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GENETIC ANALYSIS OF BLAST RESISTANCE IN SOME EGYPTIAN RICE VARIETIES USING MONOGENIC LINES AND MOLECULAR MARKERS

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ABSTRACT : *Thirty Monogenic lines carrying 23 genes were evaluated in blast nursery in three locations. Six resistant genes were effective in both natural blast nursery and artificial inoculation conditions (Pi-i, Pi-z, Pi-Z5, Pi-11, Pita and Pish), according to the reaction pattern of 5 races. Two of these genes (Pi-Z and Pi-i) were used to generate F2 segregated populations. The expected ratio of F_2 generations for blast reaction of two crosses (Sakha 101 × IRBLZFU, and Sakha 104 × IRBII-F5) was evaluated to study the inheritance of chosen blast resistant genes. The expected ratio were 15:1, and 13:3 and they showed p-value (0.90-.051) and (0.10-0.50), respectively. Simple sequence repeats (SSRs) and Intron- exon splice junctions (ISJ) were used for molecular evaluation and genetic diversity study of four Egyptian varieties. SSR markers linked to blast resistance namely RM208, RM224, and RM276 showed polymorphism with 2.3 alleles per primer with molecular weight ranged from 110 kb to 270 kb and three ISJ primers i.e. ISJ-5, ISJ-7 and ISJ-9. The number of generated bands of the three ISJ were ranged between five bands for ISJ-7 and 15 bands for ISJ-5 with average of 11 bands. The bands ISJ5-3 and ISJ5-4 were found in the resistant variety Sakha 103, while they were absent in the other genotypes, so this band might be linked to blast resistance in this variety. The band ISJ5-14 was found in the two resistant varieties Giza 177 and Sakha 103, then it might be linked to resistance in them. According to the genetic diversity study, six primer pairs (three SSRs and three ISJ) generated 39 alleles among the four varieties. Cluster diagram showed a coefficient of similarity ranged between 0.82 and 0.92; this value indicated that the varieties used were closely related in pedigree. Also, cluster diagram showed that cvs. Giza177 and Sakha103 on the same branch. This result du to the similarity in genetic components of both cvs. Giza177 and Sakha103, since cv. Giza 177 is one of the parents of cv.Sakha103 (Giza177/Suweon349).*

Key words: *Rice, blast resistance genes, monogenic lines, Egyptian varieties and molecular markers.*

INTRODUCTION

Rice blast caused by *Pyricularia oryza* was one of the most serious diseases worldwide (Valent and Chumley, 1991). Forty blast resistance genes were identified and reported (Imb and Matsumoto, 1985, Mackill and Bonman 1992, Pan *et al*. 1999, Nagato and Yoshimura 1998, Tabien *et al*., 2000, Fukuoka and Okuno, 2001). Blast races were identified by reactions to 26 Lijianxintuanheigu (LTH) monogenic lines for targeting 23 resistance genes (Mary Jeanie Telebanco-Yanoria *et al*., 2010). Relations between races and resistance genes were deliberated by Hinako Takehisa *et al.* (2009) and were understood by identifying resistance genes in Kasalath variety using monogenic lines as differential varieties carrying 24 kinds of resistance genes. Although classical plant breeding is still the foremost method for managing blast resistance however, resistance is often lost in a few years after their release because of the high variability of the rice blast races. Unstable resistance conditioned by single major genes destabilized the prominence of breading for resistant varieties unless resistance genes are carefully selected and managed (Chen *et al*., 1996). In Egypt, among the newly released rice varieties, the cv. Sakha 101 is considered as a model in yield potential (>11 ton/ha). Because of the

large spread of such mega cultivar (more than 50% of the growing area) since the year 2000, its blast resistant was recently broken (RRTC, 2006) and the cv. Sakha 104 rice variety almost has the same scenario. Still, a large portion of farmers insisted to grow these cultivars along with chemical control of the disease. This is mainly due to their superiority in yield components and quality. Thus, identifying potential blast donors with new blast resistance genes will significantly help in breeding for blast resistance using of these two varieties as recipient parents. Using molecular marker technology, has become feasible to analyze economically important traits, identify loci associating with such characters, and develop marker-assisted selection (MAS). Marker-assisted selection will help in narrowing down the possible candidate genes, lead to map-based cloning of major genes controlling the character and opening a new avenue for genetic manipulations and pyramiding of candidate genes. Achieving the above-mentioned goals will be of great importance in the efforts required for developing blast resistant genotypes using locally, adapted high-yielding background as recipient parents, that is, Sakha101 and Sakha104.

Among the marker systems developed in recent years, specific and co dominate markers are preferable in mapping and MAS because of their simplicity and high level of variability detected. SSRs markers are generally codominant and highly polymorphic. Genetically mapped SSR markers cover rice genome with at least one SSR every 16 to 20 cM (Chen *et al.,* 2005). The screening of SSR alleles would generate a database useful for variety identification and the development of molecular markers for marker-assisted selection. Also, ISJ (intron-exon splice junctions) markers are semi-specific (also known as semi-random) molecular markers proposed by Weining and Langridge (1991) and developed by Rafalski (1997). ISJ markers are based on sequences that are common in plants and partly complementary to the sequences at the intron-exon splice junctions. These junctions are highly conserved sequences. However, since the introns are generally subjected to only weak selective pressure by comparison to exons, they are usually highly variable in sequence and length. These properties would appear to make the ISJ's ideal targets for polymorphism identifications in PCR products.

