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DIVERSITY AND CORRELATION ANALYSIS IN BASMATI RICE GERMPLASM

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Abstract

Genetic diversity based on Simple Sequence Repeat (SSR) markers and seed morphological traits was assessed in a set of 50 Basmati rice accessions. Furthermore, correlation analysis was also performed for seed morphological traits. Minimum (0.344) and maximum (0.763) PIC values were observed from primers 'RM255' and 'RM249', respectively. For molecular diversity analysis data from 15 polymorphic SSR primer pairs were used to determine total number of alleles (3.53), polymorphic alleles (3.40), polymorphism % (92.77) and polymorphic information content (0.554). Correlation analysis among seed morphological traits revealed positive association of seed length (SL) with seed length-width ratio (L/W) and 1000-grain weight (0.834^{**}, 0.099^{**}, respectively). On the other hand, seed thickness showed positive significant association with seed width and 1000-grain weight with seed thickness (0.254^{**}, 0.069^{**}, respectively). Based on results, the germplasm used in this study has potential to provide genetic resources for breeding Basmati rice cultivar with improved grain yield.

Introduction

Rice (*Oryza sativa* L.) is very important cereal food crop worldwide. There are 24 species in the genus, including cultivated and wild. Two cultivated species *Oryza sativa* L. and *Oryza glabberrima* L. are widely grown due to their importance and belong to AA genome (Vaughan *et al.* 2003). Rice is also a model plant for genetic studies due to its small genome size, diploid genetics and very high genetic diverse materials (McCouch *et al.* 1998, Tanksley 1989 and Wang *et al.* 1992). At present the world rice production was 748 million tons, 1.1% more than previously reported and the area was sown 163.1 million hectares (Anonymous. 2016).

In Pakistan rice is a major source of export earnings and adds 0.6 per cent to GDP value of Pakistan. Pakistan grows high quality rice to meet both for domestic and export demands. In the year 2016, area was sown 2748 thousand hectares and production was 6811 thousand tons, while it is 2.7 per cent less than last year (Anonymous. 2016). Due to these facts the present study focuses on the screening of potential rice lines for high yield production and further developing of advance breeding lines having high commercial values. Rice seed morphological traits are important to consumers, scientists and farmers community, because these traits determine the physical appearance, quality and ultimately yield of the rice crop. Grain weight is the main component for determining the rice yield and cooking quality of the rice crop (Haung *et al.* 2013, Ishimaru *et al.* 2013). Furthermore, seed size and seed weight are the important traits for the evolution, domestication and selection process of cereals (Harlan 1992, Doganlar *et al.* 2000). On the other

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hand, grain shape, panicle length and seed length are the responsible traits for yield and cooking quality enhancement (Sun *et al.* 2016). All these parameters correlated with each other and having a positive association with grain weight that is responsible for the high yield potential of rice crop (Tan *et al.* 2000). Rice seed size, shape and some other quality parameters are genetically controlled by polygenes, which are also called quantitative trait loci (QTLs) (Tan *et al.* 2000). Some of these QTLs may have dominant effect on single grain trait or multiple grain traits. Seed length, seed width, seed thickness, seed length width ratio and seed weight are the desirable components of seed parameters that represents the variable changes in these traits depending upon the each rice variety and these components are controlled by various QTLs depending upon the nature of their genetic studies (Zhou *et al.* 2000). Some of these components are controlled by polygenes with either an additive or dominant effect. Rice grain weight is one of the most important traits for the enhancement the rice yield and the association of some additive and dominant genes may also involve under different environmental conditions (Ashfaq *et al.* 2012a). The genetic variability is very limited in cultivated germplasm for some of the desirable traits and their performance affected under stress condition. This is due to the repeated use of small core rice germplasm in breeding programs and genetic base of newly developed rice varieties has become narrow (Moncada *et al.* 2001). Introgression of desirable genes from other rice species can provide genetic improvement in rice and meet the challenges affecting rice production.

