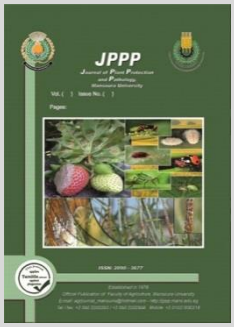
Journal of Plant Protection and Pathology

Journal homepage: www.jppp.mans.edu.eg
Available online at: www.jppp.journals.ekb.eg

Genetic Diversity Analysis and Molecular Characterization of Elite Rice Promising Lines for Yield, Blast Disease Resistance and Rice Stem Borer

Anis, G. B.[1]; A. A. Hassan[2*] and A. S. Taha[1]

[1]Rice Research Department, Field Crops Research Institute, Agricultural Research Center, Egypt
[2]Rice Pathology Department, Plant Pathology Research Institute, Agricultural Research Center, Egypt



ABSTRACT

Developing and cultivating new promising rice lines with high yield potential and blast disease resistance is an important goal of rice breeding. The study was to evaluate some developed new elite rice lines for high yield, blast resistance, effective resistance genes to *Pyricularia grisea* and rice stem borer insect as well as assessment of the genetic diversity in these lines using microsatellite markers. Significant differences were found among the studied new promising lines and their parents for yield and its component traits. Promising lines; GZ11190-3-13-1-1 and GZ11190-3-13-4-1 gave the highest grain yield compared with their parents; Giza 178 and GZ6296-12-1-2-1-1 and superiority for all studied traits. Also under this study, twenty six isolates were identified as five main groups *i.e.*, IC, ID, IF, IG and IH, but ID and IH groups were considered the most common race, *Pib* resistance gene was the most effective gene to blast fungus. Promising lines GZ11190-3-13-1-1 and GZ11190-3-13-4-1 proved resistance for all tested isolates under greenhouse condition and moderately resistant for stem borer insect. For genetic diversity analysis, twenty SSR markers were polymorphic amplifying a total of 102 alleles. Characterization of these markers showed high discriminating power with an average polymorphism information content (PIC) of 0.754. Major allele frequency ranged from 0.475 (RM590) to 0.923 (RM5 and RM5313), with an average of 0.705. The cluster analysis based on microsatellite SSR markers grouped 13 rice genotypes into clear separate groups and was able to reveal close genetic relationships between rice genotypes used in rice breeding program.

Keywords: Rice, yield, blast, disease resistance, stem borer, microsatellite.

INTRODUCTION

Rice is the most important grain crop for more than half of the world's population. In Egypt in 2018, the area cultivated with rice reached 724,000 feddans (304,000 hectares) (FAO, 2018). The overpopulation will occur in rice-consuming countries. So, the researchers have to face the challenge of better application of available resources to develop new varieties. Consequently, rice production must be enhanced by 60% in the next 20 years to satisfy needs of population (Duwayri *et al.*, 1999). The yield of inbred rice in the last decades reached a plateau. So, developing rice yield is an essential task for rice breeders for developing high yielding super rice with best grain quality and tolerant to abnormal conditions (EL-Shafey *et al.*, 2016). Developing new promising lines will be effective to avoid the undesirable linkages and will give high yielding lines. The simple sequence repeats (SSR) markers can help breeders to select genotypes carrying gene (s) of interest thus, using these markers help the breeders to best use of time and facilitate exploitation of these tools in segregating plant breeding populations (Sadat *et al.*, 2013). Rice is infected with several pathogenic species, among of which is the blast with a wide spread and yield losses under favorable conditions (Shahriar *et al.*, 2020). Blast disease, caused by *Pyricularia grisea* (Cooke) Sacc., is a serious rice disease worldwide (Zeigler *et al.*, 1994). Its repeated occurrence during several plant growth stages greatly reduces yield and grain quality by 10-30% (Skamnioti and Gurr, 2009). Development a varietal resistance is the key factor to blast management (Kalboush, 2019). Blast resistance genes must be identified for breeding efforts to be successful, and roughly 100 genes have been identified as important resistance genes in rice germplasm, as well as 350 quantitative trait loci (QTL) linked to rice blast resistance (Fukuoka *et al.*, 2014). One of the most effective, economical and environmentally sustainable ways is to incorporate resistant genes into rice cultivars by phenotyping and marker-assisted selection (Liang *et al.*, 2017). For screening rice germplasm resistance, gene identification and resistant varieties breeding, against blast disease under field condition are required (Qin *et al.*, 2021). Chen *et al.* (2019) studied the reactions rice genotypes to *Magnaporthe oryzae* infection at different growth stages of using two temperate japonica rice cultivars, M-202 and Nipponbare, and were inoculated by isolate IB-49-ZN61 under controlled conditions. Insect infestations are considered important detrimental factors for rice products. Rice plants are liable to be attacked by

* **Corresponding author.**
E-mail address: amr_ab_2028@yahoo.com
DOI: 10.21608/jppp.2021.102861.1050

several insect pests. In Egypt, the rice stem borer (*Chilo agamemnon* Bles.) is the most important insect pest, and attacks rice plants throughout the growing season. The damage of the insect appears as dead hearts in the vegetative stage and as white heads in the reproductive stage (Taha, 2017). RRTC (2004) Categorized rice genotypes as resistant, moderately resistant, moderately susceptible and susceptible, and Giza 178 was found to be moderately susceptible. Stem borers infest rice crops from the vegetative through to harvesting by attacking the tillers and panicles, particularly at panicle initiation stage (El-Hefny, 2016). The aim of this work was to evaluate some developed new elite genotypes for high yield, blast resistance, distribute the physiological races of *P. grisea*, effective resistance genes and rice stem borer infestation as well as determination of some SSR marker related with some characterization previous.

MATERIALS AND METHODS

The present work was conducted during two growing seasons; 2019 and 2020 at rice pathology laboratory, greenhouse and farm of Rice Research and Training Center (RRTC), Sakha, Egypt.

Breeding materials and field experiments

Conventional rice breeding program use phenotypic values for selection of best individuals in segregate generations. The procedure in phenotype values for selection included creating genetic variation by crossing two parents followed by several rounds of selfing to develop inbred promising lines (Fig. 1). In this investigation, eleven new promising lines, derived from a cross between two diverse Egyptian restorer genotypes; Giza178 as a female and GZ6296-12-1-2-1-1 as a male were used in the study (Fig. 1). The promising lines and their parents were transplanted at the experimental field in seven rows; 5 meters long and 20 cm x 20 cm apart between plants and rows. They were arranged in a randomized complete block design (RCBD) with three replications. The standard package of recommendation practices was adopted. Resulting inbred promising lines are tested for a range of phenotypic parameters such as days to heading (day), plant height (cm), tillers plant^{-1}, panicles plant^{-1}, panicle length (cm), 1000-grain weight (g), spikelet fertility %, grain yield/fed, hulling %, milling %, head rice % and amylose content %. All measurement techniques were based on IRRI Standard Evaluation System of rice (IRRI, 2013). The scheme for new promising lines development is illustrated in Fig. (1).

Blast sample collection and isolating the blast fungus

Typical blast lesion of rice samples were collected from locations of five governorates; Kafrelsheikh, Gharbia, Dakahlia, Sharkia and Beheira during 2020 growing season and isolated according to Shabana *et al*. (2013). Spore production was prepared for 26 isolates by growing on banana glucose agar medium under florescent light for 10 days at 28ºC. The spores were collected at a density of at least 25 per microscopic field and examined using a 10x objective (Shabana *et al*, 2013).

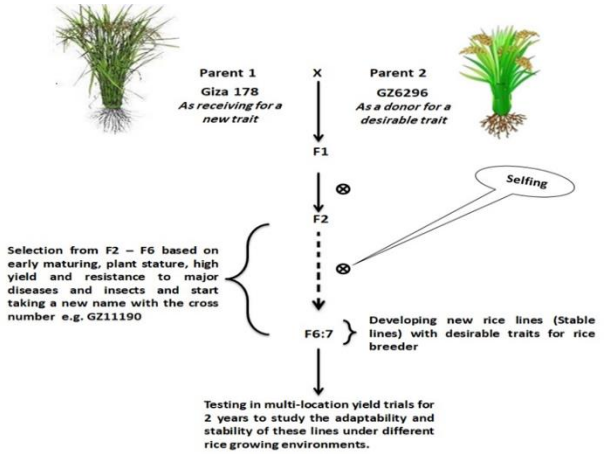


Figure 1. General scheme of a classical pure-line cultivar development in Rice Research and Training Center (RRTC), Egypt.

