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Characterization and selection of novel rice promising lines based on genetic variability, grain yield, yield components and rice stem borer susceptibility

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Abstract

This research was carried out at Sakha Agricultural Research Station, Egypt during 2020 and 2021 to investigate the genetic diversity among 14 rice genotypes for some characteristics and SSR markers linked with some specific traits. Also, the susceptibility of these genotypes to rice stem borer was assessed. Highly significant differences were found among genotypes, indicating into wide variations in genetic background. Indica/ japonica genotypes; GZ10848-1-2-2-1 and GZ10590-1-3-3-2 yielded 11.10 and11.00 t/ha., while Giza 178 and Giza 179 yielded 10.90 and 10.70 t/ha. Phenotypic coefficient of variance (PCV) was generally higher than genotypic coefficient of variance (GCV) indicating the environmental influence on the expression of traits. The highest PCV and GCV were observed in filled grains panicle^{-1} and panicles plant^{-1}. The positive correlation coefficients between grain yield and number of panicles and number of filled grains/panicle indicate that better exploration of these traits could be used to develop desired rice genotypes. Dendrogram analysis divided the 14 rice genotypes into four main clusters based on the mean values of the studied traits. Out of the 14 rice genotypes, six entries were resistant or moderately resistant to stem borer, *Chilo agamemnon* Bles. and these entries could be candidates for developing insect-resistant varieties. The dendogram examination based on DNA level succeeded in dividing rice genotypes into two primary branches agreeing to their hereditary background. Screening, utilizing SSR markers, may be an effective instrument for varietal recognizable proof and surveying hereditary differing qualities in rice.

Keywords: rice, genetic variability, correlation coefficient, cluster analysis, stem borer

Introduction

Rice (*Oryza sativa* L.) ranks second to wheat in area and production on the global level. It is a critical crop for food security in Egypt. Breeding novel rice entries with high yield potential and desirable technological and eating traits is an important task to be achieved by the breeders. Phenotypic coefficient of variance was higher than genotypic coefficient of variance for all studied traits indicating the environmental influence on the expression of these traits, Rashid *et al*. (2017) [27] and Gyawali *et al*. (2018) [13]. Higher phenotypic coefficient of variation as compared to genotypic coefficient of variation reveals the influence of environment on the expression of traits (Adhikari *et al*. 2018) [1]. Aditya and Bhartiya (2013) [2] reported that variability, genetic diversity, expected genetic advances and heritability of the traits are key basis for genetic improvement of any trait. Aly *et al*. (2000) [4] indicated that heritability is the ratio of variation due to differences among genotypes to the total phenotypic variation for a trait in a population and shows the component of a character transmitted to future generations, and added that genetic advance shows the difference between the mean genotypic values of selected population and the original population from which these were selected. The rice stem borer (*Chilo agammnon* Bles.) is the most important insect pest of rice in Egypt (Sherif *et al*. 2008) [34]. Yield losses in rice fields were estimated as about 10 % (Taha *et al*. 2021) [38]. The indica rice cultivars were found, in most cases, more susceptible to the borer than japoinca rice cultivars (Sherif and Bastawisi 1997) [33]. The symptom of white heads is more negatively affecting on rice yield as compared with the symptom of dead heart, because the dead hearts during vegetative stage could be compensated by new tillers (Sherif *et al*. 2008) [34]. Insect resistant varieties play an important role in the management of rice pests; particularly they have the unique advantage of not involving an extra cash expenditure to the farmer and provide an inherent insect control (Sherif *et al*, 2005)[35]. Evaluating rice promising lines for rice stem borer infestation is important, and is required to determine the value for cultivation and use (VCU) of the variety by the central administration for registration and approving the seeds. Simple sequence repeat (SSR) markers can assist breeders to choose genotypes carrying gene (s) of intrigued (Sadat *et al*., 2013) [31], and give the breeders productive methodologies to optimize time and assets andencourage their control in segregating plant breeding populations. These are effective instruments for numerous thinks about genome characterization, discovery of quantitative characteristic loci (QTL) for both abiotic and biotic stresses, developmental thinks about, and for marker helped determination (MAS) (Chu *et al*., 2010 and Sadat *et al*., 2013) [10, 31]. Mazal (2014) [19] concluded that characteristics like short stature, increased tillers number, reduced stem diameter and high stem silica content should be considered for developing rice cultivars resistant to stem borer. SSR markers (RM 263. RM201, RM566, RM166 and RM164) facilitate choosing cultivars to stem borer resistance. Additionally, we should rely on Sakha101, Sakha104 and Giza176 as cultivars resistant to stem borer attack in breeding programs. Therefore, this study was undertaken to get information about variability, heritability, genetic advance and traits correlation in promising rice genotypes to develop better genotype for irrigated ecology in Egypt. In addition, these genotypes were evaluated for the susceptibility of rice stem borer as an important step for developing new rice genotypes resistant to the insect borer.

Materials and Methods

This field study was conducted at the Experimental Farm of Rice Research and Training Center (RRTC) Sakha, Kafr El-Sheikh, Egypt during 2020 and 2021 growing seasons. Fourteen rice genotypes (eleven new promising lines and three commercial varieties) were evaluated. The seeds were obtained from Rice Research and Training Center (RRTC). The experiment was laid out in a randomized complete block design (RCBD) with three replications. One seedling per hill was transplanted in the permanent field after 25 days with the spacing of 20 × 20 cm between plants in 5 m long rows. All agronomic practices; fertilization, irrigation and weed and pests control were done as recommended with rice crop during both seasons. The name, type and pedigree of the studied genotypes are listed in Table (1).

Table 1: Name, type and pedigree of fourteen rice genotypes under study

Genotype	Parentage	Type
GZ10590-1-3-3-2	GZ8126-1-3-1-2/HR17570-21-5-2-5-2	Indica/Japonica
GZ10848-1-2-2-1	Giza 178/SKC23893	Indica/Japonica
GZ11190-3-13-4-1	Giza 178/GZ6296	Indica/Japonica
GZ11190-3-3-1-1-1	Giza 178/GZ6296	Indica/Japonica
GZ11453-17-7-7-5	Giza 178/Suweon 370	Indica/Japonica
GZ10101-5-1-1-1	Sakha 103/IR385	Japonica
GZ10590-1-1-3-9-1	GZ8126-1-3-1-2/HR17570-21-5-2-5-2	Indica/Japonica
GZ10686-2-1-3-2	Sakha 104/ WAB450-1-1-p-106	Japonica
GZ10804-3-1-2-2-2	GZ 7576/IR81828	Japonica
GZ11245-1-3-3-2	Sakha 102/ Yunda 01	Japonica
GZ11245-1-3-3-3	Sakha 102/ Yunda 01	Japonica
Giza 178	Giza 175/Milyang 49	Indica/Japonica
Giza 179	GZ1368-S-5-4/ GZ6296	Indica/Japonica
Giza 177	Giza 171/Yomji No.1//PiNo.4	Japonica

Data Collection

The plant characteristics, *i.e.*, duration (days), plant height (cm), number of panicles plant^{-1}, panicle length (cm), number of filled grains panicle^{-1}, grain yield (t/ha), hulling %, milling % and head rice % were measured according to the standard evaluation system (SES) of rice (IRRI, 2006) [16]. Grain yield was adjusted to 14% moisture content after one week of sun drying after threshing.