Examination of loss or gain of genetic diversity in existing breeding germplasm is essential and useful to pre-empt epidemics and abrupt environmental changes (Sajjad *et al.* 2017). Genetic diversity of different rice germplasm lines i.e. cultivar, landraces, varieties and other advance lines can be determined by using the different molecular markers (Parsons *et al.* 1997, Ram *et al.* 2007, Ashfaq and Khan 2012). Present study was carried out to screen out the available rice genotypes based on phenotyping discussed in previous paragraphs (various morphological and seed parameters traits) and genotyping (using various SSR primers at molecular level) to assess the genetic variability among the diverse rice germplasm that would be used in cross breeding programme and a helping tool for early screening and selection of the parents that responsible for determining the high yield with good desirable traits. Such fundamental practices will be very helpful on getting many breeding objectives and selection of best parents which may be used to develop new commercial hybrids. The reservoir of natural genetic variation found in a plant species is the primary tool for crop improvement. This genetic variation is not limited to cultivated species, but can also be found in wild relatives, landraces and other rice lines. Understanding, management and effective use of genetic variability observed in early cultivated varieties which can be effective to improve crops and to start up a new breeding programme.

Genetic diversity study of rice genotypes determined by the use of Simple Sequence Repeat (SSR) markers which helpful for establishing the association among various individuals even with less number of markers (McCouch *et al.* 1997). SSR markers have more beneficial than other types of markers due to of many applications in the field of plant molecular biology i.e. PCR based, co-dominant, highly polymorphic, high reproducibility, high degree of PIC (polymorphic information content), more useful for genetic studies, association mapping studies, allelic diversity, characterization of the germplasm, gene identification and ultimately the evolution of new rice lines with desirable genetic traits. For similar studies, SSR markers were used by Jin *et al.* (2010), Sow *et al.* (2014), Das *et al.* (2013), Choudhury *et al.* (2013), Ashfaq *et al.* (2012b). The SSR markers considered as a marker of choice for genomic and gene mapping studies that may be more useful to reveal the genetic diversity of the germplasm (Ishii *et al.* 2001)

The present study was designed to assess the genetic diversity in a panel of 50 basmati rice accessions using seed morphological and SSR data. Moreover, correlation analysis was also performed to determine the pattern of association among seed morphological traits. The

germplasm used in this study has sufficient morphological and molecular diversity to provide genetic resources for breeding basmati rice cultivar with improved grain yield.

Materials and Methods

The experiments were carried out at the Institute of Agricultural Sciences, University of the Punjab, Lahore under RCBD was used for the evaluation of 50 different rice genotypes including approved varieties, basmati lines and land races. Plant material with their collection site, growing area, geographical locations shown in the Table 1 and 50 diverse rice genotypes/lines were collected from Kala Shah Kaku. Different seed morphological traits and other parameters were studied at physical maturity of the each genotype. Fifteen different SSR primer pairs were used for the determination of genetic diversity of rice genotypes (Ashfaq *et al.* 2012a).

Genomic DNA extration: The fresh leaves of each genotype were collected from the paddy fields at seedling stage. The total DNA of each genotype was extracted from fresh leaves by the cetyl tri-methyl ammonium bromide (CTAB) method (Muray and Thompson 1980). The purity and concentration of extracted DNA of each rice genotypes were determined spectrophotometrically at 260 and 280 nm by using the Nano Drop (ND 1000 Spectrophotometer, Gene Ray Biotechnology, Shanghai, China). The DNA of all the genotypes was good quality. All the samples were diluted to a concentration of 40 ng/ul with ddH_2O for PCR analysis.

Recording the observations: The seed length, width and thickness of each genotype were measured in millimetre with Vernier Calliper. The 1000-grain of each genotype was separated and selected randomly for weighing purposes by using electric weighing balance in grams. Seed length width ratio was calculated by the following formula in millimetre.

$$\text{Seed length width ratio} = \frac{\text{Seed length (mm)}}{\text{Seed width (mm)}}$$

Statistical analysis: Genotyping was carried out through PCR techniques (Panuad *et al.*, 1996) by using 15 SSRs primer pairs under lab conditions for determination of the genetic variability among the rice genotypes (Table 4, Fig 1). The molecular analysis and calculations were done with the help of soft ware package Power Markers (Liu and Muse 2005). The phenotypic seed desirable traits were also analyzed through Principal Component Analysis (PCA) for their evaluation for further selections. Statistical analysis also confirms the classification of the rice germplasm lines on the basis of their genetic variability both on phenotypic and genotypic traits for future breeding studies.

Results and Discussion

The analysis of variance indicated significant variation ($p < 0.01$ and 0.05) in all the seed morphological traits (Table 2). All the genotypes showed significant differences among all the traits studied. Significant variation in all the traits including seed length, seed width, length width ratio and seed thickness studied indicated the presence of high genetic diversity among all the genotypes of rice. Data of mean values are not mentioned.