This investigation aims to the following: 1) to produce new lines carrying the resistant genes to blast thought crossing between monogenic lines and some Egyptian high yielding varieties (Sakha101 and Sakha 104, and 2) to study the genetic diversity among some of selected Egyptian rice varieties using SSR and ISJ marker related to rice blast.

MATERIALS AND METHODS

The present investigation was carried out at the experimental farm and biotechnology laboratory of Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, during the four successive growing seasons of 2007, 2008, 2009 and 2010.

The genetic materials used in this investigation involved four Egyptian commercial varieties, i.e. Giza 177, Sakha 101, Sakha 103, Sakha 104 and two monogenic lines i.e. IRBLZFU and IRBLI-F5 (exotic lines from IRRI (International Rice Research Institute) as parental lines, Table 1). The cvs. Giza 177 and Sakha 103 varieties were chosen for their highly resistance to blast and used as positive control, while cvs. Sakha 101 and Sakha 104 varieties were chosen for their high yielding and susceptibility. While, the exotic lines were used as monogenic line for blast resistant genes.

In the growing season of 2007, thirty monogenic lines were evaluated under field conditions at three locations, i.e. Sakha, Gemmeza, and Zarzoura, for blast resistance at seedling stage with natural infection at blast nursery test in addition to greenhouse test. The six parental varieties in this study were sown in the summer season of 2008 at three sowing dates, at 15 days' intervals to overcome the difference of heading date among the parental varieties.

After 30 days from sowing, seedlings of the parents were transplanted to the experimental field in three rows, 5 meters long and 20 x 20 cm apart between plants and rows. A line × tester cross was conducted among the six parents in 2008 to produce eight crosses. The hybridization technique of Jodon (1938), modified by Butany (1961), the hot water method of emasculation, was utilized. The parental varieties and the two population produced from two susceptible Egyptian varieties with two resistant monogenic lines (Sakha 101 X IRBLZFU and Sakha 104 × IRBLI-F5) and two resistant Egyptian varieties (Giza177 and Sakha103) were used as positive control. They were evaluated and arranged in a randomized complete block design experiment with three replications in 2009 as F_1 generations. Each replication contained 25 individual plants.

The F_2 populations were planted and evaluated for blast in the year 2010. Each F_2 population consisted of more than 140 individual plants.

Inculcation and disease evaluation:

1- Evaluation of monogenic lines under natural inoculation in blast nursery test:

Thirty monogenic lines were evaluated under field conditions at three locations, these are Sakha, Gemmiza, and Zarzoura, for blast resistance at seedling stage with natural inoculation at blast nursery test. The set of monogenic lines were cultivated in two seedbeds in each location. Each seedbed included 50 rows and sown with thirty monogenic lines and the rest of cv. Giza 159 (blast spreader), each five rows of the monogenic lines followed by one row of the susceptible check (Table 2).

The monogenic lines were exposed for natural blast infection at seedling stage. After forty-days from sowing, the lines were scored, according to the standard evaluation system using 0-9 scale (IRRI 1996).

2- Evaluation of monogenic lines under artificial inoculation, greenhouse test:

Seeds of monogenic lines and the local varieties, cvs. Giza 177, Sakha 101, Sakha 103, and Sakha 104 were seeded in plastic trays (30 × 20 × 15 cm.). Each tray included 20 rows representing fifteen monogenic lines and five local varieties, the rest of monogenic lines in another tray. The trays were kept in the greenhouse and fertilized with urea 46.5%N (5 g/tray). Seedlings were inoculated, about 29 days after sowing. The monogenic lines and local varieties were artificially inoculated with 5 rice blast isolates, that is, Eg 5, IG-1, IB-45, 367, and 374.

All tested lines were sprayed with spore suspension (100 ml) adjusted to 5×10^4 spores/ml. Each isolate was sprayed using electrical spray gun. The inoculated seedlings were held in a moist chamber with more than 90% R.H. and 25-28^{o}C for 24 hr. and then moved to the greenhouse. Seven days after inoculation, blast reaction was recorded according to the standard evaluation system using 0-9 scale (IRRI 1996).

Table (1): Rice genotypes, parentage, origin, grain type and blast reaction.

No.	Entries	Parentage	Origin	Type of blast reaction
1	Giza 177	Giza 171/Yu mji No.1//PiNo.4	Egyptian	R
2	Sakha 101	Giza 176/Milyang 79	Egyptian	S
3	Sakha 103	Giza 177/SUWEON 349	Egyptian	R
4	Sakha 104	GZ 4096/GZ 4100	Egyptian	S
5	IRBLZFU	IRRI LINES	IRRI	R
6	IRBLI-F5	IRRI LINES	IRRI	R

Table 2: Sowed that the thirty monogenic lines and the type of genes for blast resistance.

