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MOLECULAR DIVERSITY AND HETEROSIS ANALYSIS FOR RICE GRAIN DISCOLORATION

Muhammad Ashfaq[1,*], Muhammad Saleem Haider[1], Muhammad Ali[1], Shabnum Shaheen[3], Farah Khan [3] and Urooj Mubashar[2]

[1]Institute of Agricultural Sciences, University of the Punjab, Lahore, Pakistan; [2]Government Elementary Teachers Education College, Ghakkhar Mandi, Gujranwala, Pakistan;[3]Department of Botany, Lahore College for Women University, Lahore, Pakistan
[*]Corresponding author's e-mail: ashfaq.iags@pu.edu.pk

Grain discoloration development is becoming a major threat to rice crop. Molecular diversity among 39 genotypes was assessed using 19 SSRs primers. The average PIC value 0.619 was determined among the genotypes along with all twelve chromosomes. Six parents were selected on the basis of disease incidence/severity i.e. CB-19, CB-42, Basmati-370, Basmati-515, IR-64 and Rondo. Half diallel analyses were conducted among the genotypes to study the heterosis on the basis of agro-morphological traits. Some hybrids had good heterosis for specific traits such as productive tillers per plant (IR-64 × Bas-370), seeds per panicle (IR-64 × Rondo) and 1000 grain weight (Bas-515 × Rondo) can be used for the production and development of new plant population especially controlling the new emerging rice grain discoloration disease.
Keywords: Rice, grain discoloration, morphological traits, SSR primers, chromosomes, diallel and heterosis.

INTRODUCTION

Rice is staple food of Pakistan, 2nd most important cereal crop and export commodity. Nowadays our rice crop is subjected to many diseases which will affect its quality, reduced yield that will ultimately affect the export. In the recent years a new yield decreasing disease rice grain discoloration became a new threat to the rice growing areas. In spite of its economic importance, neither effective control measures for rice grain discoloration nor rice varieties showing complete resistance to the disease are currently available. Indeed, numerous plant pathogens with high optimal temperatures have emerged or become prevalent worldwide (Schaad, 2008).

Moreover, rice crop has a small genome size, diploid genetics and useful model experimental plant for the study of other cereal crops (Kurata *et al.,* 1994), and significant level of genetic polymorphism (Tanksley, 1989; Wang *et al.,* 1992). Landraces, cultivars, advance breeding lines, various plant populations, germplasm lines, or newly developed plant materials tested under biotic and abiotic environments can be studied through linkage disequilibrium (LD) mapping (Rostoks *et al.,* 2006; Robbins *et al.,* 2011) for the determination of new quantitative trait loci (QTL) against biotic stresses. On the other hand, the only SSR markers with significantly useful gene and strong LD will be associated to the variation in quantitative traits (Garris *et al.,* 2003). Panicle blighting has been an important sporadic problem in the southern rice production area of the United States for many years. A somewhat similar disease called "ear blight", paddy rice, or grain discoloration has been attributed to fungal causal agents (Lee, 1992a; Lee, 1992b). This problem is characterized by discoloration of the floret grains, panicle branches, usually with distinct lesions and many fungi have been described as causing this disease (Atkins, 1974; Ou, 1985; Lee, 1992b; Groth *et al.,* 1991). It was considered to be a disorder of undetermined causes as no pathogen was isolated from diseased tissues (LSU Agricultural Center, 1987; LSU Agricultural Center, 1999; Groth, *et al.,* 1991). According to Ou (1985) bacterial sheath rot and grain blighting on rice was first reported in Hungary (Klement, 1955). The disease was described as caused by *Pseudomonas oryzicola* to be a synonym of *Pseudomonas syringae* pv. syringae (Ou, 1985). Grain rot in rice was reported to be caused by several bacteria, including *Pseudomonas glumae* (*Burkholderia glumae*), but only *P. glumae* caused seedling blight on inoculated plants (Goto *et al.,* 1987). Tanii *et al.* (1974) isolated several bacteria from rice grains showing grain rot. They identified three *Pseudomonas* species, but not *P. glumae. P. glumae* was isolated from plants in nursery flats with seedling s showing bacterial seedling rot symptoms (Uematsu *et al.,* 1976). In this sense, the rice grain discoloration disease should be recognized as a potential threat to the world wide rice production; increasing reports of this disease from many rice-growing countries strongly support this notion. Phat *et al.* (2005) reported that rice yield loss due to pests has been noticed more and more seriously. Grain discoloration is considered as one of the notorious problems in Mekong Delta. Rice grain discoloration is