Correlation analysis showed significant and non-significant correlation of different seed morphological traits (Table 3). The traits showed positive and negative significant correlation with each other. All the traits mentioned in the study showed negative significant and non significant correlation with each other ($r = –0.234^{**}$, $r = –0.162^{**}$, $r = –0.452^{**}$, $r = –0.353^{**}$, $r = –0.153^{**}$

Table 1. Plant material.

Sr. No.	Germplasm number	Accession number	Variety name	Structure	Sr. No.	Germplasm number	Accession number	Variety name	Structure
1	916	0059	Basmati 443	Aromatic	26	941	0088	Kanhgra 319	Non-Aro
2	917	0060	Basmati Kamon	"	27	942	0089	Mahlar 335	"
3	918	0061	Begumi	"	28	943	0091	Rohru 414	"
4	919	0062	Hansraj 13	"	29	944	0094	N 35	"
5	920	0063	Hansraj 13 A	"	30	945	0095	RB 2	"
6	921	0064	Palman 60	Non- Aro	31	946	0097	Sufaid Munji	"
7	922	0065	Hansraj 197	"	32	947	0098	Sufaida 20	"
8	923	0065	Hansraj 197A	"	33	948	0099	Baggi Munji 22	"
9	924	0066	Begumi 302	Aromatic	34	949	0101	Kaul 29	"
10	925	0067	Lal Dhan 304	Non-Aro	35	950	0102	Bagar sugdas 34	"
11	926	0068	Mushkhan 340 A	Aromatic	36	951	0104	Sonoaitar 45	"
12	927	0070	Munji 78 B-1	Non-Aro	37	952	0106	Munji sufaid 65	"
13	928	0072	Begumi 119 A	Aromatic	38	953	0107	Mushkan 73 A	Aromatic
14	929	0074	Basmati 140	"	39	954	0108	Munji Sufaid 94	"
15	930	0075	Dhan 140 A	Non-Aro	40	955	0109	Jhona 91 A	Non-Aro
16	931	0076	Rohdu 150	"	41	956	0110	Daggar Sufaid 94	"
17	932	0077	Chipet A 200	"	42	957	0111	Coarse 99	"
18	933	0078	Chaklala 201	"	43	958	0112	Dhan Kasarwala 102-4	Aromatic
19	934	0079	K 202-17	"	44	959	0113	Dhan Kasarwala 108-31	"
20	935	0080	EB 204	"	45	960	0114	Jhona 109	Non-Aro
21	936	0081	Chaklala 211	"	46	961	0115	Kasarwala & Mundar	Aromatic
22	937	0082	Chaklala 214	"	47	962	0119	Jhona 129A	Non-Aro
23	938	0083	Chaklala 234	"	48	963	0120	Dhan Kasarwala	Aromatic
24	939	0086	Dhan 300	"	49	964	0121	Dhan 133	"
25	940	0087	Bamla Red 310-6	"	50	965	0122	Begumi 135	"

and 0.0508^{NS}). However, positive correlation was observed between seed width and seed thickness (r = 0.254**), seed length and length width ratio (r = 0.834**), seed length and 1000-grain weight (r = 0.099**), seed thickness and 1000 grain weight (r = 0.069*). All these traits lead to increasing the high yield potential of the rice crop and also provide information about of seed size and seed shape that showed genetic variability among the genotypes.

Table 2. Analysis of variance of different seed morphological traits of various rice genotypes and their mean square values.

Source of variation	D.F	SL	SW	ST	L/W	1000GW
Genotypes	49	3.196**	0.966**	1.689**	0.049**	0.301**
Replications	2	0.003	1.265	0.028	0.0001	0.0018
Error	98	0.060	0.470	0.020	0.0051	0.1550

Level of significance p < 0.05 = * and p < 0.01 = **. SL = Seed length, SW = Seed width, ST = Seed thickness, L/W = Length/width ratio and 1000-grain weight.

Table 3. Correlation among different seed morphological traits of rice.

Traits	SL	SW	ST	L/W	1000GW
SL	1.00				
SW	–0.234**	1.00			
ST	–0.162**	0.254**	1.00		
L/W	0.834**	–0.452**	–0.35**	1.00	
1000 GW	0.099**	0.0508NS	0.069*	–0.153**	1.00

Level of significance p < 0.05 = * and p < 0.01= **. SL = Seed length, SW = Seed width, ST = Seed thickness, L/W = Length/width ratio and 1000-grain weight.