No.	Monogenic lines	Resistance gene
1	IRBLA-A	Pi-a
2	IRBLA-C	Pi-a
3	IRBLI-F5	Pi-i
4	IRBLKS-F5	Pi-k-s
5	IRBLKS-S	Pi-k-s
6	IRBLKKA	Pi-k
7	IRBLKP-K60	Pi-k-p
8	IRBLKH-K3	Pi-k-h
9	IRBLZFU	Pi-z
10	IRBLZ5-CA	Pi-z-5 (=bi2)(t)
11	IRBLZT-T	Pi-z-t
12	IRBLTA-K1	Pi-ta (=pi4)(t)
13	IRBLTACT2	Pi-ta
14	IRBLB-B	Pi-b
15	IRBLT-K59	Pi
16	IRBLSH-S	Pi-sh
17	IRBLSH-B	Pi-sh
18	IRBL1-CL	Pi-1
19	IRBL3-CP4	Pi-3
20	IRBL5-M	Pi-5(t)
21	IRBL7-M	Pi-7(t)
22	IRBL9-W	Pi-9(t)
23	IRBL12-M	Pi-12(t)
24	IRBL19-A	Pi-19(t)
25	IRBLKMTS	Pi-k-m
26	IRBL20-IR24	Pi-20
27	IRBLTA2-PI	Pi-ta-2
28	IRBLTACP1	Pi-ta
29	IRBL11-ZH	Pi-11(t)
30	IRBLZ5-CA(R)	Pi-z-5

3- Permanent field (adult stage under natural condition):

The derived F_2 populations of the two crosses, Sakha 101 × IRBL FU (Pi-z), Sakha 104× IRBL I-F5 (Pi-i), were tested for blast reaction at adult stage in Sakha farm under natural infection condition. One hundred forty-one plants of the first cross were evaluated according to blast and the disease pattern showed according to their reaction. Also, one hundred sixty plants of the second cross were evaluated, and the chi-squared (χ^2) test was computed according to Gomez and Gomez, (1976). The T-test was used to examine the existence of genetic variance between parental means.

Molecular analysis:

DNA was isolated from the twenty-four rice accessions according to CTAB method (cetyl-tetramethyl ammonium bromide) according to Murray and Thampson (1980). A 100-ml extraction buffer was prepared (5 M NaCl (28 ml), 1 M Tris-HCL, pH8.0 (10 ml), 0.5 M EDTA, pH 8.0 (4 ml), CTAB (2 gm), B-mercaptoethanol 200 ml and H_2O (57.8 ml)). Approximately 100 ml TE (Tris-EDTA) buffer was prepared from H_2O (98.8 ml), Tris base pH 8.0 (1.0 ml), and EDTA (200 ml). Also, TAE (Tris base, Acetic acid glacial, and EDTA) was prepared from TAE buffer for 1 liter, Tris base (242 gm.), acetic acid glacial (57.1 ml), 0.5 M EDTA, pH 8.0 (100 ml), and H_2O up to 11. The polymerase chain reaction (PCR) started initial amplification at 94°C for 5 min, with 35 cycles of amplification under the following parameters: template denaturation at 94°C for 1 min, primer annealing at 55°C for 1 min, and primer extension at 72°C for 2 min by the end of the 35th cycle; final extension at 72°C for 7 min was given, followed by storage at 4°C. Three SSR (RM208, RM228, and RM276) and PCR reaction were applied, 10-*µl* volume containing DNA (2.00 *µl*), H_2O (3.45 *µl*), 10 × PCR buffer (1.00 *µl*), $MgCl_2$ (0.80 *µl*), dNTPs (0.60 *µl*), forward primer (1.00 *µl*), reverse primer (1.00 *µl*), and Taq polymerase (0.06 *µl*).

DNA amplified fragments were loaded in 1.2% agarose gel containing ethidium bromide (2 *µl*/100 ml). The 0.5X TAE was used as running buffer and 100 bp DNA ladders (0.5 µg/*µl*, fermentas) as molecular weight marker. Electrophoresis was conducted at 70 Volts, 50 mA for 3 hours. Then, gels were photographed and analyzed using BioDoc Analysis software (Biometra, Germany). Also, three ISJ primers, these are ISJ-5, ISJ-7, and ISJ-9, were used to study the selected genotypes. PCR reactions for ISJ primers were carried out in 10-*µl* volume containing the following: total genomic DNA (15 ng/*µl*) 1.00 *µl*, d.d.H_2O (4.35 *µl*), 10 × PCR buffer (1.00 *µl*), $MgCl_2$ (25 mM) (0.80 *µl*), dNTPs (1 mM) (0.10 *µl*), Taq DNA polymerase (5 U/*µl*) (0.25 *µl*), and ISJ primer (30 ng/*µl*) (2.50 *µl*). The following profile suggested by El-Moghazy (2008) was used: initial denaturation at 94°C for 3 min, 45 cycles of amplification under the following parameters: template denaturation at 94°C for 1 min, primer annealing at 48°C for 1 min, and extension at 72°C for 2.30 min by the end of the 45th cycle, final extension at 72°C for 7 min followed by storage at 4°C. The banding pattern was then scored and used to prepare the matrix. Employing the computer package NTSYS .pc (Rohlf, 2000), similarity coefficients were calculated and used to establish genetic relationship among the genotypes based on unweighted pair group method of arithmetic averages (UPGMA) and sequential agglomerative hierarchical nested (SAHN) clustering.

