Full Length Research Article
Advancements in Life Sciences – International Quarterly Journal of Biological Sciences

ARTICLE INFO

Date Received: 20/09/2022;
Date Revised: 15/06/2023;
Date Published Online: 30/06/2023;

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How to Cite:
Ashfaq M, Chaudhry FA, Javed A, Ali M, Rasheed A, et al., (2023). Genetic Diversity and Screening of Rice Germplasm Lines against Grain Discoloration Disease. Adv. Life Sci. 10(2): 186-192.

Keywords:
Oryza sativa; DNA; SSR; Germplasm; Traits; Diversity

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Genetic Diversity and Screening of Rice Germplasm Lines against Grain Discoloration Disease

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Abstract

Background: The research was conducted to screen out the diverse rice germplasm lines against grain discoloration disease by using 10 SSR primer pairs and on the basis of various morphological characters to evaluate the diversity of the genotypes at Faculty of Agricultural sciences, University of the Punjab, Lahore.

Methods: Seed morphological traits were measured and recorded under Randomized Complete Block Design (RCBD) experiment. DNA extraction and PCR analysis was done to measure the genotypic characteristics of rice. Genotypic and phenotypic variability was measured by using ANOVA and correlation. Microsatellite markers, also called simple sequence repeats (SSR) were used for screening and to check the genetic diversity of rice.

Results: The PIC values ranged from 0.204 of RM-07 to 0.640 of primer RM-22 along with other genetic characters i.e., number of alleles, percentage of polymorphism chromosomal location etc. The higher PIC (polymorphism information content) value showed the greatest genetic diversity among the genotypes. The polymorphic SSRs produced 3-5 alleles with an average unit of 3.3. The PIC values ranged from 0.204 to 0.640 with an average mean of 0.51. All the genotypes showed significant differences in all the studied traits which gave the significant results and non-significant results at significance level of $p<0.05$= *; $p<0.01$=** respectively. A significant association was also observed between seed length with length width ratio, 1000 grain weight (r= 0.1251*; r= 0.1922**) and seed thickness with 1000 grain weight (r=0.1205*). All these traits showed higher variations for different rice lines.

Conclusions: From the study it was concluded that some rice found to be highly resistant (Blue Nile, IR 50, Line 9 (4x15) and Line 12 (4x23) against grain discoloration that could be more useful for the production new rice lines with diverse genetic characteristics.

Introduction

Rice has a small genome sequence of 430 Mb than other cereals crop. The complete genome sequence of the rice has been studied. Rice is used as model plant for study purposes due to its precise genome sequencing and specific molecular markers [1]. Rice is diploid and a self-pollinating crop. It is widely distributed and grown under different ecological conditions. In Asia cultivated species are highly consumed which constituent the largest population of the world. Rice also has medicinal properties and is used against different human disease like blood pressure, skin diseases, headache, epilepsy, arthritis, paralysis and colon cancer [2]. There are four types of rice glutinous, japonica, Indica and aromatic. Rice has highly genetic variations which are useful to check the resistance or susceptibility against diseases of rice. Rice is the 2nd largest cereal crop after wheat. Globally 11% of arable land is used for cultivation of rice annually. The genome size of rice is small. It has diploid genetics and is mainly used as experimental plant for the study of cereal crops [1, 3], and also for genetic polymorphism [4, 5].

Rice germplasm is important to check the diversity and for the security of food. Rice germplasm has large genetic diversity as compared to other species. The rare three Sub species of rice indica, japonica and javanica which contain the large reservoir of germplasm including cultivars and local landraces [6, 7]. There are some wild relatives which have the possible resources used for the enhancement of cultivated rice [8]. In the breeding programs rice germplasm is used genetic resources for the development of new resistant rice against biotic and abiotic factors. In commercial rice germplasm high levels of genetic resemblances were found worldwide. To breeding programs variations in germplasm and genetic relationship of genotypes are very important considerations for further developing, enhancing and multiplication of germplasm with advanced characteristics. Some high yielding varieties show the homozygosity due to the limited variations while landraces have the high variations having medicinal properties and unrivaled qualitative traits. Landraces are also important genetic resources that resist pests and diseases. Heterogeneity is also present in local landraces. Indigenous and exotic rice germplasm is used to check the genetic diversity and to screen the germplasm against rice discoloration disease [9].

In rice germplasm genetic diversity is quite large as compared to other species of crops i.e., indica, japonica, and javanica are three subspecies of rice which is a large source of rice germplasm and variety of local landraces and cultivars are also included [6,7]. Rice consumption increased by 40% between 1961 and 2002 [10]. Rice is highly consumed in Asia, and concluded its consumption is higher than 80 kg/person/year. While average per capita consumption is between 30 and 60 kg/person/year in the subtropics such as South America, Africa, and the Middle East. In the developed West countries such as Europe and the United States people consume less than 10 kg/person/year.