Pathogenicity test, identification of pathogen physiological races and determination effective resistant blast genes under greenhouse condition

Pathogenicity tests and race identification studies were carried out on the obtained fungal isolates under greenhouse condition with artificial infection. Eight international differential varieties (IDVs) (Atkins *et al.*, 1967) were used to identify blast physiological races. In addition, ten international Japanese differential varieties (JDVs) *i.e*., Shin 2 (*Pik-^{s}*), Toride 1 (*Piz-^{t}*), Tusyake (*Pik-^{m}*), Kanto 51(*Pik*), Fukunishiki (*Piz*), Ishikarishiki (*Pii-Pik-^{s}*), BL-1(*Pib*), Yashiro-Mochi (*Pita*), Pi No. 4(*Pita-^{2}*), Aichi Asahi (*Pia*) were used to determine effective resistant blast genes. As well as, eleven advanced rice lines; GZ11190-3-1-1-1, GZ11190-3-1-2-1, GZ11190-3-1-2-3, GZ11190-3-3-1-1, GZ11190-3-7-2-2, GZ11190-3-8-2-1, GZ11190-3-8-2-2, GZ11190-3-8-2-3, GZ11190-3-13-1-1, GZ11190-3-13-4-1, GZ11190-3-13-4-2 and parentage Giza178 as a female and GZ6296-12-1-2-1-1 as a male were used to evaluate its resistance level. Sakha101 was used as a susceptible check. All tested entries were seeded in plastic trays (30cm). The trays were kept in the greenhouse at 25-28ºC, and fertilized with Urea 46.5%N (5g/tray). Seedlings were inoculated with spore suspension (100 ml) adjusted to approximately 5 x 10^4 spores/ml after 3-4 weeks of sowing. The inoculated seedlings were kept in a moist chamber with at least 90% relative humidity and temperatures between 25 and 28^{0}C for 24 hours before being moved to the greenhouse.

Blast disease assessment

Under greenhouse condition and seven days after inoculation, blast reaction, as the typical blast lesions was scored as 0-9 scales according to the Standard Evaluation system (IRRI, 2013).

Rice Stem Borer (*Chilo agamemnon* Bles.) assessment under field condition

Genotypes were evaluated for rice stem borer infestation. This evaluation was conducted in 2019 and 2020 rice seasons, at the experimental farm of RRTC. Samples were taken twice; 35 days after transplanting for assessing the symptom of dead heart, and three weeks prior to harvest for assessing the symptom of white head. The sample size was 25 hills each time. In each sample, numbers of total tillers and tillers with either symptom

were recorded to count the percentages of dead hearts and white heads. The reaction of evaluated genotypes was classified into five categories according to the standard evaluation of RRTC, Sakha, Egypt as follows: Resistant (R): 0 – 3 % white head (WH), Moderately resistant (MR): > 3 – 6 % WH, Moderately susceptible (MS): > 6 – 9 % WH, Susceptible (S): > 9 – 12 % WH, and Highly susceptible (HS) >12 % WH according to Taha (2017).

DNA extraction, PCR and SSR molecular markers

DNA was extracted from the fresh rice seedlings of each genotypes, using CTAB methods (Luo *et al.,* 2005). The PCR amplification was conductec in a 10-µL reaction volume containing 1.5 µL of 20.0 ng/µL template genomic DNA, 1.0 µL of 10× PCR buffer, 0.25 µL of 1.0 pmol/µL dNTPs, 1.5 µL of 2.0 pmol/µL primer pairs, 0.06 µL of 5.0 U/µL Taq DNA polymerase and 5.69 µL of ddH_2O. The amplification technique consisted of an initial denaturation step (94 °C for 5 min), followed by 32 cycles of 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min. The reactions were completed with a final extension step of 72 °C for 7 min. The PCR products were separated by electrophoresis on 8% non-denaturing polyacrylamide (Creste *et al.*, 2001). A total of 27 SSR primer pairs were selected at random for the genetic diversity analysis of the thirteen rice genotypes and tested on the parents Giza 178 and GZ6296. Primers that showed polymorphic banding patterns were selected for further study whereas primers that showed monomorphic banding patterns were excluded (Table 1).

Table 1. Marker name, chromosome number and sequences of SSR markers

Marker	Chromosome	Size (bp)	F primer	R primer
RM7405	1	108	TTGGCTCGCCCATATATAGG	CAGTCAGTCATCACTGGTAGTCG
RM5	1	76	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
RM3850	2	109	AAGTTGAGAATGAGGGACAA	TTCGGAAGTGAAAAGGTAAT
RM3392	3	125	GTCCAATGATTCGTTCCCAC	CTTCACCGTTCACCAATTCC
RM3204	3	92	GCAACCCTTTCTTCCTCCTC	CCAAGGAGAGCGCACTAGC
RM289	5	49	TTCCATGGCACACAAGCC	CTGTGCACGAACTTCCAAAG
RM163	5	130	ATCCATGTGCGCCTTTATGAGGA	CGCTACCTCCTTCACTTACTAGT
RM6467	6	113	GGCAATCTCTCCGAATCTTC	CTAGCTGCTCTGCTCTGCTG
RM3805	6	71	AGAGGAAGAAGCCAAGGAGG	CATCAACGTACCAACCATGG
RM3394	7	201	CCCTTACGTGCAGTACATTG	ATGCAGGCTACTTACTAGCG
RM20897	7	154	TTTACACACATGCTCCTTCTGC	AAAGCAACCACCTCCATTATCC
RM248	7	82	TCCTTGTGAAATCTGGTCCC	GTAGCCTAGCATGGTGCATG
RM6471	8	82	TCTCCCATCTCCCATCTCAC	TGGTGATTGTGACAGATCGC
RM23001	8	115	CAGTTCCTCTCCTCCACCACTTCG	TGGTGGACTGGAGGGCTACTGC
RM205	9	155	CTGGTTCTGTATGGGAGCAG	CTGGCCCTTCACGTTTCAGTG
RM590	10	102	CATCTCCGCTCTCCATGC	GGAGTTGGGGTCTTGTTCG
RM202	11	137	CAGATTGGAGATGAAGTCCTCC	CCAGCAAGCATGTCAATGTA
RM26652	11	169	CAATCCATTGCTGGTTGATGC	CAAGATCTCCAAGGTGCTGAGG
RM5313	12	187	AATTCCTCCTCTTTCTCCGC	GAGAACATCACGGTGGCC
RM1227	12	137	CATGGTAGCACACACCCTTG	CATCGACATGTGGACCACTC

Data analysis: Data were recorded and subjected to statistical analysis using standard statistical analysis with MSTATC. in the table of main treatments, Duncan Multiple Range, T. (1955) was used to compare the significantly different averages. The statistical analysis including the number of alleles per locus, major allele frequency and polymorphism information content (PIC) values were analyzed using Power Marker-3.25 (Liu and Muse 2005). Using the Paleontological Statistics (PAST) software tool and the mean performance of the genotypes tested, a genetic distance tree was constructed (Hammer *et al.*, 2001).

RESULTS AND DISCUSSION

Mean performance of rice genotypes

Performance of agronomic traits for the thirteen new promising lines including their two parents; Giza 178 and GZ6296-12-1-2-1-1, were evaluated during two rice seasons (2019 and 2020) under field condition and the results shows no significantly between two seasons so, the average data between two seasons are presented in Fig. (2, 3 and 4) and showed a wide range of variability for all studied traits. This wide range reflects the variation among the tested genotypes. Regarding to crop growing period (days to 50 % flowering), desirable values for earliness were obtained from the parental line GZ6296 (92 days) followed by the new promising lines; GZ11190-3-13-4-1 (88.3 days), GZ11190-3-13-1-1 (90.3 days) and GZ11190-3-13-4-2 (91.0 days) (Fig. 2A). Plant heights varied from 92.0 cm to 106.0 cm among studied genotypes (Fig. 2B). The promising lines; GZ11190-3-1-1-1, GZ11190-3-1-2-3, GZ11190-3-3-1-1 and GZ11190-3-8-2-1 were not statistically different in plant height (with 92.0 cm) which indicates that they may have similar gene(s) expression for such trait, while Giza 178 and GZ11190-3-13-4-1 produced the tallest plants with 106.0 cm and 101.7 cm, respectively. Concerning the number of tillers and panicles/plant, significant differences were found among the studied genotypes, ranging from 22 to 26 tillers/plant and form 20 to 25 panicles/plant. The highest number of tillers and panicles plant^{-1} were those of GZ11190-3-8-2-2, whereas the lowest were those of GZ11190-3-7-2-2 (Fig. 2 C and D). The differences among the rice genotypes in tillering ability are mainly attributed to genetics of variety.

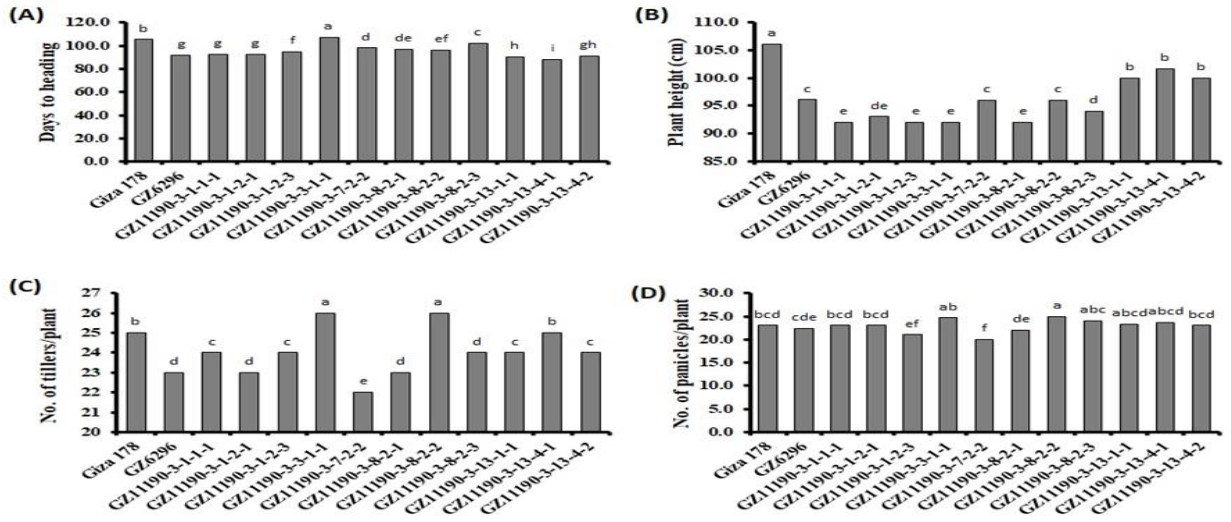


Figure 2. Mean performance values for four agro-morphological traits (A: days to heading; B: plant height; C: number of tillers/plant and D: number of panicles/plant) of rice genotypes through 2019 and 2020 (average data). In the column means followed by a common letter are not significantly different at the Duncun test with P< 0.05 probability.