becoming a serious threat to the Pakistan and Asian and is becoming more severe with the passage of time (Arshad *et al.,* 2009; Phat *et al.,* 2005). Rice grain discoloration affect the qualitative and quantitative traits (Sumangata *et al.,* 2009, Tariq *et al.,* 2012) that ultimately responsible for yield reduction. These diseases also responsible to affect the grain quality, broken of rice during milling, weight loss, exports, postharvest losses, crop yield and ultimately badly affect the economy of Pakistan (Ghazanfar *et al.,* 2013). The pathogens associated with discolored rice grain disease have also been reported by many scientists (Khan *et al.,* 2000 and Javid *et al.,* 2002). Rice yield reduction is caused by many rice pathogens Worldwide estimated about 14-18% (Mew and Gonzales, 2002; Mew *et al.,* 2004) and some areas resulting heavy yield losses ranging from 50 to 90% (Hajano *et al.,* 2012; Jabeen *et al.,* 2012; Agrios, 2005) and only in Tamil Nadu India yield losses up to 39% (Shanmugam *et al.,* 2006; Saeed *et al.,* 2014; Rajappan *et al.,* 2001). Rice molecular markers also play a very important role for screening, selection and identifying the new resistant rice lines against diseases and other biotic and abiotic stresses (Choudhary *et al.,* 2013; Pinta *et al.,* 2013). Molecular markers used as a helping tool for new genes identification and selection of resistant rice lines along with conventional breeding techniques and ultimately leads to the development new resistant genetic material (Mizobuchi *et al.,* 2013; Yu *et al.,* 2008). Molecular markers also associated with the screening and identification of plant pathogens (Mannan and Hameed, 2013). The most susceptible period for floret infections appears to be during panicle emergence and flowering. Inoculation during flowering gives the highest rates of floret infection and production of diseased spikelets (Pinta *et al.,* 2013). Fungi and bacteria associated with discolored grains affect germination ability (Misra *et al.,* 1990; Ou 1985). Bacteria are found associated with 28-32% of discolored seed (Misra *et al.,* 1990; Baldacci and Corbetta, 1964). It has been established that pathogenic bacteria exist on the phylloplanes of rice plants during the growing season (Hikichi *et al.,* 1993), on or in rice seeds stored at room temperature during the winter (Tsushima *et al.,* 1987; Tsushima, 1989), on weeds in the field and in rice tissues from the previous crop buried in soil (Sogou and Tsuzaki, 1983). An effective control measure for plant diseases is to eradicate sources of contamination with the pathogen.

The objectives of the study were to screen out of rice germplasm on the basis of various genotypic and phenotypic traits leading to the identification of disease incidence levels.

MATERIALS AND METHODS

***Plant materials evaluation under field and lab condition*:** Different rice lines, land races, approved varieties, strains and advanced breeding lines were collected from different sources i.e. United States Department of Agriculture (USDA), Arkansas USA, Rice Research Institute (RRI), Kala Shah Kaku, Lahore, Pakistan and different countries of origin. Different seed and agro-morphological traits were studied under lab and field conditions in Randomized Complete Block Design with three replications. The study includes the 39 diverse genotypes of Rice (*Oryza sativa* L.) as shown in the Table 1.

***Morphological traits measurement*:** Various seed morphological traits were measured with the help of Vernier Caliper to study the size and shape of the genotypes. Seed length, width and thickness of each grain were measured and recorded the observation separately. The grain weight of each genotype was measured with the help of weighing balance in grams. Seed length width ratio can be determined by using the following formula:

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

***Growth conditions*:** The genotypes were grown in plastic trays under controlled temperature and humidity in the growth chamber for the collection of fresh leaves. The leaf samples were collected from 20 days old seedling for extraction of DNA of each genotype. The leaves of each genotype were cut with the help of sterilized scissor and kept in to the eppendorf tubes on the ice. All the samples were stored in -80ºC in the refrigerator.