Evaluation of Genotypes to the Stem Borer (*Chilo agamemnon* Bles.)

Forty days after transplanting, the rice plants were examined to determine the dead heart symptom. From each plot, five random hills were cut at the soil surface. The total number of tillers and those having dead hearts were counted, thus, the dead heart percentage was calculated. Three weeks prior to harvest, white head percentage was estimated. Five hills per plot were cut at the soil surface, the total number of tillers and those having white heads were recorded, and thus, the white head percentage was calculated. The evaluated entries were categorized as follows: 0 - 3% white heads (WH), resistant, > 3-6% WH, moderately resistant, > 6-9% WH, moderately susceptible, > 9-12% WH, susceptible, > 12% WH, highly susceptible (RRTC, 2016) [29].

Genomic DNA Isolation

Total genomic DNA was extracted from 15 day-old seedlings after crushing in liquid nitrogen in microfuge tubes using CTAB system as described by Murray and Thompson (1988) [21]. The quantity and quality of DNA were assessed with 0.8% agarose gel electrophoresis using diluted uncut lambda phage DNA as a size standard. The concentration of DNA was adjusted to approximately 15 ng/ µl for PCR reaction.

PCR Amplification and Electrophoresis

Genetic diversity for 14 genotypes was screened using 21 SSR markers. Primer names, sequences and chromosome number are listed in Table (2). PCR amplification reactions were performed in 10 µl reaction mixtures, containing 1µl of template DNA, 2 µl of each forward and reverse primers, 3 µl ddH$_2$O and 5 µl of 2X *GoTaq* Green Master Mix (Promega, USA.). The reaction mixture was first denatured for 5 min at 95°C, followed by 35 cycles of denaturation for 1 min at 94°C, annealing at T_m - 2°C for 30 seconds and elongation at 72°C for 1min, and a final extension at 72°C for 10 min. PCR amplification was loaded in 3% agarose gel containing Ethidium Bromide for electrophoresis in 1X TAE (pH 8.0). DNA ladder (100bp) was used for determination of size of amplicons. The gel was run at 60 volts (2.5V/cm) for 3 hrs and photographed using Biometra gel documentation unit (BioDoc, Biometra, Germany). Scoring of amplified bands was done as present (1) or absent (0) for each genotype and primer pair. To identify the informative SSR marker, polymorphism information content (PIC) value for each SSR marker was analyzed using the software package, NTSYS-pc version 2.11 (Rohlf, 1988) [28]. The total number of amplified bands, number of polymorphic alleles, number of amplified alleles and polymorphism ratio (P %) and Polymorphism information content (PIC) were calculated to assess the diversity of alleles of marker locus (Anderson *et al.*, 1993) [5]. Genetic similarity coefficients were used to construct a dendrogram using the Unweighed Pair Group Method with Arithmetic Average (UPGMA) sequential agglomerative hierarchal nested (SHAN) cluster.

Statistical Analysis

The observations and mean values were recorded and subjected to statistical analysis. Analysis of variance was computed for each season assuming that the genotypes under study are random. As the error variances of the experiments were statistically homogeneous, the two experiments were statistically combined over the two seasons. The descriptive statistical analysis, analysis of variance (ANOVA) of yield and its component traits and correlation coefficients were worked out using SPSS software (version 20.0). The estimates of genotypic variance (GV), phenotypic variance (PV), phenotypic coefficient of variations (PCV%) and genotypic coefficient of variations (GCV%) were computed according to the formula suggested by Burton (1952) [8]. Broad sense heritability was calculated using formula suggested by Hanson *et al* (1956) [15]. The estimates of genetic advance were obtained and categorized as high, medium and low as suggested by Johnson *et al.* (1955) [18]. The genetic distance tree construction was expressed by using the Paleontological Statistics (PAST) software package using the mean performance of the studied genotypes (Hammer *et al.* 2001) [14].

Table 2: List of SSR markers sequences and chromosome number

Primer name	Chromosome number	Sequence	
		Forward	**Reverse**
RM5	1	TGCAACTTCTAGCTGCTCGA	GCATCCGATCTTGATGGG
RM148	3	ATACAACATTAGGGATGAGGCTGG	TCCTTAAAGGTGGTGCAATGCGAG
RM164	5	TCTTGCCCGTCACTGCAGATATCC	GCAGCCCTAATGCTACAATTCTTC
RM166	2	GGTCCTGGGTCAATAATTGGGTTACC	TTGCTGCATGATCCTAAACCGG
RM201	9	CTCGTTTATTACCTACAGTACC	CTACCTCCTTTCTAGACCGATA
RM208	2	TCTGCAAGCCTTGTCTGATG	TAAGTCGATCATTGTGTGGACC
RM224	11	ATCGATCGATCTTCACGAGG	TGCTATAAAAGGCATTCGGG
RM246	1	GAGCTCCATCAGCCATTCAG	CTGAGTGCTGCTGCGACT
RM263	2	CCCAGGCTAGCTCATGAACC	GCTACGTTTGAGCTACCACG
RM315	1	GAGGTACTTCCTCCGTTTCAC	AGTCAGCTCACTGTGCAGTG
RM430	5	AAACAACGACGTCCCTGATC	GTGCCTCCGTGGTTATGAAC
RM440	5	CATGCAACAACGTCACCTTC	ATGGTTGGTAGGCACCAAAG
RM510	6	AACCGGATTAGTTTCTCGCC	TGAGGACGACGAGCAGATTC
RM512	12	CTGCCTTTCTTACCCCCTTC	AACCCCTCGCTGGATTCTAG
RM555	2	TTGGATCAGCCAAAGGAGAC	CAGCATTGTGGCATGGATAC
RM585	6	CAGTCTTGCTCCGTTTGTTG	CTGTGACTGACTTGGTCATAGG
RM3586	3	GAAGAGAGAGCCAGAGCCAG	ACACGATCGAGCTAGAAGACG
RM3735	4	GCGACCGATCAGCTAGCTAG	ATAACTCCTCCCTTGCTGCC
RM5687	4	GATCGCTGGCGATTGATC	GACTTGTGGGGTGGTTTTTG
RM7601	7	GCCTCGCTGTCGCTAATAC	CAGCCTCTCCTTGTGTTGTG
RM527	6	GGCTCGATCTAGAAAATCCG	TTGCACAGGTTGCGATAGAG

Results and Discussion

Analysis of Variance

Analysis of variance for studied traits was highly significant indicating that a wide of variation among the rice genotypes in their genetic background (Table 3).