A number of factors are involved in reducing the rice grain production per unit area but in all these diseases are one of the main factors. Diseases which attack rice are 76 in number caused by fungi, bacteria, mycoplasma like organism, viruses and nematodes. Disease symptoms of rice are rots, blotch, Discoloration, blights, due to infection caused by certain microbes on the glumes, kernels, or both [11].

In Pakistan the produced quantity of rice is 6 million tones every year that is 30% on average of world paddy rice. Rice grain discoloration disease which is new major emerging threat in Pakistan. This disease is caused by both fungus and bacteria which cause the deterioration of grain texture and quality. The severity of disease can be major or minor in different ecological zones of Pakistan with the changing climatic conditions. Grain discoloration damages the grain morphology like it affects the grain shape & size as result reduce rice yield. Grain discoloration reduces the grain weight and ultimately effect milling, shelling and processing of rice crop. In Pakistan massive loss is predicted because of this disease. It is expected that it can also cause massive loss in major rice growing countries worldwide. It's a serious threat for all rice producing countries due to its complexity. To solve this problem some strategies can be adopted for the better use of genetic resources through molecular breeding programs. Moreover, the identification of its specific pathogen should be done, and agronomic practices should be improved which will be helpful to cope up with this disease. The objectives of the study were to check the genetic diversity and screening of rice germplasm by using molecular markers; to screen out the rice germplasm lines on the basis various phenotypic and genotypic traits that are resistant to rice grain discoloration disease.

Methods

Plant Material

The experiment was performed at Faculty of Agricultural Sciences of University of the Punjab Lahore under Randomized Complete Block Design (RCBD) with three replication. Total 10 SSR based markers were used on 18 rice lines (collected from RRI and United States Department of Agriculture (USDA), USA) to check the genetic diversity of exotic and indigenous rice germplasm against rice discoloration disease.

Sample collection

The discolored rice sample was collected from the experimental area. The panicle samples of each rice line were collected from the field where plants were transplanted or grown. The samples were placed into the plastic bag with tag for the identification and recording of various traits. The discolored seeds were also used as samples (Figure 1).

Figure 1: Discolored rice panicle samples of different rice varieties.

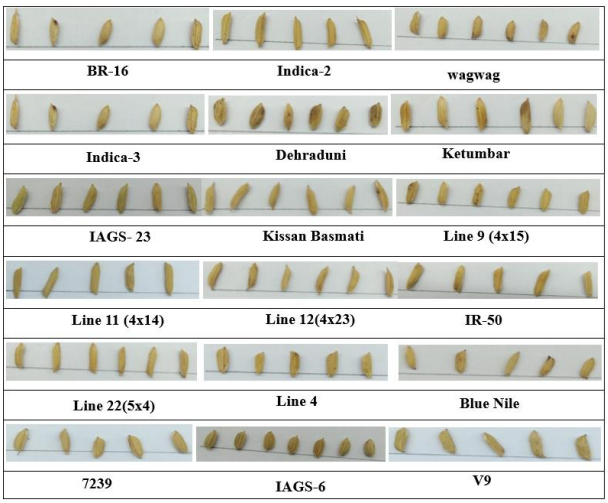

Figure 2: Seed morphology of coarse and fine varieties/lines of rice used in the study.

DNA EXTRACTION

The total 18 rice lines was used for study. DNA extraction was done by using Cetyl trimethyl ammonium bromide (CTAB) method with some amendments [14].

Grinding of leaf samples

The leaf samples were sectioned into pieces then crushed in the pestle and mortar by using liquid nitrogen to obtain the finely grinded powder. DNA extraction depends on the plant material used. Any mechanical mean or degradation was done to break the membranes and cell wall to contact the nuclear material. For this purpose, liquid nitrogen is used to break the cell wall to access the DNA without the harms of enzymes or chemicals they remain inactivated. The leaf tissues which are grinded than resuspended into the CTAB buffer. The particulates which are insoluble are removed through centrifugation to pure the DNA. The pellet is washed thoroughly to remove the contamination of salts. The DNA which is purified than stored in sterile water or TE buffer. This method is widely used to extract the DNA from plant tissues. After extraction of DNA its quality is checked by agarose gel electrophoresis which is marked with ethidium bromide and visualized the bands under UV LIGHT [15].