***Genomic DNA extraction*:** 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 at 260 and 280 nm by using the Nano Drop (ND 1000 Spectrophotometer). Good quality DNA was extracted from all the genotypes. For PCR analysis samples were diluted to a concentration of 40 ng/µl double distilled water (ddH2O).

***SSR primers selection*:** Nineteen SSRs primers pairs were selected from published rice microsatellite framework map for the genetic divergence study of germplasm. Chromosomal positions and original source of these markers can be found from rice genome database (http://www.gramene.org). All the primers were obtained from Gene ray biotechnology company Shanghai China and selected on the basis of their polymorphic characteristics.

***Electrophoresis of amplified products*:** Gel electrophoresis (3% agarose gel) was used for analyzing the PCR amplified products at 100 V for 1 hour and 30 minutes. To compare the molecular weight of each amplified PCR products a 100 bp DNA ladder was used. To see the fine bands on gel tris base Acetic acid (EDTA) buffer was used for of each germplasm line stained with ethidium bromide. All PCR amplified products with specific genotypes were observed under UV light for further scoring.

***Allele scoring*:** Agarose gels staining ethidium bromide generally revealed a multiple number of bands. From microsatellite analyses alleles were scored qualitatively for

Table 1. Plant Materials used in the experiment

Sr. No.	Variety Name	Origin	Disease incidence%	Accession No.	Taxon
1.	CB-19	Pakistan	5	4029-3	*O. sativa*
2.	Basmati-198	Pakistan	10	Approved variety	*O. sativa*
3.	Basmati-385	Pakistan	20	Approved variety	*O. sativa*
4.	Shaeen basmati	Pakistan	30	Approved variety	*O. sativa*
5.	Basmati 515	Pakistan	10	Approved variety	*O. sativa*
6.	KSK-133	Pakistan	7	Approved variety	*O. sativa*
7.	Basmati-370	Pakistan	8	Approved variety	*O. sativa*
8.	Basmati-Pak	Pakistan	40	Approved variety	*O. sativa*
9.	IR-6	Philippines, IRRI	25	Approved variety	*O. sativa*
10.	CB-13	Pakistan	50	1053-1-2	*O. sativa*
11.	CB-14	Pakistan	55	1053-2-2	*O. sativa*
12.	CB-15	Pakistan	45	1053-2-4	*O. sativa*
13.	CB-17	Pakistan	15	4029 A	*O. sativa*
14.	CB-21	Pakistan	50	4048-11	*O. sativa*
15.	CB-33	Pakistan	55	4365	*O. sativa*
16.	CB-42	Pakistan	50	33797-1	*O. sativa*
17.	IR-64	Philippines, Luzon	9	GSOR 311793	*O. sativa*
18.	Chaoyang No. 01	China	25	PI 615219	*O. sativa*
19.	Rondo	United States	20	PI 657830	*O. sativa*
20.	CB-32	Pakistan	55	4361	*O. sativa*
21.	Sathi Basmati	Pakistan, Punjab	20	GSOR 311134	*O. sativa*
22.	Frooz	Iran	50	584569	*O. sativa*
23.	Stg567989	United States, Arkansas	50	GSOR 311705	*O. sativa*
24.	Acroni	Suriname	11	GSOR 311179	*O. sativa*
25.	IR-773-A1-3621	Philippines	7	345823	*O. sativa*
26.	Yangzi-95	China	7	PI 614977	*O. sativa*
27.	KBNT-1	United States, Arkansas	8	PI 632282	*O. sativa*
28.	Arabi	Egypt	40	GSOR 311315	*O. sativa*
29.	W-1193	Brazil	20	GSOR 311702	*O. rufipogon*
30.	V20B	Philippines, Luzon	17	PI 452281	*O. sativa*
31.	GP-103	Pakistan	45	0167	*O. sativa*
32.	ZiriPalman	Pakistan	10	385821	*O. sativa*
33.	Palman-246	Pakistan	15	247885	*O. sativa*
34.	Gui-99	China	9	GSOR 311443	*O. sativa*
35.	GP-79	Pakistan	50	0137	*O. sativa*
36.	Rexoro	USA, Louisiana	55	1779	*O. sativa*
37.	H256-76-1-1-1	Argentina	10	596914	*O. sativa*
38.	CR-833	China	9	----	*O. sativa*
39.	CR-832	China	9	----	*O. sativa*