Table 3: Mean squares of fourteen rice genotypes for nine agro-morphological traits

Source	Replications (d.f - 2)	Treatments (d.f - 13)	Error (d.f - 26)
Duration(day)	1.88	30.12**	0.36
Plant height (cm)	2.95	39.95**	1.33
Number of panicles/plant	0.21	9.39**	0.62
Panicle length (cm)	0.38	4.97**	0.44
Number of filled grains/panicle	16.38	281.46**	26.07
Grain yield (t/ha)	0.00	0.64**	0.01
Hulling %	0.31	6.49**	0.54
Milling %	0.21	4.75**	0.5
Head rice %	0.5	7.96**	0.94

Mean Performance

Data in Table (2) showed that the indica/ japonica rice genotypes; GZ10590-1-3-3-2, GZ10848-1-2-2-1, GZ11190-3-13-4-1, GZ11190-3-3-1-1-1 and GZ11453-17-7-7-5 are earlier than Giza 178 by around 10 days, shorter in plant height except GZ11190-3-3-1-1-1. GZ11190-3-3-1-1-1, GZ10590-1-3-3-2 and GZ11190-3-13-4-1 which recoded the highest values of number of panicles plant^{-1} and panicle^{-1} length compared with Giza 178 as indica/ japonica type. GZ10590-1-3-3-2 indica/ japonica gave number of filled grains panicle^{-1} higher than Giza 178. For japonica types; GZ10101-5-1-1-1, GZ10590-1-1-3-9-1, GZ10686-2-1-3-2, GZ10804-3-1-2-2-2, GZ11245-1-3-3-2 and GZ11245-1-3-3-3 exhibited the highest values of number of panicles plant^{-1}, panicle length and number of filled grains panicle^{-1} compared to the japonica type Giza 177, in addition to the promising lines that are shorter than Giza 177 (Table 3). There is no difference between the japonica promising lines and Giza 177 in duration trait (Table 4).

Table 4: Duration, plant height, number of panicles, panicle length and number of filled grains for studied rice genotypes

Genotype	Duration (day)	Plant height (cm)	No. of panicles/plant	Panicle length (cm)	No. of filled grains/panicle
GZ10590-1-3-3-2	123.3	94.7	24.7	23.8	165.0
GZ10848-1-2-2-1	124.3	94.0	24.0	20.9	147.7
GZ11190-3-13-4-1	123.3	99.0	24.7	22.0	146.3
GZ11190-3-3-1-1-1	124.3	100.3	25.0	22.2	151.3
GZ11453-17-7-7-5	124.7	87.0	23.3	20.8	133.3
GZ10101-5-1-1-1	124.3	98.0	22.0	22.9	130.0
GZ10590-1-1-3-9-1	124.7	97.0	22.3	23.8	136.7
GZ10686-2-1-3-2	125.3	97.0	21.3	20.2	142.0
GZ10804-3-1-2-2-2	125.3	98.3	22.3	22.6	137.7
GZ11245-1-3-3-2	124.3	93.3	23.3	22.3	137.7
GZ11245-1-3-3-3	125.0	93.0	23.3	22.3	140.7
Giza 178	135.3	100.0	23.3	21.2	150.3
Giza 179	121.0	96.0	23.3	20.2	142.7
Giza 177	125.0	100.0	18.0	19.9	128.0
LSD = 0.05% = 0.01%	0.85 1.23	1.61 2.34	1.10 1.60	0.93 1.35	7.11 10.34

For grain yield, the indica/ japonica rice genotypes GZ10848-1-2-2-1 and GZ10590-1-3-3-2 gave 11.10 and 11.00 t/ha., while, Giza 178 and Giza 179 gave 10.90 and 10.70 t/ha, respectively (Table 5). GZ10590-1-3-3-2, GZ10848-1-2-2-1, GZ11190-3-13-4-1, GZ11190-3-3-1-1-1 and GZ11453-17-7-7-5 showed the highest values of milling % and head rice % compared with the indica/ japonica Giza 179. Regarding to japonica rice varieties, GZ10686-2-1-3-2, GZ10804-3-1-2-2-2, GZ11245-1-3-3-3 and GZ11245-1-3-3-2 recorded 10.7 and 10.6 t/ha compared with the japonica variety Giza 177 which gave 9.7 t/ha.

Table 5: grain yield, hulling %, milling % and head rice % for some of promising lines and commercial rice varieties

Genotype	Grain yield (t/ha)	Hulling (%)	Milling (%)	Head rice (%)
GZ10590-1-3-3-2	11.0	80.3	71.3	67.3
GZ10848-1-2-2-1	11.1	79.7	70.7	64.3
GZ11190-3-13-4-1	10.5	78.7	70.3	64.7
GZ11190-3-3-1-1-1	10.5	79.3	70.7	65.3
GZ11453-17-7-7-5	10.2	78.3	71.0	66.0
GZ10101-5-1-1-1	9.7	82.3	72.3	68.3
GZ10590-1-1-3-9-1	9.8	80.0	71.7	66.3
GZ10686-2-1-3-2	10.7	82.0	72.3	65.7
GZ10804-3-1-2-2-2	10.7	82.7	72.7	68.7
GZ11245-1-3-3-2	10.6	80.3	71.7	66.3
GZ11245-1-3-3-3	10.7	80.3	71.7	66.7
Giza 178	10.9	79.0	70.0	67.0
Giza 179	10.7	79.0	68.0	62.3
Giza 177	9.7	82.3	72.7	66.0
LSD = 0.05% = 0.01%	0.15 0.22	1.02 1.49	0.98 1.43	1.35 1.96

Estimates of Genetic Parameters

Parent's means, estimates of genotypic and phenotypic variance, phenotypic coefficient of variance (PCV), genotypic coefficient of variability (GCV), heritability in broad sense and genetic advance% are presented in Table (6). Analysis of variance showed a wide range of variability among studied rice genotypes for all studied characters over the two seasons, where mean squares were highly significant. Therefore, the selection would be effective among the rice genotypes for these characters. Similar results were obtained by Sedeek *et al*. (2009) [32]. Data in Table (5) exhibited that the phenotypic coefficient of variance was generally higher than genotypic coefficient of variance for all studied traits indicating the environmental influence on the expression of these traits, but the biggest portion of P.C.V was contributed by the genotypic component, less than by environmental component. The highest phenotypic and genotypic variances were observed in number of filled grains panicle^{-1}.The highest values of phenotypic coefficient of variability and genotypic coefficient of variability were recorded for number of filled grains panicle^{-1} and number of panicles plant^{-1}. Heritability percentage is estimated as the ratio between the genotypic variance and the total of phenotypic variance. Heritability in broad sense was high for most of studied characters with values of 96.42% for duration and 71.44% for head rice % (Table 5). High heritability coupled with high genetic advance (Table 5) was observed for number of filled grains panicle^{-1} and days to heading. This indicates that selection process for these traits would certainly bring improvement in the genotypes. Burton (1952) [8] and Dhakal *et al*. (2020) [11] concluded that genotypic coefficient of variance, together with heritability estimates would give a clear image about the value of advance expected from the selection. Thus, the expected gain from selection would be a better indication for selection response. All the traits showed relatively high G.C.V with high heritability estimates in broad sense. Accordingly, the genetic advance (g%) from selection appeared to be effective for these characters. The highest genetic advance was recorded for number of filled grains panicle^{-1}, plant height, and duration, while the lowest values of genetic advance were observed for grain yield. The genetic advance % was exhibited for number of panicles plant^{-1}, number of filled grains panicle and panicle length while the lowest values were those of milling% and hulling %.