SSR Analysis

Ten SSR primers were used to study the genetic diversity and screening of exotic and indigenous rice germplasm against discoloration disease of rice. These SSR primers used for study were got from GRAMENE website. The SSR markers that were used for the PCR amplification are listed below (Table 1).

Statistical Analysis

The two-way analysis of variance and correlation of different seed morphological traits were done by using SAS version 9.2 [16].

Sr#	Primer name	Sequence	Annealing temperate	Chromosomal location
1	RM07	**F** TTCGCCATGAAGTCTCTCG **R** CCTCCCATCATTTCGTTGTT	56	3
2	RM211	**F** CCTCCCATCATTTCGTTGTT **R** CTTCACGAGGATCTCAAAGG	56	2
3	RM109	**F**GCCGCCGGAGAGGAGAGAGAG **R**CCCCGACGGGATCTCCATCGTC	67	2
4	RM135	**F** CTCTGTCTCCTCCCCCGCGTCG **R**TCAGCTTCTGGCCGGCCTCCTC	67	2
5	RM237	**F** CAAATCCCGACTGCTGTCC **R** TGGGAAGAGAGCACTACAGC	58	1
6	RM22	**F** GGTTTGGGAGCCCATAATCT **R** CTGGGCTTCTTTCACTCGTC	56	3
7	RM84	**F** TAAGGGTCCATCCACAAGATG **R** TTGCAAATGCAGCTAGAGTAC	55	1
8	RM341	**F** CAAGAAACCTCAATCCGAGC **R** CTCCTCCCGATCCCAATC	55	2
9	RM416	**F** GGGAGTTAGGGTTTTGGAGC **R** TCCAGTTTCACACTGCTTCG	56	3
10	RM449	**F** TTGGGAGGTGTTGATAAGGC **R** ACCACCAGCGTCTCTCTCTC	52	1

Table 1: List of SSR primers used in the study.

Results

Variance and correlation analysis

Different traits were studied to check the seed morphology of rice in which seed length, width, thickness, length/width ratio and 1000-grain weight were studied. All the genotypes showed significant differences in all the studied traits (Table 2). The traits were measured with the help of digital Vernier Caliper at the maturity stage of each genotype after harvesting. To observe the losses caused by grain discoloration disease. Various qualitative and quantitative traits were studied based on the panicle and grains per panicle.

Source of variation	D.F	SL	SW	ST	L/W	1000GW
Genotypes	17	4.847**	20.03NS	0.3609**	39.13NS	51.29*
Replications	2	0.7798**	25.98NS	0.0464**	26.53NS	43.85*
Error	34	0.66363**	26.1541NS	0.0481**	24.02NS	23.21*

Level of significance p<0.05=8* and p<0.01=**; SL=seed length, SW=seed width, ST=seed thickness, L/W=length / width ratio and 1000 grain weight.

Table 2: Seed morphological analysis of various traits of rice genotypes.

Traits	SL	SW	ST	L/W	1000GW
SL	1.00				
SW	0.0051NS	1.00			
ST	0.0325NS	0.0152NS	1.00		
L/W	0.1251*	-0.0925NS	-0.4704**	1.00	
1000GW	0.1922**	0.0259NS	0.1205*	0.0030NS	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.

Table 3: Association among different seed morphological traits of rice.

SSR marker	Sequence	Chromosomes location	Product Size(bp)	Total no. of alleles	No. of polymer-phic alleles	% polymer-phism	PIC
RM07	**F:** TTCGCCATGAAGTCTCTCG	3	135	3	3	100	0.204
	R: CCTCCCATCATTTCGTTGTT						
RM211	**F:** CCTCCCATCATTTCGTTGTT	2	150	3	2	75	0.620
	R: CTTCACGAGGATCTCAAAGG						
RM109	**F:** GCCGCCGGAGAGGAGAGAGAG	2	140	3	3	100	0.550
	R: CCCCGACGGGATCTCCATCGTC						
RM135	**F:** CTCTGTCTCCTCCCCCGCGTCG	2	210	4	3	75	0.630
	R: TCAGCTTCTGGCCGGCCTCCTC						
RM237	**F:** CAAATCCCGACTGCTGTCC	1	169	4	4	100	0.366
	R: TGGGAAGAGAGCACTACAGC						
RM22	**F:** GGTTTGGGAGCCCATAATCT	3	170	5	4	75	0.640
	R: CTGGGCTTCTTTCACTCGTC						
RM84	**F:** TAAGGGTCCATCCACAAGATG	1	160	4	4	100	0.633
	R: TTGCAAATGCAGCTAGAGTAC						
RM341	**F:** CAAGAAACCTCAATCCGAGC	2	252	4	4	100	0.559
	R: CTCCTCCCGATCCCAATC						
RM416	**F:** GGGAGTTAGGGTTTTGGAGC	3	163	4	3	75	0.485
	R: TCCAGTTTCACACTGCTTCG						
RM449	**F:** TTGGGAGGTGTTGATAAGGC	1	220	3	3	100	0.430
	R: ACCACCAGCGTCTCTCTCTC						
			Means	3.5	3.3	90	0.51

Table 4: Characteristics of SSR regarding alleles, PIC values for entire set of diverse rice genotypes.