absence and presence on the basis of marker allele genotype combinations. The size of amplified band for each microsatellite marker with specific genotype was compared with DNA ladder. Amplified SSR bands were treated as a unit character of the given primers. Bands were scored on the basis of presence (1) and absence (0) of observations of each genotype. Whenever an amplification product could not be detected for a particular genotype marker combination an accession was assigned a null allele for a microsatellite locus. Manually the five plants main tiller of each genotype was selected randomly for counting the infected and total number of grains per panicle. The following formula was used for the determination of disease incidence reaction of each genotype against grain discoloration. The scale used for the response of each genotype against rice grain discoloration disease in the Table 2.

$$\text{Disease incidence } \% = \frac{\text{Infected grains per panicle}}{\text{Total grains per panicle}} \times 100$$

***Development of F_1 population*:** Various rice lines that showed resistance, tolerance and susceptibility were selected after the screening and hybridization in greenhouse to develop F_1 seed i.e. IR-64, Rondo, Basmati-370, Basmati-515, CB-42

and CB-19. The F_1 population of each recombinant was evaluated in field condition for various desirable traits along with their parents for further screening and development of disease free population. Best hybrid combinations were selected on the basis of various agro-morphological traits i.e. productive tillers per plant, primary branches per panicle, seeds per panicle, seeds weight per panicle, 1000 grain weight, yield per plant and seed length width ratio. For further selection and screening of best segregate plants for the development of new homozygous plant population. To increase the homozygosity and decrease the heterozygosity plants with similar traits were selected for each next generation under field condition.

Table 2. Scale used for grain discoloration disease of rice

Infection %	Score	Response
0	0	Highly Resistant (HR)
1-10	1	Resistant (R)
10-30	3	Moderately Resistant (MR)
30-50	5	Moderately Susceptible (MS)
50-75	7	Susceptible (S)
75-100	9	Highly Susceptible (HS)

***Statistical and SSR analysis*:** Minitab, MSTAT and SAS version 9.2 were used for the analysis of variance, correlation and Principal Component Analysis studies. PCR conditions/procedures were described by Panaud *et al.* (1996) for amplification of 19 selected primer pairs used for this study. Each PCR reaction was done in total volume of 20 µl containing 0.2 µM of each primer pair, 1.5 mM MgCl2, 40 ng DNA of each genotype and 0.5 unit of Taq DNA polymerase 200 µM of deoxy ribonucleotides, 50 mM KCL and 10 mM Tris HCL (Ph 8.3). According to the annealing temperature of the primer each PCR reaction was set at 1 cycle per 2 minutes at 95°C the initial denaturation temperature and further followed by 35 cycles of 94°C for 30 seconds denaturation, annealing temperature of the each primer used (56-70°C), 30 seconds at 72°C for primer elongation and ending up with 7 minutes at 72°C for the final extension. The amplified products were stored at -20°C for later use in gel electrophoresis and gel scanning purposes. All primers used in this study were almost highly polymorphic and cover all the 12 chromosomes of rice crop for the determination (PIC) value of each SSR marker and performance of other molecular analysis by using the software package power marker (Liu and Muse, 2005).

RESULTS AND DISCUSSION

***Variance and association study of the traits*:** The two way analyses of variance of all the desirable traits were studied at various levels of significance. All the genotypes were analyzed at 1% and 5% levels of significance. All the traits showed significant differences among the genotypes (Table 3). On the other hand, association of all the desirable traits was also studied at different level of significance. The correlation studies among all the traits are shown in the Table 4.