RESULTS AND DISCUSSION

1- Evaluation of monogenic lines under different locations:

Evaluation of monogenic lines was carried out under blast nursery test in three locations (Table 3). The tested monogenic lines exhibited different reaction to the dominant races in blast nursery in natural fields according to the response of their genes (Table 3). Nine genes, presented in 11 lines were susceptible to highly susceptible under the three locations: Pi-a, Pi-k, Pi-zt, Pi-ta(=Pi4)(t), Pi-b, Pi, Pi-1, Pi-19(t), and Pi-20. The rest were highly resistant: Pi-i, Pi-ks, Pi-kh, Pi-z, Pi-ta, Pi-sh, Pi-3, Pi-5, Pi-7, Pi-9, Pi-km, Pi-ta, Pi-11 and Pi-z5

Table (3): Reaction of monogenic lines under blast nursery test in Sakha, Gemmiza, and Zarzoura locations.

Monogenic lines	Locations			Resistance gene
	Sakha	Gemmiza	Zarzoura	
IRBLA-A	6	7	6	Pi-a
IRBLA-C	5	6	5	Pi-a
IRBLI-F5	1	1	1	Pi-i
IRBLKS-F5	1	1	1	Pi-k-s
IRBLKS-S	1	1	1	Pi-k-s
IRBLKKA	5	5	5	Pi-k
IRBLKP-K60	4	4	4	Pi-k-p
IRBLKH-K3	1	1	1	Pi-k-h
IRBLZFU	1	1	1	Piz
IRBLZ5-CA	1	1	1	Pi-z-5 (=bi2)(t)
IRBLZT-T	5	5	5	Piz-t
IRBLTA-K1	4	4	4	Pi-ta (=pi4)(t)
IRBLTACT2	1	1	1	Pi-ta
IRBLB-B	4	4	4	Pi-b
IRBLT-K59	4	4	4	Pi
IRBLSH-S	1	1	1	Pish
IRBLSH-B	1	1	1	Pi-sh
IRBL1-CL	4	4	4	Pi-1
IRBL3-CP4	1	1	1	Pi-3
IRBL5-M	1	1	1	Pi-5(t)
IRBL7-M	1	1	1	Pi-7(t)
IRBL9-W	1	1	1	Pi-9(t)
IRBL12-M	2-3	2	2	Pi-12(t)
IRBL19-A	5	5	5	Pi-19(t)
IRBLKMTS	1	1	1	Pi-k-m
IRBL20-IR24	4	4	4	Pi-20
IRBLTA2-PI	1	1	1	Pi-ta-2
IRBLTACP1	1	1	1	Pi-ta
IRBL11-ZH	1	1	1	Pi-11(t)
IRBLZ5-CA(R)	1	1	1	Pi-z-5

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

Some alleles of Pi-k were resistant and others were susceptible at the same location.

2- Evaluation of the monogenic lines under greenhouse condition:

The resistance of monogenic lines was evaluated under greenhouse condition with five specific blast races. Virulence of the selected five isolates were tested and used as a differential blast isolates to identify resistance gene by reaction pattern. Isolate Eg-5 was used because it is more aggressive. Race IG-1 was specific and virulent to high-yielding variety Sakha 101, whereas IB-45 was specific race to Sakha 104. According to the reaction pattern of the five races under artificial inoculation, ten genes showed complete resistance to all isolates (Table 4). The completely resistant genes were as follows: Pi-i, Pi-z, Pi-Z5 (Pi-2), Pi-ta (=Pi4) (t), Pi-b, Pi-sh, Pi-1, Pi-ta, and Pi-11(t). Of the ten completely resistant genes, only six genes were resistant and effective at both natural blast nursery and artificial inoculation conditions (Pi-i, Pi-z, Pi-Z5, Pi11, Pi-ta and Pi-sh). Some monogenic lines were infected with only one of five isolates, and the rest have only two isolates. The isolate Eg-5 was virulent to 14 from 30 tested lines. The isolate 367 was virulent to 7 from 30 tested lines. IG-1 and IB-45 were virulent to only one line. Race 374 was a virulent and incapable of infecting with any tested lines. Therefore, the monogenic lines, which carried resistant genes (Pi-i, Pi-z, Pi-Z5, Pi11, Pi-ta and Pi-sh) to all five isolates can be recommended to be used as donors in breeding program for improving resistance to blast disease under Egyptian condition. Two genes Pi-Z , presented in IRBLZFU line, and Pi-l, presented in IRBll-F5 line, were selected to study the genetic inheritance of resistance in F_2 segregated populations and for molecular study.