All measurements related to seed were done at their maturing stage. These five traits L. W, T, L/W, 1000-seed weight were studied regarding their morphology. These parameters were studied in the lab by using the digital Vernier caliper and electric weighing balance. After recording the data of grains its statistical analysis was done. In statistical analysis significance and non-significant results were obtained.

The high variations were observed in all traits of different genotypes which are accompanying genetic diversity. The highly significant results attached with high variation which also associated with high genetic diversity in various traits studied. The association among the various traits were also studied. Some traits showed highly significant association among each other, and some traits showed non-significant behavior (Table 3). The strong association was observed between seed length with length width ratio and 1000 grain weight (r= 0.1251*; r= 0.1922**). A significant association was also observed between seed thickness and 1000 grain weight (r=0.1205*).

SSR analysis

Selected SSR primers were used for the estimation of genetic diversity of selected rice germplasm lines. All primers showed polymorphism among the genotypic traits. Different genetic characters i.e. number of alleles, polymorphism % and polymorphic information content (PIC) values etc. were calculated. The polymorphic SSRs produced 3-5 alleles with an average unit of 3.3. The PIC values ranged from 0.204 to 0.640 with average mean of 0.51 (Table 4; Figure 3). These SSR markers could be very useful for marker-trait associations (MTAs), marker assisted selection (MAS) in various segregation and mapping populations that raised from different crosses of the selected parental material /germplasm.

Disease incidence

The disease rating scale/disease incidence is defined as the ratio of infected grains over the total number grains which determine disease percentage incidence by counting the infected grains and total grains based on resistant and susceptibility. Two varieties were damaged at maturity stage and disease incidence were

determined by 16 varieties. The varieties BLUE NILE,IR-50,LINE 9(4x15), LINE 12(4x23), IAGS-23, TADUKAN, IAGS-6 showed resistance against grain discoloration from graph 0-1 bars. The varieties LINE 22(5x4), INDICA-3, BAS-5, KETUMBAR, KISSAN showed moderate resistance as shown in the graph from 1-2 bars. The varieties INDICA-2, BR-16, IAGS-23, V9, showed resistance as shown in the graph from 2-3 bars. All varieties showed resistance against grain discoloration disease but there was no susceptibility found in any variety against discoloration disease (Figure 4).

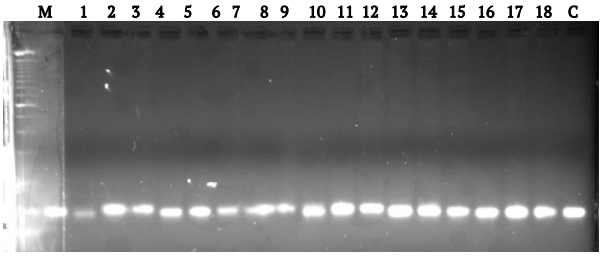

Figure 3: PCR amplification profile of RM 22 showing the range of alleles in different varieties/lines of rice. "M" represents the DNA Marker; samples 1 to 18 represent different rice genotypes and "C" H2O used as a negative control.

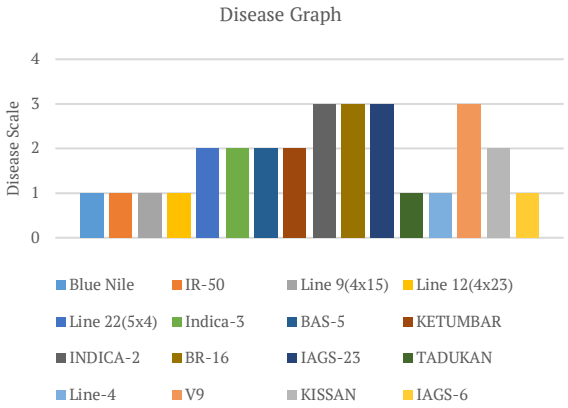

Figure 4: Disease rating scale for screening of genotypes based on disease symptoms (Where 0= with no symptom; 1= small spots on the rice glumes; 2= occurrence of 25% spots on the grain surface; 3= occurrence of 26 to 50% spots on grain surface; 4= spots exceed more than 50% on grain surface).