The mean values of panicle length of the thirteen rice genotypes varied significantly and ranged from 22.8 to 27.9 cm (Fig. 3A). In the same time, the promising lines; GZ11190-3-1-2-3 and GZ11190-3-13-4-2 had the highest mean values of 27.9 and 27.4 cm, respectively. On the other hand, the promising line GZ11190-3-13-1-1 recorded the lowest value (22.8 cm) for this trait. Regarding to the mean performance for 1000-grain weight, rice genotypes; GZ6296, GZ11190-3-13-4-1 and GZ11190-3-3-1-1 scored the highest mean values of grain weight with values of 27.5, 27.4 and 27.2 g, respectively. However, the rice commercial variety Giza 178 recorded the lowest mean value (21.2 g) for this trait (Fig. 3B). As for spikelet fertility %, the data varied significantly among the studied genotypes and ranged from 84.7 to 94.0 %. GZ6296, GZ11190-3-8-2-3 and GZ11190-3-13-4-1 had the highest value of spikelet fertility %, while the lowest value was recorded in GZ11190-3-1-2-1 (84.7 %) (Fig. 3C). Significant differences were found among the studied new promising lines and their parents for grain yield trait. The two promising lines; GZ11190-3-13-1-1 and GZ11190-3-13-4-1 gave the highest grain yield (4.25 and 4.31 t/f, respectively) compared with their parents; Giza 178 and GZ6296 (4.1 and 4.2 t/f, respectively) and these lines were superior for all traits (Fig. 3D).

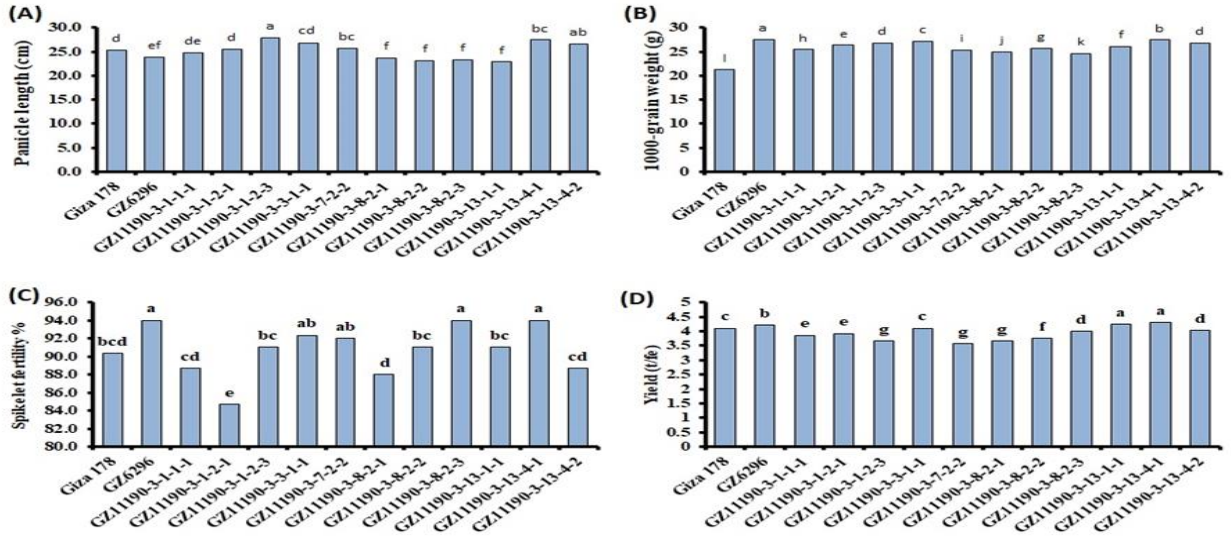


Figure 3. Mean performance values for some yield components (A: panicle length, B: 1000-grain weight, C: spikelet fertility % and D: grain yield t/f) of rice genotypes through 2019 and 2020 (average data). In the column means followed by a common letter are not significantly different at the Duncun test with P< 0.05 probability.

Concerning milling and physical characteristics, the promising lines, GZ11190-3-3-1-1 and GZ11190-3-13-4-1 recorded desirable mean values for milling characteristics as hulling, milling and head rice recovery, as indicated in Fig. (4A, B and C). The ranges of variation were as following: hulling (83-87 %), milling (68-72 %) and head rice (56-66 %). Significant negative relationships between hulling % and milling % (-0.88**, -0.58*), have been reported by Shobharani *et al.* (2003). Among the studied new promising lines and their parents, amylose content %

varied from 15.6 % to 18.79 %. The highest percentage of amylose content was estimated in GZ11190-3-8-2-3 (18.79 %) followed by GZ11190-3-13-4-2 (18.04 %) while the lowest value was in GZ11190-3-1-2-3 (15.6 %) as indicated in Fig. (4D).

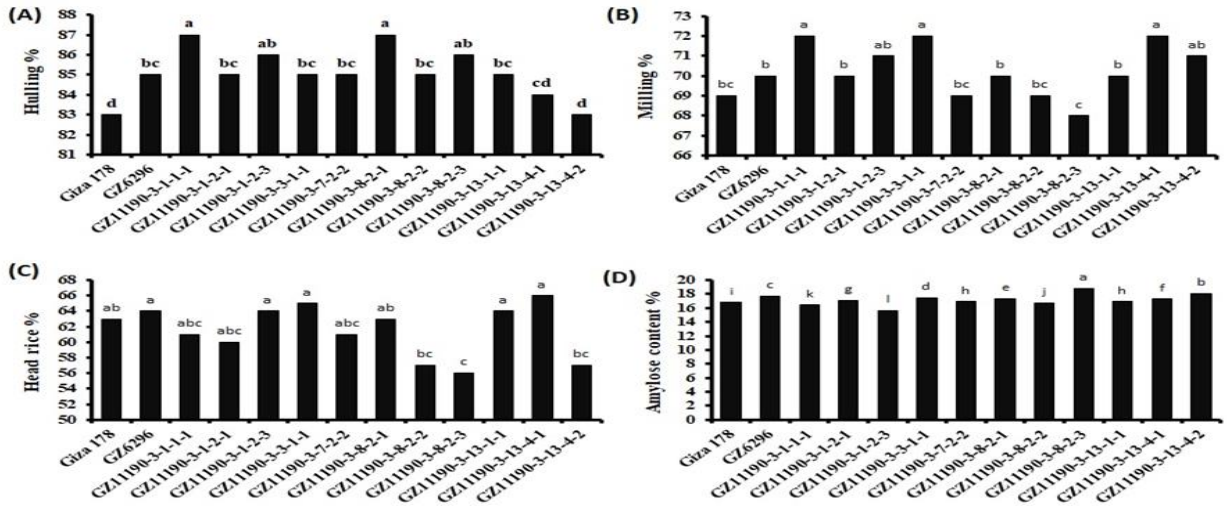


Figure 4. Mean performance values of some grain quality traits (A: hulling %, B: milling %, C: head rice % and D: amylose content %) in rice genotypes through 2019 and 2020 (average data). In the column means followed by a common letter are not significantly different at the Duncun test with P< 0.05 probability.

Isolation, Pathogenicity test and race identification

Rice samples, infected with blast disease, were collected from different rice cultivars and locations during 2020 season (Table 2). Twenty six isolates were purified by the single spore technique and succeeded to produce the fungus *P. grisea.* Isolation of *P. grisea* from plant samples number 4 (ID15), conidia preduce from incubated rice leaf (Fig.5 A), spores of *P. grisea* (Fig.5B), spore gremination after 12 hr. of incubation on water agar media (Fig.5C) and symptomes related with infected by isolate no. 4 (ID 15) on GZ11190-3-13-4-2 (Fig.5D). These isolates were identified as five main groups *i.e.*, IC, ID, IF, IG and IH using the 8 th IDV. IH and ID race groups were the most common (30.76%), while IG was the least common race group (3.87%) show in Fig. (6), these race groups included nine physiological races (two from each of IC-3, IC-11& ID-3; six from ID-15; one from each of IE-5, IE-3, IG-1; three from IF-3 and eight from IH-1) showed in Table (3). Physiological races play an important role for breakdown the new cultivars and promising lines especially when increasing the growing area of one or two cultivars. Many investigators studied the physiological races of the fungus at different rice-growing areas and the role of physiological races to breakdown the new promising lines. Also, data in Table (4) showed that eleven promising rice with two parents were resistant to *P. grisea*, except GZ6296-12-1-2-1-1 (parent) that was infected by 5 isolates while only one isolate infected promising lines GZ11190-3-7-2-2 and GZ11190-3-8-2-1. However the promising line (GZ11190-3-13-4-2) was infected by 9 isolates. These results agree with Shabana *et al*. (2013), Kalboush (2019) and Marvet *et al*. (2021) who showed the distribution of races with different rice entries and locations and this new physiological race was associated with breakdown for new rice genotypes.