To complement morphological diversity with molecular diversity 15 SSR primer pairs were used to determine the polymorphism among rice genotypes and their genotypic differences. All 15 primers were polymorphic producing 2 to 7 alleles with total 51 alleles in the germplasm. Maximum 7 alleles were produced by primer pair 'RM257' (Table 4). Each of the primer pairs 'RM250', 'RM255', 'RM25' and 'RM264' produced only 2 alleles. Minimum (0.344) and maximum (0.763) PIC values were observed from primers 'RM255' and 'RM249', respectively (Table 4).

Diversity based on some seed morphological characters, growing plants pattern that vary between the environments depending upon growth habits. Genetic marker based diversity on gene product; isozyme and protein also reveal a low level of polymorphism due the influence of environment (Ravi *et al.* 2003). On the other hand, to determine the genetic potential, genetic association, genetic distance and assessment of genetic variation among the different diverse rice germplasm lines DNA based molecular marker considered to be powerful tool reveal a high level of polymorphism without environmental influence (Xiao *et al.* 1996, Neeraja *et al.* 2006). SSR markers have more validation as compared to other markers for the evaluation and classification of rice genotypes (Singh *et al.* 2004). Genetically diverse plant materials are more likely to contribute in breeding program for the introduction of new desirable traits that involved in the yield potential

of the rice genotypes. Genetic divergence/germplasm is the base for any breeding program would be more promising for the development and enhancement of new plant materials (Arunachalam 1981, Kwon *et al.* 2002). The length, width, seed thickness, length width ratio and 1000-grain

Table 4. Characteristics of the SSR used and their chromosomes location, product size, number of polymorphic alleles, and PIC values calculated for a set of 20 genotypes of diverse rice germplasm.

SSR marker	Sequence	Chromo-somes location	Product size (bp)	Total no. of alleles	No. of polymor-phic alleles	% polymor-phism	PIC
RM529	F: CCCTCCCTTCTGTAAGCTCC R:GAAGAACAATGGGGTTCTGG	1	260	3	3	100	0.565
RM53	F: ACGTCTCGACGCATCAATGG R: CACAAGAACTTCCTCGGTAC	2	175	5	5	100	0.689
RM530	F: GCACTGACCACGACTGTTTG R: ACCGTAACCCGGATCTATCC	2	165	5	5	100	0.743
RM250	F: GGTTCAAACCAAGCTGATCA R: GATGAAGGCCTTCCACGCAG	2	165	2	2	100	0.454
RM251	F: GAATGGCAATGGCGCTAG R: ATGCGGTTCAAGATTCGATC	3	220	3	2	66.66	0.431
RM252	F: TTCGCTGACGTGATAGGTTG R: ATGACTTGATCCCGAGAACG	4	215	4	3	75.00	0.633
RM255	F: TGTTGCGTGTGGAGATGTG R: CGAAACCGCTCAGTTCAAC	4	170	2	2	100	0.344
RM249	F: GGCGTAAAGGTTTTGCATGT R: ATGATGCCATGAAGGTCAGC	5	270	6	6	100	0.763
RM25	F:GGAAAGAATGATCTTTTCATGG R: CTACCATCAAAACCAATGTTC	8	152	2	2	100	0.364
RM52	F: CTACTCGCGCGTGGAGTT R: TGTCTTACTGGTGAAGCTGG	8	175	4	3	75.00	0.654
RM256	F: GACAGGGAGTGATTGAAGGC R: GTTGATTTCGCCAAGGGC	8	245	4	3	75.00	0.502
RM264	F: GTTGCGTCCTACTGCTACTTC R: GATCCGTGTCGATGATTAGC	8	260	2	2	100	0.486
RM257	F: CAGTTCCGAGCAAGAGTACTC R: GGATCGGACGTGGCATATG	9	190	7	7	100	0.680
RM258	F: TGCTGTATGTAGCTCGCACC R: TGGCCTTTAAAGCTGTCGC	10	245	3	3	100	0.494
RM254	F: AGCCCCGAATAAATCCACCT R: CTGGAGGAGCATTTGGTAGC	11	150	3	3	100	0.522
	Mean			3.53	3.40	92.77	0.554