3- Field evaluation for F_2 segregation:

Populations produced from two crosses (Sakha 101 × IRBLZFU and Sakha 104 × IRBll-F5) were tested for blast reaction types, chi-squared (χ^2) analysis and expected ratio are presented in Table 5. One hundred forty-one plants of F_2 generations derived from the cross (Sakha 101 × IRBLZFU) and one hundred sixty plants from the cross (Sakha 104 × IRBLI-F5) were evaluated to study the inheritance of blast resistance.

Three reaction types of blast resistance, such as resistance (R), moderate resistance (MR), and susceptible (S) were recorded. Types R and MR were gathered under one class namely resistant plants .

From the first cross (Sakha 101 × IRBLZFU), 129 plants were resistant, and 12 plants were susceptible; however, in cross two (Sakha 104 × IRBLI-F5), 139 were resistant, and 21 were susceptible. The expected ratio for the first cross was 15:1, with p-value of 0.90-51.0 indicated the presence of two of leaf blast resistance segregating genes in these crosses. Each gene can express resistance in its genetic background. Also, each parent in these crosses contained one of these genes, and the allelic relation was complete dominance. These data suggested that, if the first parent is AAbb, then second parent has to be aaBB. These results were summarized in the study by El-Malky (2004).

Concerning the second cross, the expected ratio was 13:3, and the p-value was 0.10-0.50 indicated the presence of two complementary dominant gene in this cross. Similar results were obtained by Hammoud (2004).

4-Molecular analysis:

4-1. SSRs polymorphism:

In this study, three SSR markers linked to blast resistance named RM208, RM224, and RM276 were used. All of them generated polymorphic bands and the number of alleles for each primer ranged between two alleles for RM208 and RM224 to three alleles for RM276, with an average of 2.3. Figure (1) shows that RM208, two alleles were produced with two different molecular weights. The first allele (220 Kb) was found in Sakha 101 (lane 2), while the second allele (200Kb) was found in Sakha 104 (lane 3).

Table (4): Reaction of monogenic lines under artificial inoculation in greenhouse.

No.	Line	EG-5	IG-1	367	374	IB-45
1	IRBLA-A	4	2	4	2	2
2	IRBLA-C	2	2	4	2	2
3	IRBLI-F5	2	2	2	2	2
4	IRBLKS-F5	3-4	2	4	2	2
5	IRBLKS-S	4	2	2	2	2
6	IRBLKKA	4	2	2	2	2
7	IRBLKP-K60	6	2	2	2	2
8	IRBLKH-K3	4	2	2	2	2
9	IRBLZFU	2-3	2	2	2	2
10	IRBLZ5-CA	1	2	2	2	2
11	IRBLZT-T	4	2	2	2	4
12	IRBLTA-K1	1	2	2	2	2
13	IRBLTACT2	2	2	2	2	2
14	IRBLB-B	2	2	2	2	2
15	IRBLT-K59	4	4	2	2	2
16	IRBLSH-S	5	2	4	2	2
17	IRBLSH-B	2	2	2	2	2
18	IRBL1-CL	2	2	2	3	2
19	IRBL3-CP4	5	2	2	2	2
20	IRBL5-M	4	2	2	2	2
21	IRBL7-M	6	2	2	2	4
22	IRBL9-W	2	2	3	2	4
23	IRBL12-M	2	2	4	2	2
24	IRBL19-A	7	2	2	2	5
25	IRBLKMTS	2	2	5	2	2
26	IRBL20-IR24	5	2	3	2	2
27	IRBLTA2-PI	2	2	4	2	2
28	IRBLTACP1	2	2	2	2	2
29	IRBL11-ZH	2	2	2	2	2
30	IRBLZ5-CA(R)	4-5	2	5	2	2

Table (5): Reaction types chi-squared (χ^2) and expected ratio of F_2 generations for the two populations (Sakha 101 × IRBLZFu) and (Sakha 104 × IRBLI-F5).

Cross	Number of plants	Reaction type		Expected ratio	χ^2	P. value
		R	S			
Sakha101× IRBLZFU	141	129	12	15 : 1	1.22	0.90–0.51
Sakha 104 × IRBLI-F5	160	139	21	13 : 3	3.32	0.50–0.10

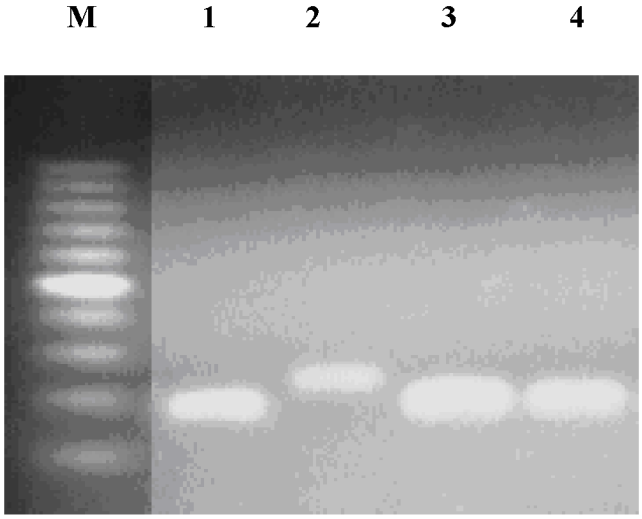

Figure (1): The electrophotogram of DNA amplified fragments using RM208 for selected genotypes. M, 100bp DNA ladder; 1, Giza177; 2, Sakha101; 3, Sakha104 and 4, Sakha 103.