Table 2. Locations of *Pyricularia grisea* isolates collected from different governorate during 2020 season

Isolate no.	Governorate	District	Rice cultivar	Race
1	Kafrelsheikh	Desouq	Sakha 101	IH-1
2	Kafrelsheikh	Desouq	Sakha 101	IC-3
3	Kafrelsheikh	Sakha	Sakha 104	ID-15
4	Kafrelsheikh	Sakha	Sakha 101	ID-15
5	Kafrelsheikh	Sakha	Giza171	IG-1
6	Kafrelsheikh	Kafrelsheikh	Sakha l04	IF-3
7	Kafrelsheikh	Kafrelsheikh	Sakha 101	IH-1
8	Dakahlia	Talkha	Sakha 101	IC-3
9	Dakahlia	Dekerns	Sakha 104	IC-11
10	Dakahlia	Dekerns	Sakha 101	ID-15
11	Dakahlia	Dekerns	Sakha 101	IE-5
12	Dakahlia	Talkha	Sakha 101	IF-3
13	Garbia	Gemmaiza	Sakha l04	ID-15
14	Garbia	Gemmaiza	Sakha 101	IH-1
15	Garbia	Gemmaiza	Giza171	IH-1
16	Garbia	Basion	Sakha 101	IC-11
17	Garbia	Qotour	Sakha 101	IE-3
18	Beheira	Elebrahimyia	Sakha 101	ID-3
19	Beheira	Mahmoudia	Sakha 101	IH-1
20	Beheira	Kafr El-Dawar	Sakha 101	ID-15
21	Beheira	Itai-El-Barood	Sakha 101	IH-1
22	Beheira	Itai-El-Barood	Sakha 104	ID-15
23	Beheira	Kafr El-Dawar	Sakha 101	IH-1
24	Sharkia	Hehia	Giza171	ID-3
25	Sharkia	Zagazig	Sakha 101	IH-1
26	Sharkia	Zagazig	Sakha 101	IF-3

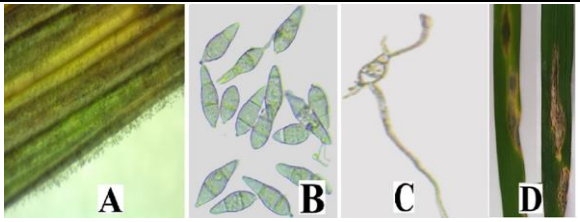


Figure 5. Isolation of *Pryicularia grisea* from plant samples number 4 (ID15),A: conidia on rice leaf, B: spores of *P. grisea*, C: spore gremination, D: symptomes on GZ11190-3-13-4-2. Bar= 10mm for (A) and 10µm for (B & C).

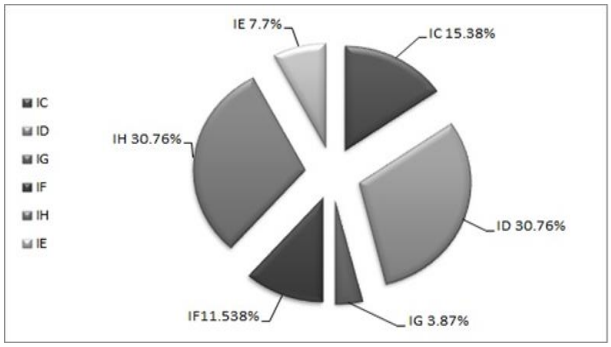


Figure 6. Distribution of different blast race groups on the International Differential Varieties under artificial inoculation with 26 isolates under greenhouse test

Effectiveness of blast resistance R-genes

In rice improvement programs the blast resistance genes are very important tools. Data presented in Table (5) and Fig. (7) show the reaction on the JDVs to rice blast fungus isolates. The frequency of resistance reactions (R) of JDV to the 26 isolates ranged from zero to 92.31%, which were depending on the effectiveness of the present resistance gene. *Pib* R-gene was the most effective to tested blast isolates (92.31 % resistance) followed by R-gene *Pii* - *Pi-k*^{s} and *Piz* (88.46% resistance), while *Pik-*^{m} and *Pik* were moderately resistant (69.23%) followed by *Pik-*^{s} and *Piz-*^{t} (50.0%). The least effective genes were *Pia*, *Pita-*^{2} and *Pita* which had 0.0, 26.93 and 42.31% resistance, respectively. These genes are recommended to be used by rice breeders as donors for blast resistance under Egyptian conditions (Sehly *et al*., 2008) or under China conditions (Wang *et al*., 2013). Shabana *et al*. (2013) recorded the reaction of monogenic lines to 132 isolates of rice blast, and found that the reaction ranged between zero and 97.76%, depending on the effectiveness of the present resistance gene. *Piz-*^{5} gene was the most effective to blast isolates followed by the gene *pita-*^{2}, *Pi5 (t)* and then *Piz*, *Pii*, *Pi9*, *Pita-*^{2}, and *Pit*. Thus, these genes are recommended to be used by rice breeders as donors for blast resistance under Egyptian condition.

Table 3. Rice blast reactions on the international differential varieties (IDVs) tested under greenhouse condtions

IDVs	Race/Isolate number/Reaction*																							
	IH-1	IC-3	ID-15	ID-15	IG-1	IF-3	IH-1	IC-3	IC-11	ID-15	IE-5	IF-3	ID-15	IH-1	IH-1	IC-11	IE-3	ID-3	IH-1	ID-15	IH-1	ID-15	IH-1	ID-3	IH-1	IF-3
	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
Raminad str.3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
Zenith	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
NP-125	R	S	R	MR	R	R	R	S	S	R	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R
Usen	R	S	S	4	R	R	R	S	S	S	MR	R	S	R	R	S	R	S	R	S	R	S	R	S	R	R
Dular	R	S	R	R	R	R	R	S	MR	R	S	R	R	R	R	MR	S	S	R	R	R	R	R	S	R	R
Kanto 51	R	HS	R	R	R	S	R	HS	HS	R	MR	S	R	R	R	HS	HS	S	R	R	R	R	R	S	R	S
CI 8970 s	R	R	R	R	S	MR	R	MR	R	R	S	MR	MR	R	R	R	R	R	R	MR	R	R	R	R	R	R
Caloro	HS	S	S	HS	HS	HS	HS	HS	S	S	S	S	HS	S	S	HS	S	S	HS	HS	S	S	S	S	HS	S

*** Reactions: R = resistant (1-2), MR = moderately resistant (3), S = susceptible (4-6), HS = highly susceptible (7-9).**

Table 4. Blast reactions for 13 rice genotypes inoculated with 26 *Pyricularia grisea* isolates under greenhouse conditions

Genotypes	Race/Isolate number/Reaction*																									
	IH-1	IC-3	ID-15	ID-15	IG-1	IF-3	IH-1	IC-3	IC-11	ID-15	IE-5	IF-3	ID-15	IH-1	IH-1	IC-11	IE-3	ID-3	IH-1	ID-15	IH-1	ID-15	IH-1	ID-3	IH-1	IF-3
	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
Giza 178	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ6296-12-1-2-1-1	R	R	S	S	S	R	S	MR	R	R	R	MR	MR	R	MR	R	R	R	R	R	S	MR	MR	R	R	R
GZ11190-3-1-1-1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ11190-3-1-2-1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ11190-3-1-2-3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ11190-3-3-1-1	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ11190-3-7-2-2	S	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ11190-3-8-2-1	R	R	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ11190-3-8-2-2	R	R	R	R	R	R	R	MR	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ11190-3-8-2-3	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	MR	R	R	R	R	R	R
GZ11190-3-13-1-1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ11190-3-13-4-1	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R	R
GZ11190-3-13-4-2	R	R	S	S	S	R	S	S	R	R	R	R	S	R	S	R	R	R	R	R	MR	S	S	R	R	R
Sakha 101	HS	S	R	HS	S	S	S	S	S	S	S	S	S	HS	S	S	S	S	HS	S	S	R	S	S	HS	S

*** Reactions: R = resistant (1-2), MR = moderately resistant (3), S = susceptible (4-6), HS = highly susceptible (7-9) IRRI, 2013**

Table 5. Rice blast reactions on the Japanese differential varieties (JDVs) tested under greenhouse condtions