Table 6: Genetic parameters for studied traits

Component	Duration (day)	Plant height (cm)	No. of panicles /plant	Panicle length (cm)	No. of filled grains/panicle	Grain yield (t/ha)	Hulling %	Milling %	Head rice %
Mean	125.0	96.26	22.93	21.80	142.10	10.49	80.31	71.21	66.07
Variance	65.36	65.36	65.36	65.36	65.36	65.36	65.36	65.36	65.36
G. V.	9.91	12.87	2.92	1.51	85.13	0.21	1.98	1.42	2.34
P. V	10.28	14.21	3.55	1.95	111.20	0.22	2.52	1.91	3.28
G.C.V	2.51	3.73	7.46	5.64	6.49	4.37	1.75	1.67	2.32
P.C.V	2.56	3.92	8.21	6.41	7.42	4.49	1.98	1.94	2.74
E. V	0.36	1.34	0.62	0.44	26.07	0.01	0.54	0.50	0.94
h b %	96.42	90.59	82.40	77.26	76.55	94.70	78.58	74.07	71.44
Δ g	6.37	7.03	3.20	2.22	16.63	0.92	2.57	2.11	2.66
Δ g %	5.09	7.31	13.94	10.21	11.70	8.76	3.20	2.96	4.03

Correlation Coefficient Analysis

Selection for specific character is known to result in correlated response in some other characters. Generally, plant breeders make selection for one or two attributes at a time. Then, it becomes important to know the effect of selection for a certain character on other characters. Improvement on grain yield per plant is the most important target character in many cereal crops, it could be achieved by direct selection through other easily observable characters. But, this needs a good understanding of association of different traits with grain yield per plant and their possible associations among themselves. The phenotypic and genotypic correlations of grain yield per plant with other quantitative characters in the studied genotypes are presented in Table (7). Data clarified the correlations among all studied traits. The number of panicles per plant was significantly and positively correlated with number of filled grains/ panicle and grain yield (t/ha), while the significant and negative correlations were found with hulling % and milling %. Regarding number of filled grains/ panicle, the results revealed highly significant and strong positive correlation with grain yield, while non-significant correlations with the rest of studied traits were found. Concerning hulling %, the results showed highly significant and positive correlations with milling %, while a significant correlation was observed with head rice %. For milling %, highly significant and positive correlations were observed with head rice %. For some traits like duration and plant height, there were no significant differences at phenotypic level with any other studied traits. The positive correlation coefficients between grain yield and number of panicles and number of filled grains / panicle indicated that the better exploration of these traits could be used to develop desired rice varieties/ lines. This was also in confirmation with the findings of Sabesan *et al*. (2009) [30], Jayasudha and Sharma (2010) [17], Oladosu *et al*. (2014) [23] and Anis *et al* (2016) [6] for yield and yield component traits.

Table 7: Phenotypic correlation coefficients between nine agro-morphological traits in the studied rice genotypes

Trait	Duration (day)	Plant height (cm)	No. of panicles/plant	Panicle length (cm)	No. of filled grains/panicle	Grain yield (t/ha)	Hulling %	Milling %	Head rice %
Duration (day)	0.1								
Plant height (cm)	0.270	0.1							
No. of panicles/plant	-0.083	-0.321	0.1						
Panicle length (cm)	-0.091	0.035	0.409	0.1					
No. of filled grains/panicle	0.101	0.094	0.707**	0.276	0.1				
Grain yield (t/ha)	0.155	-0.146	0.600*	-0.074	0.753**	0.1			
Hulling %	-0.063	0.342	-0.680**	0.069	-0.404	-0.306	0.1		
Milling %	0.040	0.056	-0.542*	0.266	-0.413	-0.403	0.784**	0.1	
Head rice %	0.380	0.083	-0.197	0.556*	-0.142	-0.213	0.570*	0.747**	0.1

Estimate of Rice Stem Borer (*Chilo agamemnon)* Infestation

The evaluation of the considered entries (Table 8) revealed that the borer infestation ranged in dead hearts between 3.79 and 17.10% (Giza 177 and GZ10590-1-3-3-2, respectively). However, the dependable evaluation is that of white heads. According to the category of RRTC (2016) [29], the three commercial cultivars; Giza 177, Giza 178 and Giza 179 performed as resistant, moderately susceptible and susceptible, respectively. Only two entries; GZ10590-1-3-3-2 and GZ11190-3-3-1-1-1 were estimated as highly susceptible. Out of the 14 rice promising lines, six entries proved to be resistant or moderately resistant to the rice stem borer, and these entries could be candidates for progress evaluations by other disciplines at Rice Research and Training Center (RRTC) for developing new varieties with desirable traits. Results of Soliman *et al*. (2016) [37] showed that yield of rice cultivars; Giza 178 and Egyptian Jasmine decreased significantly at 15-20% simulated dead hearts. This indicates that rice plants were capable of producing normal yield up to about 14% dead hearts, which emphasizes that no need to apply insecticides in most cases of dead hearts. Afzal *et al*. (2002) [3] suggested no need to apply insecticides against rice stem borer in most of cases.

Table 8: Susceptibility of rice promising lines to infestation by rice stem borer, *Chilo agamemnon*, Sakha Agricultural Research Station, average of 2020 and 2021 seasons

Genotype	Dead heart %	White head	
		%	Category
GZ10590-1-3-3-2	17.10 a	15.34 b	HS
GZ10848-1-2-2-1	8.54 de	9.76 cd	S
GZ11190-3-13-4-1	9.13 c-e	7.41 de	MS
GZ11190-3-3-1-1-1	11.73 bc	19.54 a	HS
GZ11453-17-7-7-5	5.70 f-i	4.20 fg	MR
GZ10101-5-1-1-1	6.87 e-h	4.05 fg	MR
GZ10590-1-1-3-9-1	4.17 hi	3.85 fg	MR
GZ10686-2-1-3-2	13.33 b	11.13 c	S
GZ10804-3-1-2-2-2	4.2 hi	3.60 g	MR
GZ11245-1-3-3-2	7.98 d-f	6.59 ef	MS
GZ11245-1-3-3-3	5.17 g-i	3.70 fg	MR
Giza 178	9.67 cd	8.17 c-e	MS
Giza 179	7.21 d-g	9.07 c-e	S
Giza 177	3.79 i	2.70 g	R
LSD = 0.05 %	2.752	2.983	-----

Cluster Analysis Based on Mean Performance of Traits

The mean performances of the nine agro-morphological traits were employed to calculate the Euclidean distances among eleven new promising lines and three check cultivars, and the dendrogram was constructed using these values as indicated in Figure (1). This dendrogram divided the 14 rice genotypes into four main clusters. Among the four clusters, cluster 1 was composed of four genotypes, cluster 2 had five genotypes, cluster 3 had three genotypes and cluster 4 had two genotypes. Generally, the first cluster comprised three indica-japonica promising lines (GZ10848-1-2-2-1, GZ11190-3-13-4-1 and GZ11190-3-3-1-1-1) as well as the commercial variety Giza 179 that is characterized by early maturing and high grain yield. The second cluster was the biggest one consisting of five japonica promising lines *viz.*, GZ10590-1-1-3-9-1, GZ10804-3-1-2-2-2, GZ11245-1-3-3-2, GZ11245-1-3-3-3 and GZ10686-2-1-3-2 and these lines were characterized by higher milling percentage, short plant stature and almost similar in the yield and component traits. The third cluster was composed of three genotypes (one indica-japonica line GZ11453-17-7-7-5, one japonica line GZ10101-5-1-1-1 and japonica cultivar (Giza 177) and characterized by lower filled grains/panicle and grain yield and somehow similar in some other traits. The cluster 4 consists of only two rice genotypes (Giza 178 and GZ10590-1-3-3-2) and members of this cluster were higher in filled grains/panicle and grain yield. Similar findings were reported in rice by Chandra *et al*. (2007) [9], Rajesh *et al*. (2010) [25] and Anis *et al*. (2017) [7].