***Principal component analysis of variable quantitative traits*:** Principal component analysis (PCA) was also done to check the genetic variability of all the germplasm under study on the basis of various morphological traits. Genetic variability relies on the basis of eigen value of the principal component. Those components having more than one eigen value showed more variability (Table 5). This will also give the information about the diversity of the rice lines.

***Characterization of SSR markers*:** The overall analysis showed the greatest genetic diversity of rice germplasm lines.

Table 3. 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	38	3.196**	0.966**	1.689**	0.0490**	0.3010**
Replications	2	0.003	1.265	0.028	0.0001	0.0018
Error	76	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 4. Correlation among different seed morphological traits of rice.

Traits	SL	SW	ST	L/W	1000GW
SL	1.000				
SW	-0.234**	1.000			
ST	-0.162**	0.254**	1.000		
L/W	0.834**	-0.452**	-0.350**	1.000	
1000GW	0.099**	0.051^{NS}	0.069*	-0.153**	1.000

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

Nineteen SSRs primer pairs were used for the genotyping of 39 genotypes, 19 showed distinct, clear and reproducible polymorphism fig.1. Majority of polymorphic loci had five alleles (32.2%) All the genotypes showed greatest variability along with various variable average loci traits i.e. genetic distance (0.42), linkage disequilibrium (0.296), genetic diversity (0.542), total number of alleles (4.78), polymorphic alleles (4.52), percentage of polymorphism (93.42%) and average PIC (polymorphic information content) values (Table 6). The nineteen genotypes showed polymorphism and polymorphic bands for RM-249.

***Heterosis for yield and yield related traits*:** Analysis of variance of general combining ability and specific combining ability of the parents and hybrids are given in the Table 7 and

Table 5. Principal component analysis among different seed morphological traits of rice (O*ryza sativa* L.).

Traits	PC	Eigen Value	% Variation	Cumulative %
SL (mm)	PC1	5.2067	57.9	57.9
SW(mm)	PC2	1.6451	28.3	86.1
ST (mm)	PC3	0.8532	19.5	95.6
SL/SW	PC4	0.5386	3.2	98.8
1000 GW	PC5	0.2902	1.2	100.0

Table 6. Characteristics of the SSR used and their chromosomes location, product size, number of polymorphic alleles, and PIC values calculated for a set of 39 genotypes of diverse rice germplasm.

SSR marker	Sequence	Chromosomes location	GD	LD	Gene Diversity	Product Size(bp)	Total no. of alleles	No. of polymorphic alleles	% polymorphism	PIC
RM212	F:CCACTTTCAGCTACTACCAG R: CACCCATTTGTCTCTCATTATG	1	0.1	0.80	0.65	260	5	5	100	0.565
RM53	F: ACGTCTCGACGCATCAATGG R: CACAAGAACTTCCTCGGTAC	2	0.2	0.13	0.59	175	5	5	100	0.689
RM530	F: GCACTGACCACGACTGTTTG R: ACCGTAACCCGGATCTATCC	2	0.2	0.15	0.54	165	5	5	100	0.743
RM231	F: CCAGATTATTTCCTGAGGTC R:CACTTGCATAGTTCTGCATTG	3	0.2	0.34	0.59	165	7	7	100	0.654
RM251	F: GAATGGCAATGGCGCTAG R: ATGCGGTTCAAGATTCGATC	3	0.3	0.13	0.59	220	3	2	67	0.631
RM127	F: GTGGGATAGCTGCGTCGCGTCG R: AGGCCAGGGTGTTGGCATGCTG	4	0.3	0.22	0.47	215	4	3	75	0.633
RM255	F: TGTTGCGTGTGGAGATGTG R: CGAAACCGCTCAGTTCAAC	4	0.4	0.80	0.55	170	7	7	100	0.744
RM249	F: GGCGTAAAGGTTTTGCATGT R: ATGATGCCATGAAGGTCAGC	5	0.4	0.13	0.61	270	6	6	100	0.763
RM274	F: CCTCGCTTATGAGAGCTTCG R: CTTCTCCATCACTCCCATGG	5	0.4	0.14	0.48	152	2	2	100	0.564
RM204	F:GTGACTGACTTGGTCATAGGG R:GCTAGCCATGCTCTCGTACC	6	0.4	0.27	0.55	175	4	3	75	0.554
RM248	F: TCCTTGTGAAATCTGGTCCC R: GTAGCCTAGCATGGTGCATG	7	0.4	0.43	0.38	245	4	3	75	0.502
RM264	F: GTTGCGTCCTACTGCTACTTC F:CAAAATGGAGCAGCAAGAGC	8	0.4	0.47	0.58	260	2	2	100	0.586
RM215	R:TGAGCACCTCCTTCTCTGTAG R: GATCCGTGTCGATGATTAGC	9	0.5	0.07	0.48	165	3	3	100	0.490
RM257	F: CAGTTCCGAGCAAGAGTACTC R: GGATCGGACGTGGCATATG	9	0.5	0.25	0.49	190	7	7	100	0.680
RM590	F:CATCTCCGCTCTCCATGC R:GGAGTTGGGTCTTGTTCG	10	0.5	0.36	0.75	153	3	3	100	0.650
RM258	F: TGCTGTATGTAGCTCGCACC R: TGGCCTTTAAAGCTGTCGC	10	0.6	0.14	0.50	245	6	6	100	0.494
RM206	F:CCCATGCGTTTAACTATTCT R:CGTTCCATCGATCCGTATGG	11	0.6	0.30	0.66	167	6	5	83	0.635
RM254	F: AGCCCCGAATAAATCCACCT R: CTGGAGGAGCATTTGGTAGC	11	0.8	0.25	0.34	150	5	5	100	0.522
RM235	F:AGAAGCTAGGGCTAACGAAC R:TCACCTGGTCAGCCTCTTTC	12	0.8	0.26	0.51	135	7	7	100	0.670
	Mean		0.42	0.296	0.542	193.5	4.78	4.52	93.42	0.619

