0.7965	0.7473	0.7547	0.7525	0.7423	0.7778	0.7273	1.0000		
Sigadis Milagrosa	0.7273	0.8167	0.7719	0.7925	0.8034	0.8228	0.7857	0.8500	0.8333	0.8182	0.8387	0.8261	0.8367	1.0000	
PSB RC 78	0.6200	0.7339	0.7048	0.7083	0.7184	0.7636	0.7327	0.8148	0.7952	0.7216	0.7928	0.8095	0.7765	0.9358	1.0000

Table 4. Similarity matrix for 6 rice accessions originated in Philippine.

Accession	PERYA	Magueg	Kinabuong	Camoros	Dinorado	Japayuki
PERYA	1.0000					
Magueg	0.8244	1.0000				
Kinabuong	0.8644	0.8710	1.0000			
Camoros	0.8264	0.7778	0.8319	1.0000		
Dinorado	0.7664	0.7965	0.7810	0.8627	1.0000	
Japayuki	0.7629	0.7961	07292	0.7527	0.7356	1.0000

The accessions belonging to Philippines were grouped in two clusters. Cluster B consisted of 15 accessions while 6 accessions were grouped in cluster C. In cluster B (Table 3), similarity coefficients ranged between 62.00% and 93.58%. Mutants PSB RC 78, PR 30220-1-1-25kR-300-1-1 and IR 69726-116-25kR-243-3-3 were 93.58%, 91.59% and 88.29% similar with their respective parents Sigadis Milagrosa, Pulong Humot and Matatag 1. The mutants IR 69726-116-25kR-243-3-3 and PR 30220-1-1-25kR-300-1-1developed at the same breeding center were 88.29% similar with each other though they were derived from non-aromatic and aromatic background respectively. In cluster C (Table 3), similarity coefficients ranged between 72.92% and 87.10%. Accession Kinabuong was 87.10% similar with Magueg followed by 86.44% with PERYA while accession Magueg was 82.44% similar with PERYA. The accessions Camoros and Dinorado were 86.27% similar with each other. These results are in accordance with the known genetic make-up of the accessions. From this study, it is obvious that the mutants included in cluster A showed more than 94% similarity among themselves due to the fact that these mutants were developed at the same breeding center. The accession YAU-R2-1 which shared with none of the other clusters belonged to diverse origin (China). The accessions belonging to Philippines grouped in two clusters. Most of the land races were grouped in the same cluster whereas mutants along with their parents grouped in a separate cluster. This might be an indicative of a relatively broader genetic base as compared to the germplasm belonging to Pakistan. The accessions used in this study comprised of elite mutants/lines, commercial varieties and traditional land races. The results were indicative of their genetic relationships. The genetic similarities obtained from the analysis may be used for the selection of parents to generate mapping population and help in the selection of desired parents for breeding programme.

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