JDVs	Target gene	Race/Isolate number/Reaction*																								
		IH-1	IC-3	ID-15	ID-15	IG-1	IF-3	IH-1	IC-3	IC-11	ID-15	IE-5	IF-3	ID-15	IH-1	IH-1	IC-11	IE-3	ID-3	IH-1	ID-15	IH-1	ID-15	IH-1	ID-3	IH-1	IF-3
		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
Shin 2	*Pik-s*	S	S	S	R	R	R	S	S	MR	R	R	S	MR	R	S	S	R	S	S	MR	R	R	S	R	S	S
Toride 1	*Piz-t*	R	R	S	S	S	S	S	MR	R	R	R	HS	S	R	S	R	R	R	S	S	MR	S	2	MR	S	S
Tusyake	*Pik-m*	R	S	S	R	R	R	R	HS	S	S	S	R	R	R	R	HS	R	S	R	R	R	R	R	R	R	R
Kanto 51	*Pik*	R	S	R	R	R	R	R	HS	HS	R	HS	R	R	R	R	HS	S	S	R	R	R	R	R	S	R	R
Fukunishiki	*Piz*	S	R	MR	R	R	R	R	R	R	R	S	MR	R	R	R	R	R	R	R	R	R	R	R	S	R	R
Ishikarishiroke	*Pii, PiK-s*	R	R	R	R	R	R	R	S	R	R	S	R	R	R	R	S	R	R	R	R	R	R	R	R	R	R
BL-1	*Pib*	R	R	R	R	R	R	R	MR	R	R	R	S	MR	R	R	R	R	R	R	S	R	R	R	R	R	R
Yashiro-ochi	*Pita*	S	R	S	S	S	S	MR	S	R	S	R	S	S	MR	R	R	R	R	MR	S	S	S	S	S	S	MR
Pi No. 4	*Pita-2*	HS	R	S	S	HS	S	S	S	R	S	R	S	HS	MR	S	R	S	R	HS	S	S	R	S	R	S	S
Aichi Asahi	*Pia*	S	HS	S	S	HS	HS	HS	HS	HS	HS	S	HS	HS	S	S	HS	HS	S	HS	S	S	S	S	S	S	S

*** Reactions: R = resistant (1-2), MR = moderately resistant (3), S = susceptible (4-6), HS = highly susceptible (7-9).**

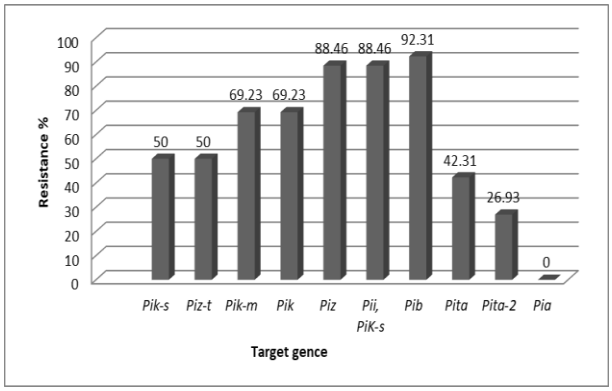


Fig. 7.Evaluation of effective resistance genes against blast pathogen isolates

Rice Stem Borer, *Chilo agamemnon* Assessment:

Data presented in Table (6) show the susceptibility of tested rice genotypes to natural infestation by the rice stem borer, *Chilo agamemnon* Bles. As for dead hearts, the values ranged between 3.75 % (Gz111 90-3-3-1-1) and 8.13 % (Gz11190-3-8-2-3). The check cultivar, Giza 178 (susceptible according to RRTC, 2004) suffered 7.83 % dead heart, and was only exceeded by Gz11190-3-8-2-3 rice genotype. Giza 178 rice cultivar was subjected to simulated dead hearts and suffered significant yield loss only at 20 % detillering, but less than this level, the plants produced the normal yield (Soliman *et al.* 2016 and Marvet, *et al*., 2021). The thirteen rice genotypes were assessed for white head infestation three weeks prior to harvest. The infestation ranged between 4.71 % (Gz11190-3-8-2-2) and 9.58 % (Gz11190-3-8-2-3). Thus, only the promising line Gz11190-3-8-2-3 surpassed Giza 178 rice cultivar (the susceptible check). Giza 178 rice cultivar and the promising line Gz11190-3-8-2-3 could be classified as susceptible (according to the category of RRTC, 2004). The symptom of white head reflects direct rice yield losses, as the plants has no chance to compensate for the attack of rice stem borer at this stage, compared to the dead hearts. Hefny (2016) concluded that economic threshold and economic injury levels of Giza 178 rice cultivar were 10 and 12 % white head, respectively.

Table 6. Susceptibility of rice genotypes to infestation with rice stem borer, average of 2019 and 2020 seasons.

Genotypes	Dead heart %	White heads	
		%	Category
Giza 178	7.83^{a}	9.05^{ab}	S
GZ6296	5.33^{cde}	6.1^{def}	MS
GZ11190-3-1-1-1	5.97^{bcd}	7.37^{bcde}	MS
GZ11190-3-1-2-1	4.53^{de}	5.65^{ef}	MR
GZ11190-3-1-2-3	7.22^{ab}	8.55^{abc}	MS
GZ11190-3-3-1-1	3.75^{e}	4.82^{f}	MR
GZ11190-3-7-2-2	5.43^{bcde}	6.98^{cde}	MS
GZ11190-3-8-2-1	6.93^{abc}	7.73^{bcd}	MS
GZ11190-3-8-2-2	3.92^{e}	4.71^{f}	MR
GZ11190-3-8-2-3	8.13^{a}	9.58^{a}	S
GZ11190-3-13-1-1	4.78^{de}	5.67^{ef}	MR
GZ11190-3-13-4-1	3.87^{e}	4.77^{f}	MR
GZ11190-3-13-4-2	5.82^{bcd}	6.98^{cde}	MS

In the column means followed by a common letter are not significantly different at the Duncun test with P< 0.05 probability.

Molecular analysis

Rice germplasm contains a large number of important genes that plant breeders can use to improve crops. Genetic diversity is fundamentally important for crop development, key factor for germplasm conservation, characterization and breeding effects. DNA primers have the ability to differentiate different rice genotypes based on the differences in their genomic region and their number of alleles. In this study, twenty seven microsatellite (SSR) markers were used to assess the genetic diversity of thirteen rice genotypes. These genotypes were analysed using 20 microsatellite (Table 7) covering 11 chromosomes. Only seven SSR markers were found monomorphic and showed only one allele among all genotypes and were discarded. The level of divergence varied among different rice genotypes for 20 microsatellite loci. All the 20 markers could amplify a total of 102 alleles, varying from 2 to 9 alleles with an average of 5.1 alleles per marker, which is higher than the reports of Sivaranjani, *et al*. (2010) who detected 61 alleles in 46 aromatic cultivars with 26 SSR markers. Maximum number of alleles was observed for the marker RM26652 with 9 alleles followed by 8 alleles each for RM6467 and RM248. Similarly, RM5 and RM289 were detected with 7 alleles, RM3392, RM3805 and RM202 with 6 alleles, RM163, RM3394, RM205 and RM590 with 5 alleles. In the same time, 4 alleles were detected for the markers RM7405,

RM5313 and RM1227 followed by 3 alleles each for RM3850, RM3204 and RM23001. RM20897 and RM6471 could amplify 2 alleles each. Major allele frequency ranged from 0.475 (RM590) to 0.923 (RM5 and RM5313), with an average of 0.705. Polymorphism information content (PIC) is the measure which reflects the discriminating power of the markers. Polymorphism of 13 rice genotypes was assessed by calculating PIC values for each of the 20 SSR markers. The PIC value in this study ranged from 0.247 (RM23001) to 2.118 (RM26652) with a mean of 0.754 Table (7). The PIC value, detected in or study, is higher than the earlier reports of Siwach, *et al*. (2004), Wei, *et al*. (2009), Yuan, *et al*. (2007) and Anand, *et al.* (2012). The variability parameters, including number of alleles, major allele frequency and Polymorphism information content (PIC) indicated the suitability of markers chosen for diversity analysis and molecular characterization of rice genotypes. Also, the results for SSR markers related for different characters such as blast resistant gene, makers *i.e.* RM289, RM590 and RM202 related for (R-genes *Pi 26*(t), *Piz* (the most effective genes under this study) and R- gene *Piy*(t), respectively). The same markers were related for R-genes (Goncharova, *et al*.,2020, Eizenga, *et al*.,2009, Xing, *et al*., 2015 and Wang, *et al*., 2014), RM163 and RM3394 related for identified blast resistance to blast genotype (Hasan, *et al*., 2018 and Wang, *et al*., 2015), as show in Table (7). Six SSR markers (RM3392, RM289, RM6467, RM23001, RM205 and RM5313) were determined for grain yield of thirteen promising lines. The same markers were related for grain yield (Zihe, *et al*., 2019, Gaikwad, *et al*., 2014, Wang, *et al*., 2020, Niu, *et al*., 2020, Bernier, *et al*., 2007 and Solis, *et al*., 2018). While, five SSR markers (RM7405, RM5, RM3204, RM163, RM6471) were determined for grain quality and some characters were selected such as heading date, day to flowering traits and root growth by SSR markers (Table 7). The results obtained from marker-assisted diversity analysis provided some useful rice genotypes and could be selected as parents for further breeding programs. Our study provides some SSR markers for improving rice yield and blast resistance by MAS and contributes to the understanding of its molecular mechanisms. This will bring about greater diversity, which will lead to a high productive index in terms of increase in yield and overall quality.