Table 7. General and specific combining ability analysis under field conditions.

Source of variation	d.f	T/P	PB/P	S/P	SW/P	1000SW	Y/P	LWR
Rep.	2	0.10	0.200	3.33	0.001	0.22	0.34	0.051
GCA	5	9.74**	1.150**	941.60**	0.110**	18.64**	8.57**	0.101**
SCA	15	3.15**	1.250**	664.53**	0.319**	7.56**	42.21**	0.201**
Error	40	0.12	0.031	1.20	0.002	0.08	0.13	0.010

Level of significance at 1% and 5 %

T/P; Tillers per plant, PB/P; Primary branches per panicle, S/P; Seed per panicle, SW/P; Seed weight per panicle, 1000SW; 1000 seed weight, Y/P; Yield per plant, LWR; Seed length width ratio

Table 8. Heterosis studies over mid-parent and better parent for yield and yield related traits under field conditions.

Crosses	Productive tillers/plant		Primary branches/panicle		Seeds/panicle		Seed weight/panicle		1000 seed weight		Yield/plant		Length-width ratio	
	Ht%	Hbt%	Ht%	Hbt%	Ht%	Hbt%	Ht%	Hbt%	Ht%	Hbt%	Ht%	Hbt%	Ht%	Hbt%
CB-19×CB-42	-7.82*	-13.62*	3.29*	-	-25.70*	-36.61*	-15.63*	-30.82*	-10.04*	-20.08*	-18.25*	-36.03*	-	-
Bas-370×CB-42	-3.12*	-6.27*	2.01*	-	-53.76*	-67.87*	-2.87*	-6.74*	-13.56*	-26.92*	0.39*	-0.78*	0.89*	-
Rondo×CB-42	10.43*	-	-3.15*	-5.79*	-16.71*	-29.53*	-18.60*	-33.37*	-7.33*	-14.38*	-22.29*	-38.81*	-1.03*	-2.98*
IR-64×CB-42	-3.32*	-6.37*	-4.45*	-8.20*	-19.13*	-32.89*	-21.29*	-38.56*	-4.12*	-8.54*	-23.05*	-39.69*	4.99*	-
Bas-515×CB-42	17.25*	-	1.00*	-	-45.37*	-59.06*	-30.86*	-50.87*	-24.00*	-45.15*	-28.45*	-45.28*	-5.02*	-10.10*
Bas-370×CB-19	20.16*	-	-0.72*	-1.64*	-10.94*	-20.93*	-9.03*	-18.12*	-3.00*	-6.69*	10.17*	-19.74*	-3.80*	-7.34*
Rondo×CB-19	12.16*	-	-0.70*	-1.58*	-26.03*	-42.97*	-32.03*	-49.11*	-2.50*	-5.69*	-26.09*	-51.24*	-10.30*	-19.90*
IR-64×CB-19	-4.26*	-9.00*	2.83*	-	-10.53	-21.84	-25.14*	-35.13*	-4.17*	-8.00*	-21.16*	-41.36*	3.86*	-
Bas-515×CB-19	16.14*	-	5.33*	-	-25.12	-41.98	-20.45*	-37.16*	-	-	-27.23*	-44.89*	3.64*	-
Rondo×Bas-370	20.33*	-	10.60*	-	-31.73	-48.19	-21.10*	-35.88*	-5.38*	-10.00*	-21.45*	-43.1*	-4.76*	-9.10*
IR-64×Bas-370	29.80*	-	-3.17*	-7.02*	-29.34	-46.08	-18.10*	-34.88*	-20.00*	-39.00*	-22.62*	-44.5*	4.50*	-
Bas-515×Bas-370	5.42*	-	8.80*	-	-29.63	-46.34	-20.65*	-36.55*	1.97*	-	-24.82*	-41.67*	-4.76*	-8.47*
IR-64×Rondo	-6.82*	-12.51*	-0.73*	-1.92*	24.05*	-	-14.65*	-26.84*	-2.86*	-4.56*	-20.75*	-40.68*	0.41*	-
Bas-515×Rondo	5.94*	-	-6.83*	-11.79*	-24.31*	-42.13*	-26.98*	-43.93*	19.00*	-	-29.18*	-53.2*	-3.29*	-6.23*
Bas-515×IR-64	19.48*	-	-8.92*	-16.05*	-37.52*	-54.43*	-31.75*	-52.89*	-5.88*	-10.11*	-33.64*	-66.36*	3.61*	-

Level of significance at 1% and 5%; Ht% = Heterosis; Hbt% = Heterobeltosis