For RM224, Figure (2) shows that two alleles were found; the first one (130 Kb) was found in Sakha 104 (lane 3), whereas the second one (120Kb) was found in Sakha 101 (lane 2).

For RM276, Figure (3) showed that two alleles. The first one (130 Kb) was found in Sakha 104 (lane 3), whereas the second one (110 kb)was found in sakha 101 (lane 2).

4-2. ISJ Markers:

In this study, three ISJ primers linked to blast resistance namely; ISJ-5, ISJ-7 and ISJ-9 were used. The number of generated bands were ranged between five bands for ISJ-7 and 15 bands for ISJ-5 with average of 11 bands (Figure 4,5 and 6).

For ISJ-5, Figure (4) indicated that fifteen bands were generated, three of them (ISJ5-3, ISJ5-4 and ISJ5-14) were polymorphic bands, while the other bands were monomorphic revealing polymorphic ratio of 13.3%. The bands ISJ5-3 and ISJ5-4 were found in the resistant variety Sakha 103, while they were absent in the other genotypes, so this band might be linked to blast resistance in this variety. The band ISJ5-14 was found in the two resistant varieties Giza 177 and Sakha 103, then it might be linked to resistance in them.

For ISJ-7, Figure (5) showed five bands were generated, two of them (ISJ7-2 and ISJ-3) was polymorphic band, while the other three bands were monomorphic revealing polymorphic ratio of 10%. The bands ISJ7-2 was absent in the resistant variety Giza 177, while it was found in the other genotypes. The band ISJ7-3 was found in all varieties except Sakha 103.

For ISJ9, Figure (6) indicated twelve bands, five of them (ISJ9-1, ISJ9-2, ISJ9-3, ISJ9-7 and ISJ9-11) were polymorphic bands, while the other bands were monomorphic revealing polymorphic ratio of 27.08 %. The bands ISJ9-1 and ISJ9-2 were found in the variety Sakha 104, while they were absent in the other genotypes. The band ISJ9-3 was found in the resistant variety Sakha 103. The band ISJ9-7 was found in the variety Sakha 101, while it was absent in the other varieties. The band ISJ9-11 was found in all varieties, while it was absent in Sakha104.

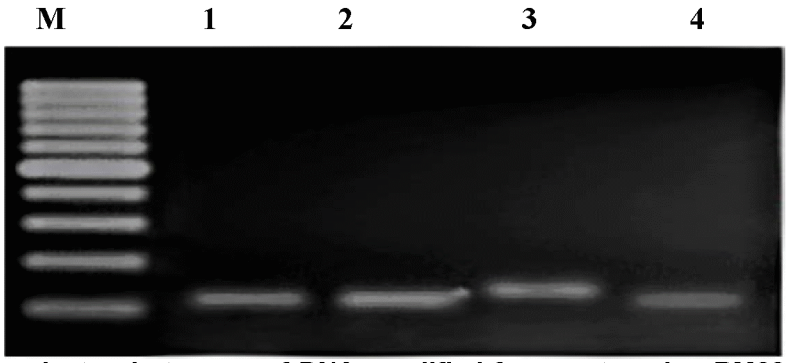

Figure (2): The electrophotogram of DNA amplified fragments using RM224 for selected genotypes. M, 100bp DNA ladder; 1, Giza177; 2, Sakha101; 3, Sakha104 and 4, Sakha 103.

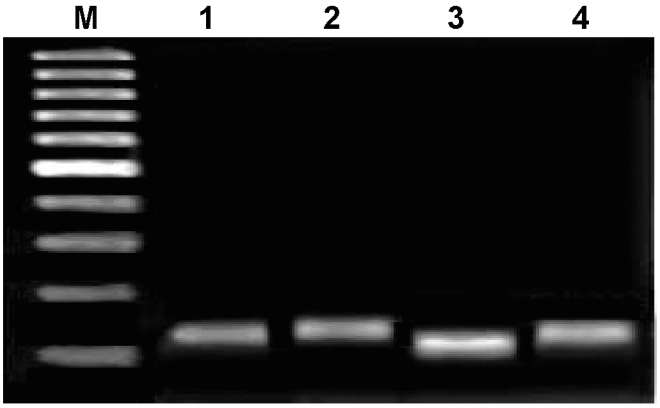

Figure (3): The electrophotogram of DNA amplified fragments using RM276 for selected genotypes. M, 100bp DNA ladder; 1, Giza177; 2, Sakha101; 3, Sakha104 and 4, Sakha 103.