Cluster analysis based on SSR markers

The dendrogram on Fig (9) is divided into two dendrograms. The first one based on analysis for 20 microsatellite SSR markers and was constructed to understand the relationship pattern and the genetic distances among 13 rice genotypes (Fig.9c). The cluster analysis grouped the studied rice genotypes into two main groups (I and II) at the cut of 3.60 genetic distance level. Seven rice genotypes; Giza 178, GZ11190-3-13-4-1, GZ11190-3-1-2-3, GZ11190-3-7-2-2, GZ11190-3-8-2-2, GZ11190-3-8-2-1 and GZ11190-3-8-2-3 were grouped in cluster I, whereas, six rice genotypes; GZ11190-3-3-1-1, GZ11190-3-13-1-1, GZ6296, GZ11190-3-13-4-2, GZ11190-3-1-1-1 and GZ11190-3-1-2-1 were grouped in cluster II. At the cut of 3.20 genetic distance level, the cluster analysis grouped the rice genotypes into four clusters (Fig. 9c). The rice genotypes; Giza 178 as a parent and Gz11190-3-13-4-1 as a promising line was developed through a cross between Giza 178 and GZ6296, and were closely clustered together with the genetic distance of 0.310 C (sub group III). These two rice genotypes could be considered as similar in most studied traits, such as blast resistance and high yield. Also, the second dendrogram based on analysis for five microsatellite SSR markers was constructed to understand the relationship between resistance to blast with the 13 rice genotypes. Cluster analysis divided the studied rice genotypes into two main groups (A and B). Group A was divided into two sub clusters containing A_1, the resistance genotypes except the susceptible line GZ11190-3-13-4-2 and sub cluster A_2 containing the susceptible genotypes as shown in (Fig. 9D). Rice genotypes GZ11190-3-8-2-2 and GZ11190-3-8-2-3 were moderately resistant and GZ11190-3-1-2-3 rice genotype was resistant. Also, group B in Fig. (9D) consisted of the most resistant rice genotypes. Cluster analysis showed that the rice genotypes resulting from genetically similar parents were clustered together. Similar findings of genetic divergence based on SSR were reported earlier in rice by Kanawapee *et al* (2011), Ghaley *et al* (2012) and Kumbhar *et al* (2015).

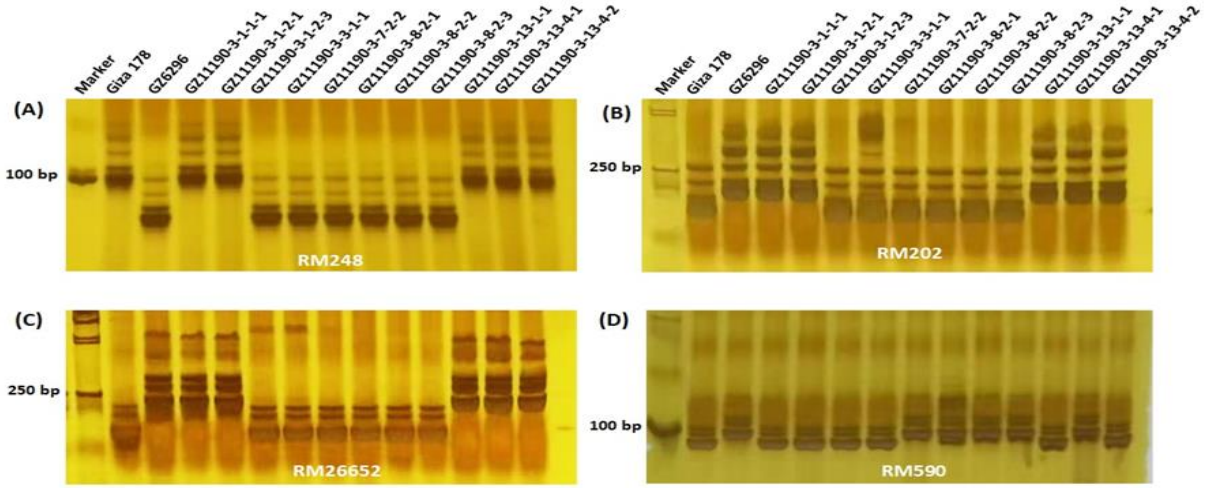


Figure 8. The banding patterns of (A) RM 248, (B) RM202, (C) RM2665 and (D) RM 590 among the 13 genotypes, M: 100 bp ladder.

Table 7. Variability parameters of twenty polymorphic SSR markers used in this study

Marker	Repeat motif	No. of alleles	Major allele frequency	Polymorphism information content (PIC)	Determinates for some characters such as grain yield , quality and blast resistant	Reference
RM7405	(GATG)8	4	0.692	0.852	Grain colour	Maeda, *et al*.,2014
RM5	(GA)14	7	0.923	0.994	Grain width	Steele, *et al.*, 2006
RM3850	(GA)24	3	0.769	0.532	heading date	Hori, *et al.*, 2015
RM3392	(CT)17	6	0.692	0.875	grain Yield	Zihe, *et al.* (2019)
RM3204	(CT)12	3	0.769	0.568	Milling Yield	Zhang, *et al.*, 2020
RM289	G11(GA)16	7	0.538	1.656	Yield component , R gene *Pi 26*(t) and R gene *Piz*	Gaikwad, *et al*., 2014 , Goncharova, *et al*.,2020 and Eizenga,*et al*.,2009
RM163	(GGAGA)4(GA) 11C(GA)20	5	0.538	0.497	Milling quality, grain shape \ identified blast resistance to blast genotype	Zheng, *et al*.,2007 & Hasan, *et al.*, 2018
RM6467	(GCC)8	8	0.725	0.319	grain yield	Wang, *et al.*, 2020
RM3805	(GA)19	6	0.638	0.355	grain yield and days to flowering traits	Venuprasad, *et al.*, 2012
RM3394	(CT)17	5	0.825	0.264	Blast resistant and yield component	Wang, *et al.*, 2015
RM20897	(CTG)7	2	0.665	0.544	root growth angle	Uga, *et al*., 2015
RM248	(CT)25	8	0.538	1.739	Flowering Time, Tiller number on day,	Ranawake and Mori, 2014
RM6471	(GCC)9	2	0.729	0.343	Grain quality, amylose content, protein content	Liu, *et al*., 2011
RM23001	(CCA)9	3	0.825	0.247	Number of grain/ panicle	Niu, *et al*., 2020
RM205	(CT)25	5	0.775	0.287	Grain Yield	Bernier, *et al*., 2007
RM590	(TCT)10	5	0.475	0.531	Blast resistant gene	Xing, *et al*., 2015
RM202	(CT)30	6	0.538	0.994	R- gene *Piy*(t)	Wang, *et al*.2014
RM26652	(AAG)9	9	0.846	2.118	Early root vigour	Wang, *et al*., 2018
RM5313	(TC)12	4	0.923	0.520	Grain Yield under drought	Solis, *et al.*, 2018
RM1227	(AG)15	4	0.692	0.863	spikelet number per panicle	Sasaki, *et al*., 2017
Average		5.1	0.705	0.754	-	-

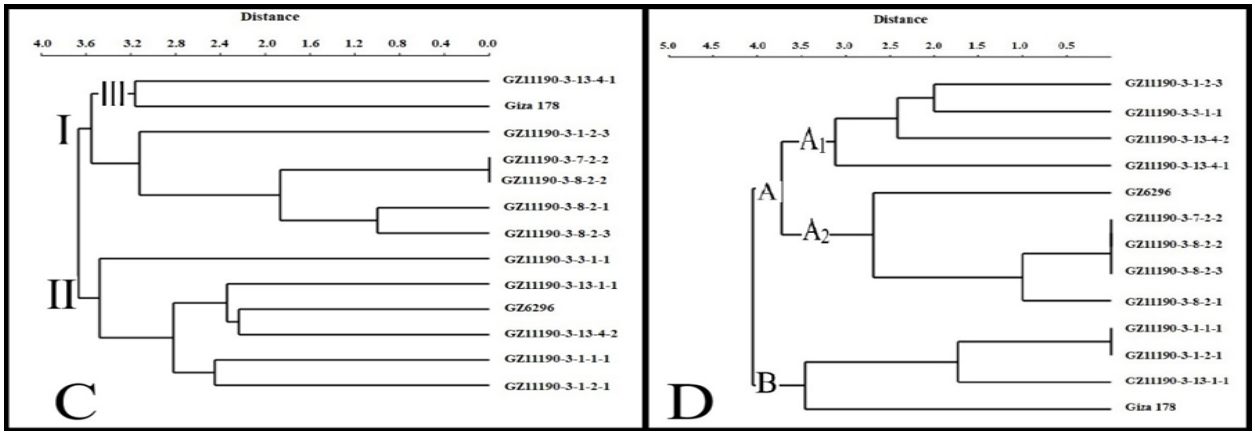


Figure 9. Dendrogram of the thirteen rice genotypes, (C) using 20 SSR markers to all traits and (D) using five SSR markers; RM 289, 163, 3394, 202, and 590 related to blast resistance genes.