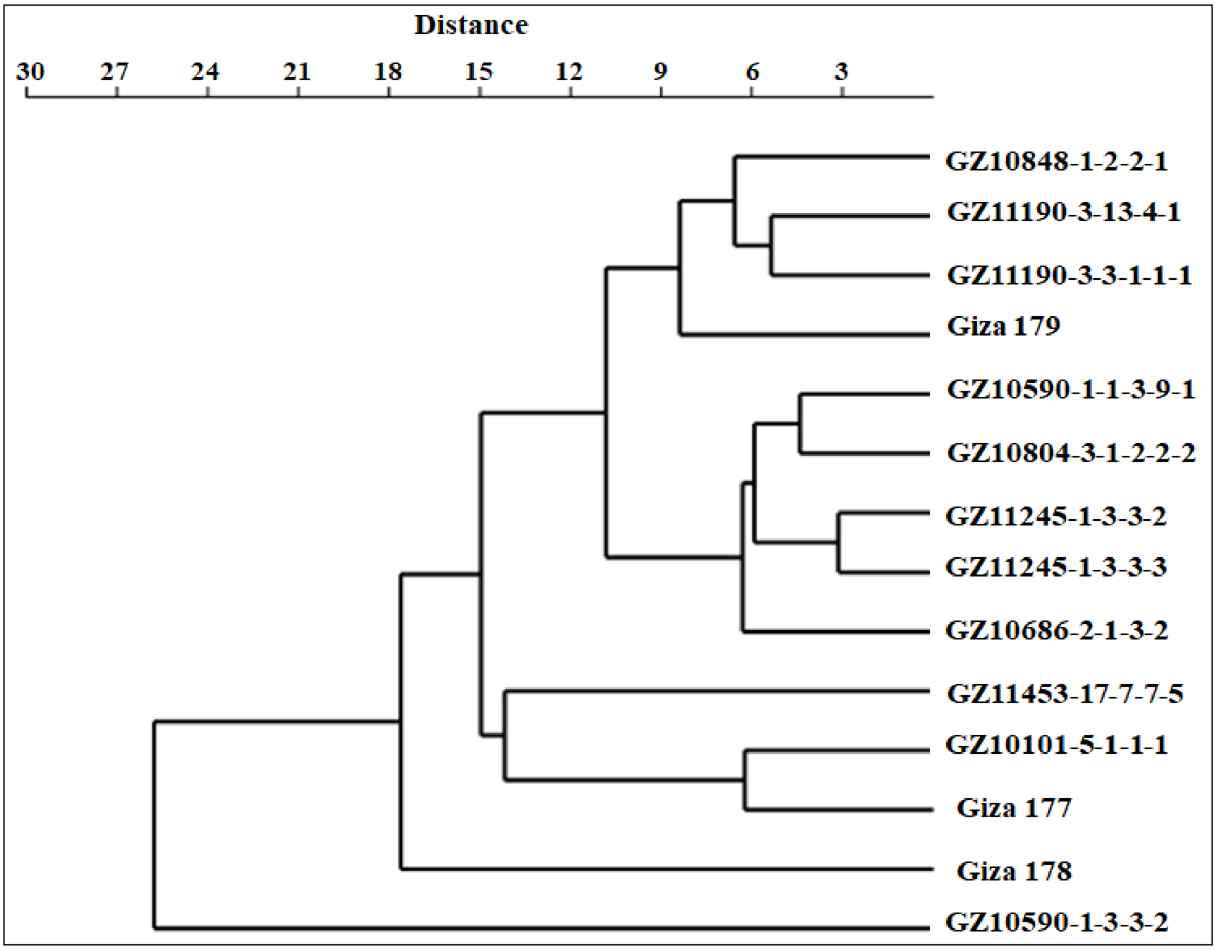

Fig 1: Dendrogram of 14 rice genotypes based on nine agro-morphological traits constructed by means of paired group and genetic distance

Cluster Analysis Based on Molecular Level

The genetic similarity among 14 promising rice genotypes displayed in a dendrogram based on Jaccard's similarity index and UPGMA method (Figure 2) gives a clear resolution of relationships among examined varieties. At 30% similarity, the UPGMA cluster chart clearly appeared two major groups with an additional sub-cluster within each group. This dendogram appeared that the cultivars are determined from hereditarily comparable sort clustered together. Group A involved the seven indica/ japonica rice genotypes; Giza178, GZ10590-1-3-3-2, GZ10848-1-2-2-1, GZ11190-3-13-4-1, GZ11190-3-3-1-1-1, GZ11453-17-7-7-5 and Giza179 that high yield, early maturity, high number of panicle, shorter plant stature, susceptible and moderate susceptible to stem borer, whereas the group B involved the other seven japonica rice genotypes; GZ10101-5-1-1-1, GZ10590-1-1-3-9-1, GZ10686-2-1-3-2, GZ10804-3-1-2-2-2, GZ11245-1-3-3-2, GZ11245-1-3-3-3 and Giza177 were high yielding and moderately resistant to stem borer. Genotypes in this group are intrigued to rice breeders since a few of them as a benefactor for breeding stem borer resistance and high yield. Finally, clustering genotypes within the developed dendogram was generally divided according to their hereditary foundation and origin were summarized in different studies. El-malky (2007) [12], Mazal (2014) [19] Ramadan *et al* (2017) [26] and Mazal (2021) [20] found that all japonica Egyptian rice genotypes were assembled in one cluster whereas all indica/ japonica Egyptian rice genotypes formed another cluster. Cluster analysis based on molecular level confirmed the agronomic information. From this study, we are able based on five new lines:GZ11453-17-7-7-5 (HR), GZ10101-5-1-1-1(MR), GZ10590-11-3-9-1(MR), GZ10804-3-1-2-2-2(MR) and GZ11245-1-3-3-3(MR) in rice breeding program were stem borer resistance, short stature, early maturity and high yield compared with check variety resistant stem borer Giza177. Representative banding design for 14 rice genotypes.