heterosis studies of the yield and yield related traits of specific combinations are also studied. Some combinations showed positive significant heterosis and some showed negative significant heterosis. The results are shown in the Table 8. All the quantitative traits showed significant results along with specific hybrids. The high yielding hybrids (IR-64 × Bas-370), (IR-64 × Rondo) had significant heterosis and contributing in high yield potential of the crop.

Genetic studies play a very important role to evaluate the genetic potential of the various genotypes for their future utilization in the breeding experiments for enhancing the yield, controlling diseases and improvement of quality traits. Furthermore, it tell us about the genetic diversity of the genotypes and their evolutionary history relating origin, investigation of new genes responsible for improving qualitative and quantitative traits and improving the plant characteristics by using modern plant molecular genetics approaches (Choudhary *et al.,* 2013; Pinta *et al.,* 2013). Genetic variability is the main step to increase the potential ideo-type traits in to a newly developed plant generations ultimately to increase the production. It is responsible for the stability, maintenance, uniformity and purity of the crop passing through selection and having resistance against insect pest and diseases for the improvement of yield. Screening of rice genotypes is the basic step to evaluate the germplasm material having great genetic resistance against rice diseases especially rice grain discoloration disease (Mizobuchi *et al.*, 2013; Yu *et al.,* 2008). Rice grain discoloration is a major threat to the rice crop in the current scenario for the responsible of yield losses and economy of the country (Arshad *et al.,* 2009; Phat *et al.,* 2005). In the present study, different rice genotypes were screened on the basis of their various morphological and genetic traits for the further evaluation and development of potential resistant lines in to the next generations. To overcome these problems the screening of best resistant lines will be the possible solution for increasing yield and other yield attributing traits. Our study is more beneficial and helpful for the other researchers in this era and relating to the future prospects. This will also provide the gateway to the scientific community for organizing, planning and contributing more in this field. All studied traits showed significant differences among the genotypes in the analysis of variance. Some traits showed positive association and some showed negative association. Positive association was identified between various important traits (seed length with length width ratio and 1000 grain weight r= 0.834**, 0.099**, seed thickness with seed width and 1000 grain weight r= 0.254**, 0.069*). The eigen value of first two components contributed 88.2% in genetic variation and leading to the information of genotypic