REFERENCE

Anand, D., Prabhu, K. V. and Singh, A. K. (2012). Analysis of molecular diversity and fingerprinting of commercially grown Indian rice hybrids. *J. Plant Biochem. Biotechnol*, 21(2):173–179.

Atkins, J.G., Robert, A. L., Adair, C. R., Goto, K., Kozako, T., Yanagida, R., Yamada, Y. and Matsumoto, S. (1967). An international set of rice varieties for differentiating races of *Pyricularia oryzae*. *Phytopathology*, 57: 298-301.

Bernier, J., Kumar, A., Ramaiah, V., Spaner, D. and Atlin, G. N. (2007). A large-effect QTL for grain yield under reproductive-stage drought stress in upland rice. *Crop Sci*., 47: 507–516. Doi: 10.2135/cropsci2006.07. 0495

Chen, X., Jia, Y. and Wu B. M. (2019). Evaluation of rice responses to the blast fungus *Magnaporthe oryzae* at different growth stages, *Plant Disease*, 103 (1):132-136.

Creste, S., Tulmann, N. A. and Figueira, A. (2001). Detection of single sequence repeat polymorphisms in denaturing polyacrylamide sequencing gels by silver staining. *Plant Mol Biol Rep.*, 19 (4): 299-306.

Duncan, M. R.T. (1955). Multiple range and multiple F. test. Biometrics, 11: 1-42.

Duwayri, M., Tran, D.V. and Nguyen, V.N. (1999). Reflection on yield gaps in rice production. Intr. Rice Commission Newsletter. FAO, Rome. (48): 13-25.

Eizenga, G. C., Agrama, H. A., Lee, F. N., Jia, Y. (2009). Exploring genetic diversity and potential novel disease resistance genes in a collection of rice (Oryza spp.) wild relatives. *Genet Resour Crop Evol* ., 56: 65–76. DOI 10.1007/s10722-008-9345-7

El-Hefny, A. S. (2016). Economic threshold and economic injury levels for rice stem borer, using simulated white heads in rice. *Egypt. J. Agric. Res*., 94(2): 353 – 364.

EL-Shafey, R.A., Anis, G.B. and Elmoghazy, A.M. (2016). evaluation of some developed high yielding rice genotypes resistant to blast, bakanae and white tip nematode. *J. Plant Production, Mansoura Univ.,* 7 (3): 317- 329.

FAO, rice market monitor (2018). VOLUME XXI ISSUE No. 1 , 38 pp.

Fukuoka, S., Yamamoto, S.I., Mizobuchi, R. and Yamanouchi, U. *et al*. (2014). Multiple functional polymorphisms in a single disease resistance gene in rice enhance durable resistance to blast. *Sci. Rep*, 4: 4550.2

Gaikwad, K.B., Singh, N., Bhatia, D., Kaur, R., Bains, N.S., *et al*. (2014). Yield-enhancing heterotic QTL transferred from wild species to cultivated rice *Oryza sativa* L. *PLoS ONE* 9(6): e96939. Doi:10.1371/journal.pone.0096939

Ghaley B. B., Christiansen J. L., Andersen S. B. (2012). Genetic diversity for blast resistance of Bhutan rice landraces. *Euphytica*, 184 (1): 119–130.

Goncharova, J., Bragin, O., Goncharov, S., and Kharitonov, E. (2020). Loci that determine long-term resistance to blast of Russian rice varieties. *XI International Scientific and Practical Conference "Biological Plant Protection is the Basis of Agroecosystems Stabilization*, 21, 00014, 1-6.

Hammer, O., Harper, D.A.T., P.D. Ryan (2001). PAST: Paleontological statistics software package for education and data analysis. Paleontol. Electron., 4:1-9.

Hasan, M. M, Rafii, M. Y., Ismail, M. R., Mahmood, M., Rahim, H. A., Latif, M. A., Amirul Alam Md., Fahim Ahmad & Malek M. A. (2018). Genetic analysis of the resistance to rice blast in the BC2F1 population derived from MR263 × Pongsu Seribu1. *Biotechnology & Biotechnological Equipment*, 32(5): 1134-1140. DOI: 10 .1080 /13102818. 2018. 1506266

Hori, K., Nonoue, Y., Ono, N., Shibaya, T., Ebana, K., Matsubara K., Ogiso-Tanaka, E., *et al* (2015). Genetic architecture of variation in heading date among Asian rice accessions. *BMC Plant Biology* 15(115): 1-16. DOI 10.1186/s12870-015-0501-x

IRRI, (2013). Standard evaluation system for rice (SES). Manila*: International Rice Research Institute*.

Kalboush, Z.A. (2019). Resistance of rice genotypes to the blast fungus and the associated biochemical changes. *Egypt. J. Agric. Res*., 97 (1): 39-55.

Kanawapee N., Sanitchon J., Srihaban P., Theerakulpisut P. (2011). Genetic diversity analysis of rice cultivars (Oryza sativa L.) differing in salinity tolerance based on RAPD and SSR markers. *Elect J Biotech*, 14(6): e1379.

Kumbhar, S. D., P. L. Kulwal, J. V. Patil, C. D. Sarawate, A. P. Gaikwad, A. S. Jadhav (2015). Genetic diversity and population structure in landraces and improved rice varieties from india. *Rice Science*, 2015, 22(3): 99-107

Liang, Y., Yan, B.Y., Peng, Y. L., Ji, Z.J., Zeng, Y.X., Wu, H.L. and Yang, C.D. (2017). Molecular screening of blast resistance genes in rice germplasms resistant to *Magnaporthe oryzae*. *Rice Sci*., 24:41-47.

Liu, K. and Muse, S.V. (2005). Power marker: integrated analysis environment for genetic marker data. *Bioinformatics*, 21:2128–2129.

Liu, X., Xiangyuan, W., Xiaodong, M., and Jianmin, W. (2011). Dissecting the genetic basis for the effect of rice chalkiness, amylose content, protein content, and rapid viscosity analyzer profile characteristics on the eating quality of cooked rice using the chromosome segment substitution line population across eight environments. *Genome*, 54:64-80. Doi:10.1139/G10-070

Luo, Z.Y., Zhou, G., Chen, X.H., Lu, Q.H., Hu, W.X. (2005). Isolation of high-quality genomic DNA from plants. *Bull Hunan Med Univ*. 26: 178-180,

Maeda, Y., Omoteno, T., Fujita, M., Iyama, K., Morikawa, O., Mukaino, K. and Ebitani, H. M., *et al*., (2014). Genetic dissection of black grain rice by the development of a near isogenic line. *Breeding Science*, 64: 134–141. Doi:10.1270/jsbbs.64.134

Marvet, M. Awadallah, Taha A. S. and Tahoon A. M. (2021). Genetic variability, insect and disease resistance of some promising rice genotypes. *J. of Plant Protection and Pathology, Mansoura Univ*., 12 (6): 413 – 421.

Niu, X., Zhu Y., Sun Z., Yu S., Zhuang J. and Fan Y. (2020). Identification and validation of quantitative trait loci for grain number in rice (Oryza sativa L.), *Agronomy*, 10 (180):1-15. Doi:10.3390/agronomy10020180

Qin, P., Xiaochun H., Jiang N., Bai Z., Liu T., Fu C., Song Y., Wang K. and Yang Y. (20201). A Procedure for inducing the occurrence of rice seedling blast in paddy field. *Plant Pathology Journal*, 37(2):200-203.

Ranawake, A. L. and Mori N. (2014). Study on flowering time SSR marker RM 248 in traditional rice accessions in Sri Lanka. *British Biotechnology Journal*, 4(11): 1165-1174.

RRTC, (2004). Rice Research and Training Center. The 8th national rice program workshop (final results of 2003 season) March 2014, PP 125-142, Rice Research and Training center, Agricultural Research Center, Ministry of agricultural and land Reclamation, Egypt.

Sadat, S., Saeid K.A., Bihamta M.R. and Lotfali Ayeneh G.A. (2013). Marker assisted selection for heat tolerance in bread wheat. *World Applied Sciences Journal*, 21(8):1181-1189.

Sasaki, K., Fujita D., Koide Y., Lumanglas P.D., Gannaban R. B., Tagle A. G., Obara M., Fukuta Y., Kobayashi N. and Ishimaru T. (2017). Fine mapping of a quantitative trait locus for spikelet number per panicle in a new plant type rice and evaluation of a nearisogenic line for grain productivity. *Journal of Experimental Botany*, 68(11): 2693–2702. Doi:10.1093/jxb/erx128

Sehly, M. R., El-Wahsh S. M., Badr E. A. S., EL-Malky M.M. H, EL-Shafey R. A. S. and Aidy I.R. (2008). Evaluation of certain Egyptian rice cultivars to blast disease incidence during fourteen years in Egypt, *J. Agric. Sci., Mansoura Univ*., 33 (4): 2533 – 2547.

Shabana, Y. M., El-Wahsh S. M., Abdelkhalik A. F., Fayzalla1 S. A. and Hassan A. A. (2013). Physiological races of rice blast and host resistant genes under Egyptian condition, *J. Plant Prot. and Path., Mansoura Univ*., 4 (8): 709 – 720.

Shahriar, S.A., Imtiaz A.A., Hossain M.B., Husna A., Eaty M.N. (2020). Rice Blast Disease. *Annual Research & Review in Biology* 2(1):50-64.