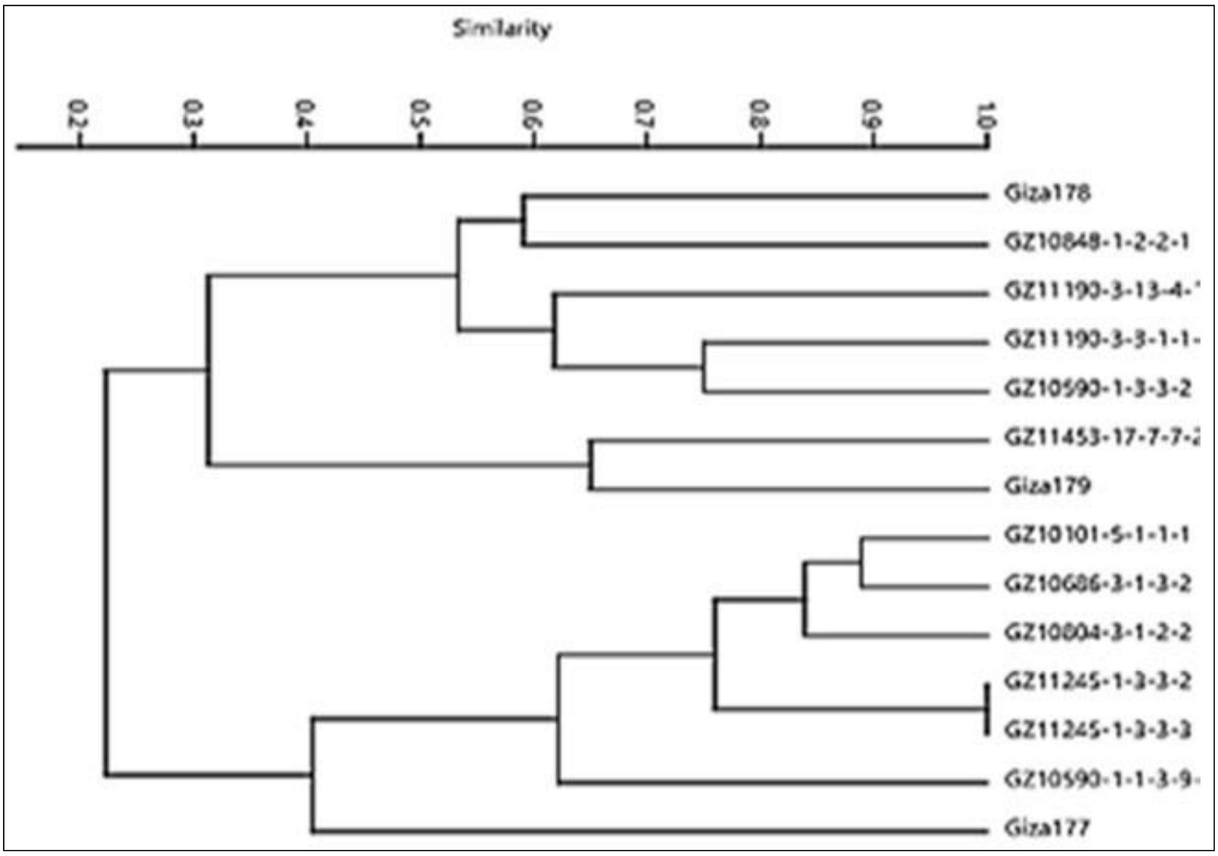

Fig 2: Dendrogram explaining the genetic relationships among tested rice genotypes using SSR markers employing UPGMA method.

The obtained comes about in Table (9) and Figure (3 and 4) based on the analysis of the 14 rice genotypes utilizingpolymorphic SSR loci to distinguish genetic variability under the molecular level.

Table 9: Summary of molecular analysis for tested genotypes using SSR markers

SSR marker	# of amplified bands	# of amplified alleles	# of polymorphic alleles	Polymorphism %	PIC value
RM263	13	2	2	100	0.497
RM148	14	2	2	100	0.50
RM166	18	2	2	100	0.444
RM224	13	2	2	100	0.473
RM208	14	2	2	100	0.408
RM246	14	2	2	100	0.245
RM315	14	2	2	100	0.490
RM430	14	2	2	100	0.50
RM3526	14	2	2	100	0.245
RM5687	14	2	2	100	0.592
RM7601	14	2	2	100	0.490
RM201	10	3	3	100	0.560
RM164	14	3	3	100	0.449
RM440	14	2	2	100	0.510
RM510	14	2	2	100	0.337
RM585	16	2	2	100	0.625
RM555	14	1	0	0	0
RM512	14	1	0	0	0
RM3735	14	1	0	0	0
RM527	14	2	2	100	0.245
RM5	14	2	2	100	0.408
Total	308	42	38	1800	8.018
Average	14	1.91	1.73	81.8	0.364

Among the 21 SSR markers utilized for genotyping, three markers (RM3735, RM555, and RM512) were monomorphic, whearse 18 SSR markers created polymorphic alleles among the considered genotypes and revealed diverse levels of polymorphism. Based on the banding sizes and patterns, a UPGMA dendrogram was produced to illustrate the genetic relationships between the tested genotypes. A total of 308 amplified bands with an average of 14 amplified bands/primer. The number of alleles varied from 1 to 2 alleles / locus, and 42 amplified alleles were found to be polymorphic with an average 1.91.the polymorphic alleles play important roles for variety differentiation, diversity characterization and conservation potential parents (Pradhan, 2016 and Singh *et al*., 2016) [24,36]. Polymorphism information content (PIC) values reflection of allele diversity and frequency among genotypes also varied from locus to another and provide an estimate of the discriminating power of the marker (Nagy *et al*., 2012) [22]. PIC value among the polymorphic markers varied from 0.00 for RM3735, RM555 and RM512 to 62 for RM585 with an average of 0.36. The identified polymorphism reflects the amount of diversity among the tested genotypes and thus the possibility of genetic improvement using such a set of genotypes in breeding programs since genetic diversity is the prerequisite for such effective programs.