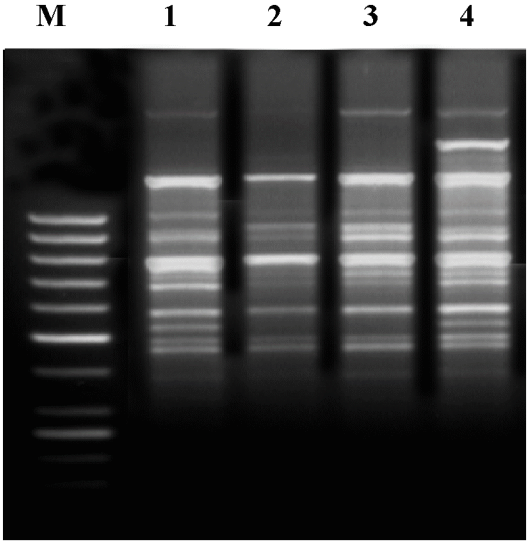

Figure (4): The electrophotogram of DNA amplified fragments using ISJ5 for selected genotypes. M, 100bp DNA ladder; 1, Giza177; 2, Sakha101; 3, Sakha104 and 4, Sakha 103.

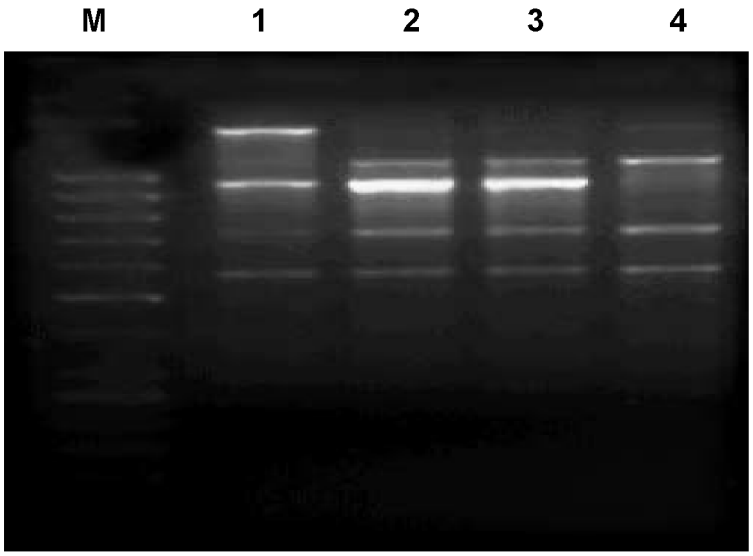

Figure (5): The electrophotogram of DNA amplified fragments using ISJ7 for selected genotypes. M, 100bp DNA ladder; 1, Giza177; 2, Sakha101; 3, Sakha104 and 4, Sakha 103.

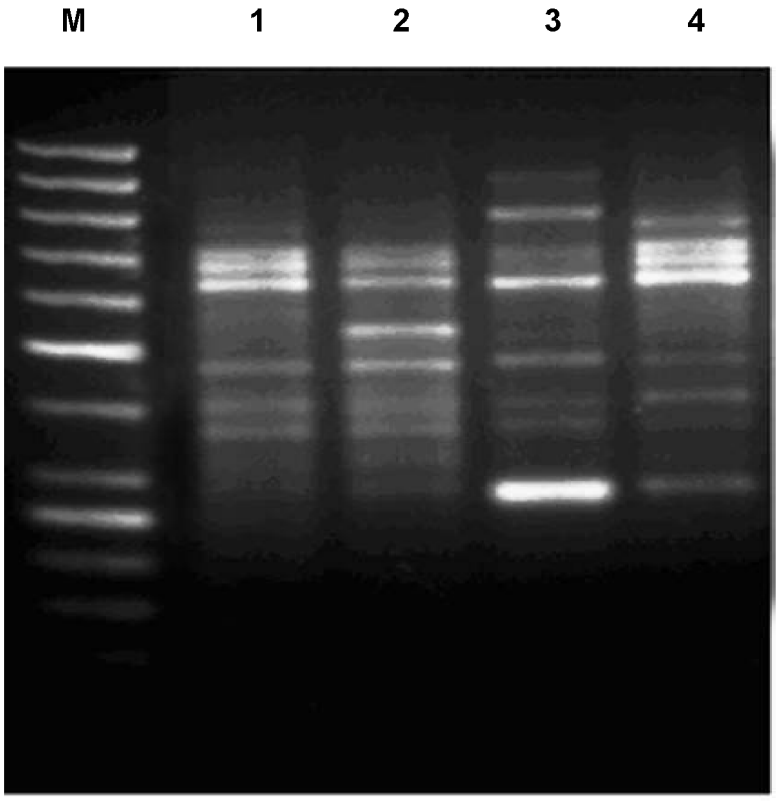

Figure (6): The electrophotogram of DNA amplified fragments using ISJ9 for selected genotypes. M, 100bp DNA ladder; 1, Giza177; 2, Sakha101; 3, Sakha104 and 4, Sakha 103.