differences among the germplasm (Ashfaq *et al.*, 2012; 2013). Grain discoloration is a threatening disease spreading more in Pakistan may be due to high moisture content, pathogens attack and unfavorable environmental conditions. To achieve the good results and overcome to this disease the production and development of new breeding lines through advanced molecular breeding techniques. Marker assisted selection (MAS) is very useful for early screening of resistant rice lines against this disease (Mannan and Hameed, 2013).

Genotypic and phenotypic characteristics of diverse rice lines and their genetic diversity provides the information for selection screening and developing of new resistant breeding material against this disease. Diverse germplasm is the basic source for increasing yield potential, resistant against any disease, development of new traits/genes, investigation and determination of new QTLs that control specific traits located on different chromosomes. On the other hand, marker trait association provides information for the investigation of new QTLs that leads to the screening of resistant rice lines and enhancement of yield potential. This will also provide the new insight in the field of plant molecular breeding and genetics for further investigations in this emerging field (Ashfaq *et al.*, 2012, 2014). A total of 91 alleles were identified from the given set of genotypes. Thirty Simple sequence repeats (SSRs) markers were used for the screening and identifying the new genes from the given set of germplasm. The polymorphic information content (PIC) of all the markers showed greatest variability for the entire set of genotypes/germplasm. This will provide the information about the genetic diversity of the rice lines. The average PIC value from the present was obtained 0.619, which presents the variation among the genotypes for further screening, classification and breeding of new rice lines (Ashfaq *et al.*, 2014; Table 6). Marker trait association that locate specific quantitative trait loci (QTL) in a particular germplasm for controlling the specific traits through linkage disequilibrium mapping also called association mapping and very useful in molecular breeding experiments. Its efficient use will lead to facilitate gene discovery, loci identification, classification and connecting the challenges of sequence diversity with heritable phenotypic and genotypic differences. Genome-wide LD mapping is a powerful technique to identify genomic regions linked to specific phenotypic trait (Mackay and Powell, 2006; Saeed *et al.*, 2014). The association of the markers with genetic distance between pairs of loci in O. sativa was shown in figure 2. Power Marker software was used for detecting and determination of marker trait association. Some microsatellite markers showed strong linkage disequilibrium and could be very useful for plant germplasm traits studies and associations between them for the identification of new QTL with specific gene. The markers that closely associated with each other and linked with various morphological traits may be useful for the evaluation of rice germplasm and identification of new QTL i.e. RM-249, RM-274, RM-254 and RM-235 respectively located on chromosomes 5, 11, and 12.Six parental lines were screened for the development of new hybrid progenies in a half diallel fashion (Griffing, 1956) and ultimately for the production of new resistant rice varieties. Parental lines that involved in diallel analysis for the production and development of resistant plant material were shown in figure 3. Furthermore these crosses along with their parents were tested for further screening against rice grain discoloration disease. Some hybrids had good heterosis for specific traits such as productive tillers per plant (IR-64 × Bas-370), seeds per panicle (IR-64 × Rondo) and 1000 grain weight (Bas-515 × Rondo) can be used for the production and development of new plant population against rice grain discoloration disease and ultimately for increasing the yield potential of the crop (Ashfaq *et al.*, 2012, 2013).

Conclusion: The proposed plan will contribute to identify new rice varieties which are resistant against rice grain discoloration disease. From the study different rice genotypes were screened out for further development of rice population. The genotypes i.e. IR-64, Rondo, Basmati-370, Basmati-515, CB-42 and CB-19 were screened on the basis of disease incidence/severity and various morphological traits for further development of homozygous population in the next growing season. Germplasm collection and enhancement is also a major step for the development of new genetic material. We will produce new varieties with new genes which will be responsible to increase the rice production to full fill the food requirements of human being. On the other hand, disease free rice grains will also the major step to increase the yield potential of the rice crop.

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