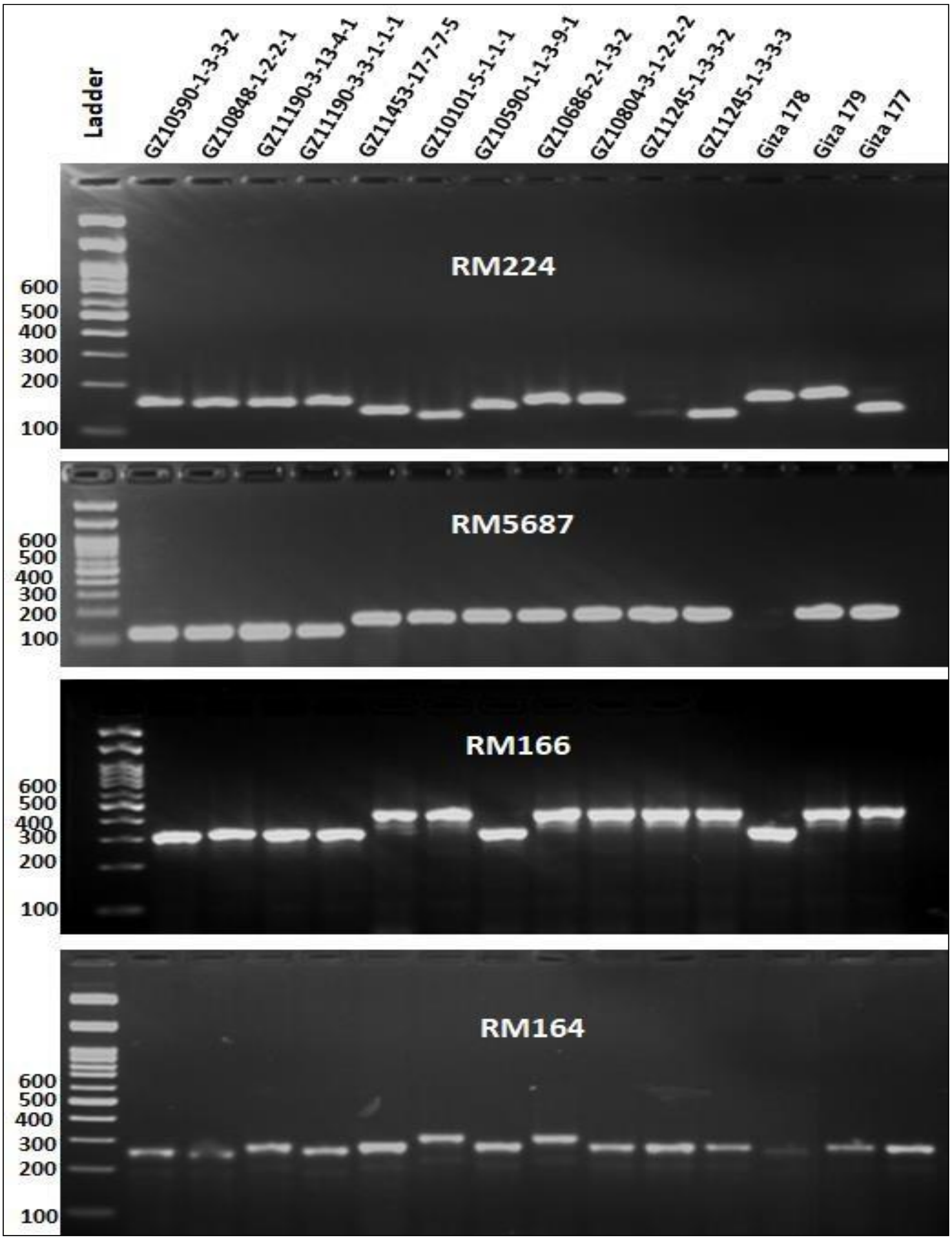

Fig 3: Banding pattern of 14 rice genotypes amplified by RM224, RM5687, RM166 and RM164 marker

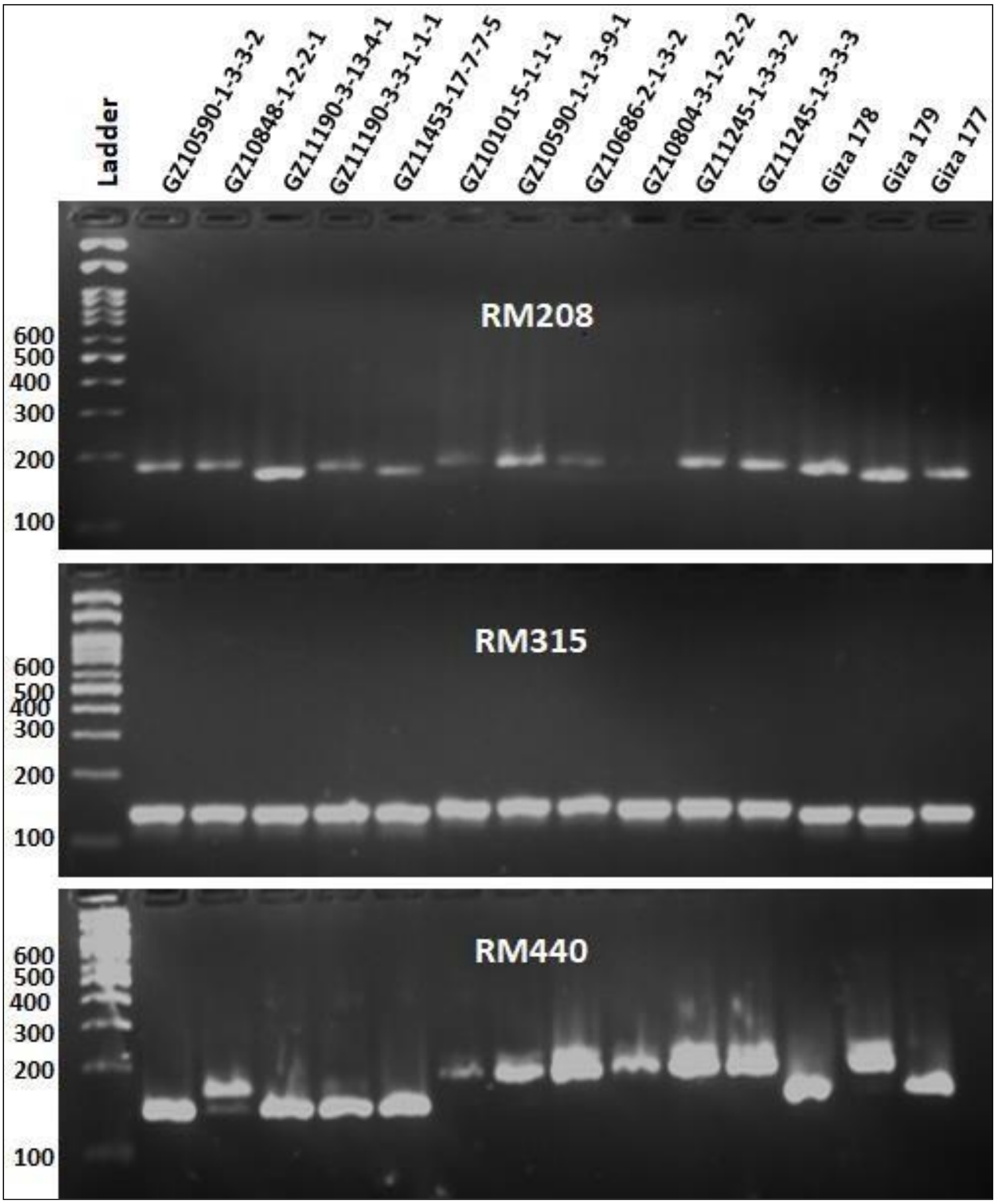

Fig 4: Banding pattern for 14 rice genotypes amplified by RM208, RM315 and RM440 markers

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References

1. Adhikari BN, Joshi BP, Shrestha J, Bhatta NR. Genetic variability, heritability, genetic advance and correlation among yield and yield components of rice (*Oryza sativa* L.). Journal of Agriculture and Natural Resources,2018:1(1):149-160.
2. Aditya JP, Bhartiya A. Genetic variability, correlation and path analysis for quantitative characters in rainfed upland rice of Uttarakhand hills. Journal of Rice Research,2013:6(2):24-34.
3. Afzal M, Yassin M, Sherwat SM. Evaluation and demonstration of economic threshold level (ETL) for chemical control of stem borers, *Scirpopgaga incertulas* WIK. and *S. innotata* WIK. International J. Agric. & Biol,2002:4(3):323-325.
4. Aly SS, Jafri SJH, Khan TZ, Mahmoud A, Butt MA. Heritability of yield and yield components of rice. Pak. J. Agri. Res,2000:16:89-91.
5. Anderson JA, Churchill GA, Autrique JE, Tanksley SD, Sorrels ME. Optimizing parental selection for gentic linkage maps. Genome,1993:38(1):181-186.