weight is one of the quantitative measures for grain size and grain shape. Grain morphology i.e. colour, size and shape having unique position for the breeders during the selection and evaluation process (Kasem *et al.* 2009 and Bai *et al.* 2010). In the present study all the genotypes showed significant variations with respect to their desirable seed morphological traits. It is thought to relate the largest shape variation in small grain crops. On the other hand, length width ratio is the major genetic variation of rice grain shape and highly associated with the quantitative traits parameters and can be used in the breeding program for the improvement of the rice varieties (Iwata *et al.* 2010). All these quantitative traits are most important for measuring the yield of agriculture crops and leads to increasing their yield potential. Scientists studied different yield and

yield contributing traits i.e. grain yield per plant, 1000-grain weight, productive tillers per plant, grains per panicle, grain length, grain width, panicle length and plant height. Some traits showed a positive significant association with grain yield per plant both at genotypic and phenotypic levels (Sabesan *et al.* 2009, Kanbar *et al.* 2009, Nandan *et al.* 2010, Ashfaq *et al.* 2012b). Significant association of the various morphological traits played a very important role for the enhancement of yield and yield contributing traits (Salam *et al.* 2009). It may be fruitful for improving seed health, vigour that ultimately leads to increase the germination rate and yield potential of the crop. On the other hand, various associated desirable quantitative traits also play a very important role for hybrid seed production that leads to the increase in yield and development of new high yielding varieties with good characteristics (Ashfaq *et al.* 2013, Yan *et al.* 2009).

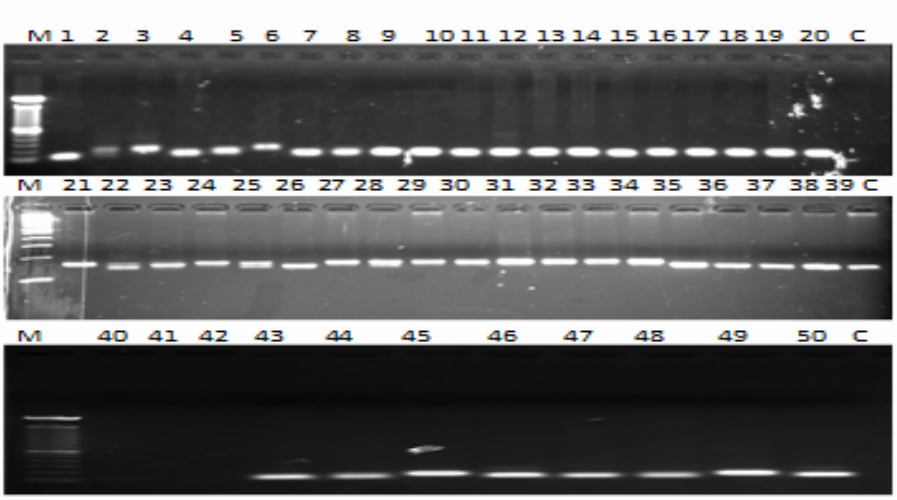

Fig. 1. PCR profile of RM 5302 showing the range of alleles in diverse rice genotypes; "M" represents the DNA marker; samples 1 to 50 represent different rice genotypes; "C" is H_2O used as a negative control.

The information generated from the experiment would allow us to select the diverse parents for the creation of new genetic materials, which can be used for the development of new rice varieties with high yield potential under adverse environmental conditions. The practice of estimation of genetic diversity is very useful for germplasm enhancement and making of new potential lines through conventional and molecular breeding approaches. A set of diverse genotypes i.e. Basmati kamon, Basmati-140, Begumi 119 A, Mushkan-73A, Palman 60, Coarse-99, EB-204 and K-202-17 were selected for further recombination, developing and selection of the new breeding populations with variable desirable genotypic and phenotypic traits. These genotypes can be used for improvement in yield potential of the rice crop. We have higher yield in coarse varieties and high quality in basmati rice germplasm, we can combine such desirable traits varieties in to one place through recombination and mutation to create the new variable genetic materials for breeding purposes. Such types of genetic studies may be fruitful for the breeders, scientists, research students and farmer's community for the development and enhancement of new germplasm material for strengthen future breeding programme to increase the economy.

From the study it was concluded that, 15 SSR primers were sufficient enough to determine the genetic diversity among 50 diverse rice germplasm lines. Some rice lines showed more polymorphism and other showed less polymorphism along with entire set of SSR primers. On the other hand, such types of marker based identification, screening, classification and selection of the diverse genotypes could be helpful both for breeders, scientific community and farmers for further improvement, development and enhancement of new germplasm material for future breeding studies of new high yielding rice varieties production. The correlation analysis revealed 1000-grain weight can be used as selection criterion for bold grain size in Basmati rice.

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