Discussion

Grain discoloration in rice is very destructive disease that causes a very huge yield loss every year in yield and its severity increasing in all parts of the world during rice season. In the present study different sets of rice lines were studied on the basis of various phenotypic and pathological evaluation. The examination of these diverse rice lines and can be utilized in breeding program for further screening and selection of resistant rice against grain discoloration [17].

All the traits studied showed significant differences among all the entire set of genotypes at the level of 1% and 5% significance. Genetic variability among the genotypes that leads toward the improvement and development of new rice lines with diverse characteristics and having more potential against biotic and abiotic factors. On the other hand, a strong association between studied traits and the genotypes that provides the information for the screening and selection of rice lines. A strong association is also very helpful in selection and identification process for startup a new breeding research program that more reliable in further screening of suitable genotypes in a climatic changing environment. A positive highly significant association was observed between seed length and 1000 grain weight (r= 0.1922**). On the other hand, a positive association was observed between seed length with seed length width ratio (r=0.1251*) and seed thickness with 1000 grain weight (r=0.1205*).

Various molecular traits or diversity indices were studied to evaluate the genetic diversity amongst the rice lines used in the current study i.e., heterozygosity, polymorphic information content and chromosomal location etc. Rice genotypes were identified by using molecular markers that are considered as qualitative characters which are less effected by environmental changes [18]. The PIC values were RM 109 0.18 to RM 07 0.75. PCR analysis also showed that there is no co relation between heterozygosity and polymorphic information content. The lower PIC value 0.04 were showed by RM 449 and RM 84. The primer RM 7 and RM 22 showed the higher PIC values which means these primers were highly informative. The PIC values of other primers were RM 135 0.05, RM 211 0.14, RM 237 0.59, RM 341 0.44, RM 341 0.44. The heterozygosity was also checked of all these SSR primers in which RM 237 and RM 22 showed the same heterozygosity which was 0.62 and highest was showed by RM 7 which was 0.77 [19-21] .

Grain discoloration disease is affected by the involvement of complexity of pathogens this can be a major threat to rice crop production in coming years along with facing climate change affects. Mostly, disease appeared in the reproductive and maturity stage of the crop that badly affected the yield of rice crop along with other environmental factors [18].

The DNA markers which are more reliable and less costly were used to study the rice lines. Different SSR primers were used to check the genetic diversity of diverse rice genotypes on the basis of various genotypic traits. All the primers showed polymorphism among genotypes that enhance the screening and selection process of rice lines. Molecular markers are used to study genetic variations and genetic relationships

between and within species. The techniques of DNA profiling are studied worldwide for breeders' rights and variety registration of plants [22].

The genotypes were analyzed by using the molecular markers for their characterization. The molecular markers cover the complete genome than conventional markers [23]. The molecular markers are powerful tools because by using these markers we can differentiate the similar verities which are difficult to distinguish by using conventional markers. These markers are stable in different environment and have rapid work techniques like microsatellites. The molecular markers have many advantages like used for the characterization of many varieties, check the distinction among verities and also used in future protection [24]. A total 10 SSR primers were used for molecular analysis of 18 rice genotypes. The PIC (polymorphism information content) was considered to be informative for each primer of the SSR markers [24-26].

The purpose of the study was to determine the genetic diversity of indigenous and exotic rice lines against grain discoloration disease of rice. On the basis of morphological and molecular characterization different rice lines were screen out against grain discoloration disease. Such types of morphological and molecular techniques are very helpful for further screening and selection of rice germplasm lines on basis of various genotypic and phenotypic traits [27, 28].

From the study it was concluded that some rice lines i.e. Blue Nile, IR 50, Line 9 (4x15) and Line 12 (4x23 showed resistant against grain discoloration that could be more useful for the production new rice lines with great genetic potential. These lines could also be very useful in breeding for high yield and grain discoloration disease resistant cultivars. On the other hand, SSR markers showed more diversity among the rice germplasm lines that could be very fruitful for the evolution of new crop species. The findings of this study are equally beneficial for farmers and scientific community to start up a new breeding research program.

Competing Interest

The authors have no conflict of interest

Author Contributions

MA and AJ conceived the idea. FAC, MA, AR, MJ and AS designed and performed the experiments. All authors contributed to the article and approved the submitted version.

Acknowledgement

The authors would like to very thankful to Dr. Harold Bockelman from USDA, USA for providing the rice germplasm lines and University of the Punjab for providing the funds to complete the research timely.

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