6. Anis G, El-Sabagh A, El-Badry A, Barutçular C. Improving grain yield in rice (*Oryza sativa* L.) by estimation of heterosis, genetic components and correlation coefficient. Inter. J. Curr. Res,2016:8:25080-25085.
7. Anis G, El-Mowafi H, El-Sherif A, Freeg H, Arafat E, EL-Sabagh A. Utilizing two line system in hybrid rice (*Oryza sativa* L.) and potential yield advantage under Egyptian conditions. Agri. Adv,2017:6:398-406.
8. Burton GW. Quantitative inheritance in pearl millet (*Pennisetum glaucum*). Agronomy Journal,1952:43:409-417.
9. Chandra BS, Reddy TD, Ansari NA. Genetic divergence in rice (*Oryza sativa* L.). Res. Crops,2007:8:600-603.
10. Chu CG, Chao S, Friesen TL, Faris JD, Zhong S, Xu SS. Identification of novel tan spot resistance QTLs using an SSR-based linkage map of tetraploid wheat. Mol. Breed,2010:25:327-338.
11. Dhakal A, Sharma S, Pokrel A, Poudel A. Variability and heritability estimate of 30 rice landraces of Lamjung and Tanahun districts, Nepal. Indonesian J. of Agric. Sci,2020:21(1):1-10.
12. El-Malky MMH. Combining ability and genetic analysis of some quantitative characters in rice. Proc.5th Conf. Egypt. J. Plant breed,2007:11:581-592.
13. Gyawali S, Poudel A, Poudel S. Genetic variability and association analysis in different rice genotypes at mid hill of western Nepal. Acta Scientific Agriculture,2018:2(1):6-76.
14. Hammer O, Harper DAT, Ryan PO. Phenological statistics software pakage for education and data analisis. Palaeontologia Electronica,2001:4(1):9-16.
15. Hanson CH, Rohbrnson HF, Comstock RE. Biometric studies in segregating population of Korean ispeds. Agron. J,1956:48:268-272.
16. IRRI. International Rice Research Institute. Standard Evaluation System (SES) for Rice. P.O. Box 933.1099, Los Banos, Manila, Philippines, 2006.
17. Jayasudha S, Sharma D. Genetic parameters of variability, correlation and path-coefficient for grain yield and physiological traits in rice (*Oryza sativa* L.) under shallow lowland situation. Elect. J. Plant Breed,2010:1:33-38.
18. Johnson HN, Robinson HF, Comstock RE. Estimate of genetic and environmental variability in soybean. Agronomy Journal,1955:27:314-318.
19. Mazal TM. Genetic studies on stem borer resistance in rice. Ph. D. Thesis, Fac. Agricultural, Tanta University, 2014.
20. Mazal TM. Field evaluation and genetic diversity for heat tolerance using stress indices and SSR Markers in rice. Menoufia J. Plant Prod,2021:6:267-287.
21. Murray AA, Thompson WF. Rapid isolation of high molecular weight plant DNA. Nucleic Acid Res,1988:8:4321-4325.
22. Nagy S, Poczai P, Cernák I, Gorji AM. PICcalc: An online program to calculate polymorphic information content for molecular genetic studies. Biochem Genet,2012:50:670-672.
23. Oladosu Y, Rafii MY, Abdullah N, AbdulMalek M, Rahim HA, Hussin G. Genetic variability and selection criteria in rice mutant lines as revealed by quantitative traits. Scient. World J, 2014, 1-12.
24. Pradhan SK, Saumya RB, Sahoo A, Mohapatra S, Nayak DK, Mahender A. Population structure, genetic diversity and molecular marker-trait association analysis for high temperature stress tolerance in rice. PLOS one,2016:11(1):1-23.
25. Rajesh T, Paramasivam K, Thirumani S. Genetic divergence in landraces of rice. Elect. J. Plant Breed,2010:1:199-204.
26. Ramadan EA, Anis GB, Gawish M, Mostafa ME. Fingerprinting of some Egyptian rice genotypes using Intron-exon Splice Junctions (ISJ) markers. J Plant Mol Breed,2017:5(2):38-49.
27. Rashid MM, Muruzzaman M, Hassan L, Begum SN. Genetic variability analysis for various yield attributing traits in rice genotypes. Journal of the Bangladesh Agricultural University,2017:15(1):15-1.
28. Rohlf FJ. NTSYS-pc: Numerical Taxonomy and Multivariate Analysis System. Exeter Publishing Ltd, New York, 1988.
29. RRTC. Rice Research and Training Center. Technical Recommendations for Rice Field Crops Research Institute, Ministry of Agriculture. and land Reclamation, 2016.
30. Sabesan T, Suresh R, Saravanan K. Genetic variability and correlation for yield and grain quality characters of rice grown in coastal saline lowland of Tamiluadu. Elect. J. Plant Breed,2009:1:56-59.
31. Sadat S, Saeid KA, Bihamta MR, Torabi S, Salekdeh SGH, Ayeneh GAL. Marker assisted selection for heat tolerance in bread wheat. World Appl. Sci. J,2013:21(8):1181-1189.
32. Sedeek SEM, Hammoud SAA, Ammar MH, Metwally TF. Genetic variability, heritability, genetic advance and cluster analysis for some physiological traits and yield and its components in rice (*Oryza sativa* L.). J. Agric. Res., Kafr EL-Sheikh Univ,2009:35(3):858-878.
33. Sherif MR, Bastawisi AO. Comparative observations on infestation of japonica, indica and japonica × indica rices by rice stem borer and rice leaf miner and their population fluctuations. Journal of Agricultural Science, Mansoura University,1997:22(3):907-915.
34. Sherif MR, Hendawy AS, EL-Habashy MM. Utilization of *Trichogramma evenescens* West. for controlling the rice stem borer, *Chilo agamemnon* Bles. in rice fields in Egypt. Proc. of 2nd Arab Conference of Applied Biological Pest Control, Cairo, Egypt, 2008.

35. Sherif MR, Hendawy AS, EL-Habashy MM. Management of rice insect pests. Egypt. J. Agric. Res,2005:83(5A):111-130.
36. Singh N, Choudhury DR, Tiwari G, Singh AK, Kumar S, Srinivasan K *et al*. Genetic diversity trend in India rice varieties: An analysis using SSR markers. BMC Genetics,2016:17(1):1-13.
37. Soliman AM, Hendawy AS, El-Hefny AS, Sherif MR. Determination of economic threshold level for *Chilo Agamemnon* Bles. infestation in rice plants, based on simulated dead hearts. Egypt. J. Agric. Res,2016:94(1):59-68.
38. Taha AS, Saleh HA, Eman M Bleih. Influence of sowing dates and nitrogen fertilization levels on rice yield and insect infestation of Sakha super 300 rice cultivar. Egypt. Acad. J. Biolog. Sci,2021:12(1):75-86.