Shobharani, N., Rama Prasad, A. S., Prasad, G. S. V. and Pati, D. (2003). Relationship of hull content with milling traits in scented rices, *Journal of Food Science and Technology*, 40: 663-665.

Sivaranjani, A.K.P., Pandey M.K., Sudarshan I., Ram K.G., Sheshu M.M., Sundaram R.M., Varaprasad G.S. and Shobharani N. (2010). Assessment of genetic diversity among basmati and non-basmati aromatic rices of India using SSR markers. *Current Science*, 99(2): 221-226.

Siwach, P., Jain S., Saini N., Chowdhury V.K. and Jain R.K. (2004). Allelic diversity among Basmati and non-Basmati long-grain indica rice varieties using microsatellite markers. *Journal of Plant Biochemistry and Biotechnology*, 13: 25-32.

Skamnioti, P. and Gurr, S. J. (2009). Against the grain: safeguarding rice from rice blast disease. *Trends Biotechnol*., 27:141- 150.

Soliman, A. M., A. S. Hendawy, Amany S. El-Hefny and M. R. Sherif (2016). Determination of economic threshold level for *Chilo agamemnon* Bles. infestation in rice plants, based on simulated dead hearts. *Egypt. J. Agric. Res*., 94(1): 59 – 69.

Solis, J., Gutierrez A., Mangu V., Sanchez E., Bedre R., Linscombe S. and Baisakh N. (2018). Genetic mapping of quantitative trait loci for grain yield under drought in rice under controlled greenhouse. *Front. Chem*., 5(129):1-12. Doi: 10.3389 /f chem. 2017.00129

Steele, K. A., Price A. H., Shashidhar H. E. and Witcombe J. R. (2006). Marker-assisted selection to introgress rice QTLs controlling root traits into an Indian upland rice variety. *Theor Appl Genet*., 112: 208-221. DOI 10.1007/s00122-005-0110-4

Taha, A. S. (2017). Effect of silica and nitrogen fertilization on rice yield and stem borer infestation. Ph. D. Thesis, Fac. Agric., Kafr El-Sheikh Univ.

Uga, Y., Kitomi Y., Yamamoto E., Kanno1 N., Kawai1 S., Mizubayashi1 T. and Fukuoka S. (2015). A QTL for root growth angle on rice chromosome 7 is involved in the genetic pathway of DEEPER ROOTING 1 *Rice*, 8(8): 1-8. DOI 10.1186/s12284-015-0044-7

Venuprasad R., Bool M. E., Quiatchon L., Atlin G. N. (2012). A QTL for rice grain yield in aerobic environments with large effects in three genetic backgrounds. *Theor Appl Genet*, 124: 323–332. DOI 10.1007/s00122-011-1707-4

Wang, F., Longkumer T., Catausan S. C., Calumpang C. L. F., Tarun J. A., Cattin-Ortola J., Ishizaki T., Tanaka J. P., Rose T., Wissuwa M. and Kretzschmar T. (2018). Plant cell environ genome-wide association and gene validation studies for early root vigour to improve direct seeding of rice. *Plant Cell Environ*, 41: 2731 –2743. DOI: 10.1111/pce.13400

Wang, X., Seonghee L., Wang J., J. Ma, Tracy B. and Yulin J. (2014). Current advances on genetic resistance to rice blast disease. Chapter7 Rice - Germplasm, Genetics and Improve, 195-217. DOI: 10.5772/56824

Wang, X., Melissa H. J., Pooja ., Fleet N. L., and Jia Y. (2015). Genome-wide association of rice blast disease resistance and yield-related components of rice. American Phytopathological Society, 28(12): 1383–1392. http://dx.doi.org/10.1094/MPMI-06-15-0131-R

Wang, Y., Wang J., Zhai L., Liang C., Chen K., Xu J. (2020). Identify QTLs and candidate genes underlying source, sink, and grain yield-related traits in rice by integrated analysis of bi-parental and natural populations. *PLoS ONE* 15(8): 0237774. https://doi.org/10.1371/journal.

Wang, J.C. , Y. Jiab, J.W. Wen, W.P. Liu, X.M. Liu, L. Li, Z.Y. Jiang, J.H. Zhang, X.L. Guo and Ren, J.P. (2013). Identification of rice blast resistance genes using international monogenic differentials. *Crop Protection*, 45:109-116.

Wei, X., Yuan X., Yu H., Wang Y., Xu Q. and Tang S. (2009). Temporal changes in SSR allelic diversity of major rice cultivars in China. *Journal of Genetics and Genomics*, 36: 363–370.

Xing, J., Jia M. H., Correll J. C., Yuan L., Deng H. and Jia Y. (2015). Confirming and identifying new loci for rice blast disease resistance using *Magnaporthe oryzae* field isolates in the US. *Crop Sci*., 55: 2620–2627. Doi: 10.2135/cropsci2015.02.0077

Yuan, X.P., Wei X.H., Hua L., Yu H.Y., Wang Y.P., Qun X.U. and Tang S.X. (2007). A Comparative study of SSR diversity in chinese major rice varieties planted in 1950s and in the recent ten years (1995–2004). *Rice Science* 14: 78–84.

Zeigler, R. S.; Thome J.; Nelson J.; Levy M. and Correa F. (1994). Linking blast population to resistance breeding: A proposed strategy for durable resistance. Pages 267–292 in: Zeigler, R.S., Leong S.and Teng P.S. (eds.). Rice Blast Disease, CAB International, Wallingford, UK.

Zhang, H., Zhu Y.-Jun, An-Dong Zhu, Ye-Yang Fan, Ting-Xu Huang, Jian-Fu Zhang, Hua-An Xie and Jie-Yun Zhuang (2020). Identification and verification of quantitative trait loci affecting milling yield of rice. *Agronomy*, 10, 75. doi:10.3390/agronomy10010075

Zheng, T. Q., Xu J. L., Li Z. K., Zhai H. Q. and Wan J. M. (2007). Genomic regions associated with milling quality and grain shape identified in a set of random introgression lines of rice (*Oryza sativa* L.), *Plant Breeding*, 126: 158-163.

Zihe, L.I., *et al*. (2019). Quantitative trait loci for rice yield-related traits using chromosomal segment substitution lines. *Rice Science*, 26(5): 261-264.

تحليل التنوع الوراثى والتوصيف الجزيئي لمحصول سلالات الارز المبشرة، ومقاومتها لمرض اللفحة وثاقبة ساق الارز

جلال بكر انيس[1]، عمرو عبدالبارى حسن[2] و احمد سمير طه[1]

[1]معهد بحوث المحاصيل الحقلية- مركز البحوث الزراعيه- مصر

[2]معهد بحوث امراض النباتات- مركز البحوث الزراعيه- مصر

يعد استنباط وزراعة سلالات أرز جديدة مبشرة ذات إنتاجية عالية ومقاومة لمرض اللفحة هدفًا مهمًا لتربية الأرز. تهدف الدراسة الي تقييم بعض سلالات الأرز الجديدة للإنتاجية العالية، مقاومة مرض اللفحة، وتحديد جينات المقاومة الفعالة ل *Pyricularia grisea* والمقاومة لحشرة ثاقبة ساق الأرز بالإضافة الى تقييم التنوع الوراثي في هذه السلالات باستخدام المعلمات الجزيئية. وجد أن هناك فروقا معنوية بين سلالات الأرز الجديدة المدروسة والآباء فيما يتعلق بالمحصول والصفات المكونة له. أعطت السلالات GZ11190-3-13-1-1 و GZ11190-3-13-4-1 أعلى إنتاج للحبوب مقارنة بابائهم جيزة 178 و GZ6296. هذه السلالات هي أيضا متفوقة فى معظم الصفات الأخرى المدروسة. تحت الدراسه تم عزل ستة وعشرين عزلة تنتمى لخمس مجاميع رئيسية هى IC، ID، IF، IG و IH ، لكن مجموعة ID و IH كانت الأكثر شيوعًا. كان جين المقاومة *Pib* هو الجين الأكثر فاعلية لفطر اللفحة. كما أظهرت الدراسة ان السلالات GZ11190-3-13-1-1 و GZ11190-3-13-4-1 مقاومة لستة وعشرين سلالة من *P. grisea* تحت ظروف الصوبة الزجاجية ومقاومة متوسطة لحشرة ثاقبة الساق. لتحليل التنوع الجيني ، تم استخدام عشرين معلم جزيئى SSR متعددة الأشكال بمتوسط 102 من الأليلات. أظهر توصيف هذه المعلمات قوة تمييز عالية بمتوسط محتوى معلومات متعدد الأشكال (PIC) يبلغ 0.754. تراوح تردد الأليل الرئيسي من 0.475 للمعلم الجزيئى RM590 الى 0.923 للملعم الجزيئى RM5 و RM5313 بمتوسط 0.705 . قسم التحليل العنقودي المستند إلى المعلمات الجزيئية التراكيب الوراثية الثلاثة عشر المدروسة الى مجموعات منفصلة واضحة وكان قادرًا على الكشف عن العلاقات الوراثية الوثيقة بين التراكيب الوراثية حتى يمكن استخدامها في برنامج تربية الأرز .