4-3. Diversity study:

All six primer pairs (three SSRs and three ISJ) generated multiple fragments in this group of rice varieties. The primer pairs will be referred to as loci, and the fragments generated by each primer pair amplification reaction will be called alleles. The total number of alleles detected was 39 among the four varieties. Four varieties (Giza177, Sakha101, Sakha104, and Sakha103) were representative of the RRTC germaplasm and used as commercial varieties. However, pedigree information can provide a direct and preliminary suggestion of the genetic relations among the four varieties.

SSRs and ISJ alleles were used to construct a matrix, which were then used to calculate the genetic similarity among the four varieties (Table 6). Furthermore, a phenogram was generated by UPGMA to show genetic relations of the varieties studied and presented in Figure 6. The UPGMA cluster diagram showed the genetic variation pattern, and a coefficient ranged between 0.82 and 0.92; this value indicates that the varieties used were relative in pedigree. Also, cluster diagram showed Giza177 and Sakha103 on the same branch and a coefficient of about 0.9152 (Table 5). This result may be du to the similarity in genetic components of both Giza177 and Sakha103, since Giza 177 is one of the parents of Sakha103 (Giza177/Suweon349) as well as both share many important characters especially resistant to blast infection, and early duration. The second group includes two susceptible, medium duration and high yielding varieties, Sakha 101 and Sakha 104. However, Sakha 104 is 10-days earlier than Sakha 101 in duration. (Figure 7). This result was in agreement with Ansari, *et. al.*, (2006) and. El-Wahsh, and M.H. Ammar (2007)

Table (6): Similarity matrix of four varieties based on three SSRs and three ISJ markers.

Rows/culms	Giza 177	Sakha 101	Sakha 104	Sakha 103
Giza 177	1.0000000			
Sakha 101	0.8771930	1.0000000		
Sakha 104	0.4821053	0.8275862	1.0000000	
Sakha 103	0.9152542	0.8333333	0.8000000	1.0000000

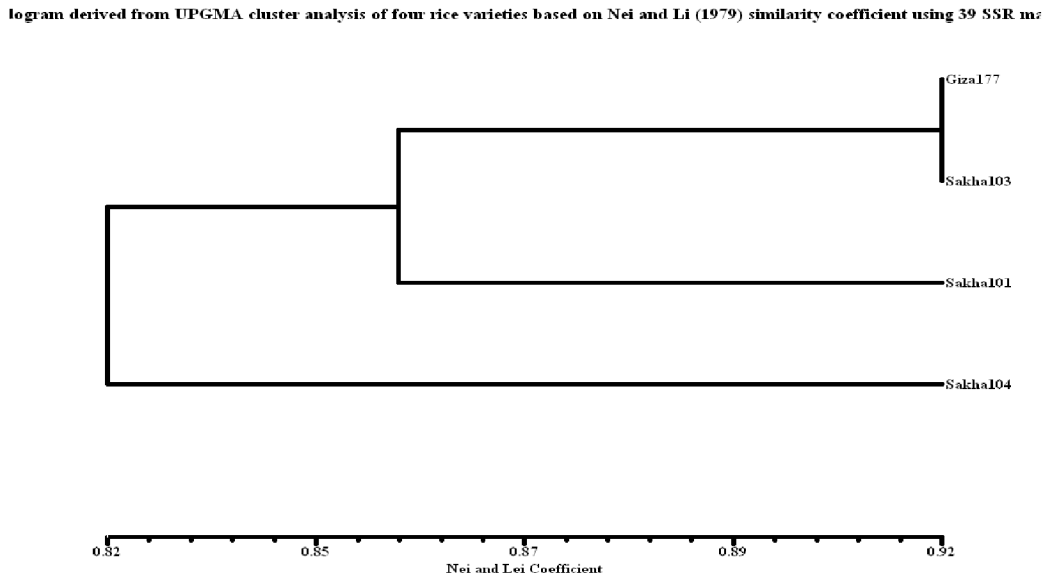

Figure (7): Dendrogram showing a cluster analysis of four Egyptian rice varieties based on polymorphism of three SSR markers and three ISJ markers.

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التحليل الوراثي لجينات مقاومة اللفحة فى بعض أصناف الأرز المصري باستخدام السلالات أحادية الجين والمعلمات الجزئية

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الملخص العربي

تم استخدام ثلاثين من سلالات الأرز أحادية الجين لمقاومة مرض اللفحة والتي تحمل ٢٣ جين للمقاومة فى حقل اختبار اللفحة فى ثلاث مواقع مختلفة ووجد أن ستة جينات مقاومة تحت الظروف المصرية فى المواقع المختلفة وهى (Pi-i, Pi-z, Pi-Z5, Pi-11, Pita and Pish) وكانت فعالة تحت ظروف العدوى الصناعية لخمسة سلالات ممرضة وأيضا تحت العدوى الطبيعية. ومن بين هذه الجينات اثنان هما (Pi-z and Pi-i,) وهما فى السلالاتين IRBLZFU و IRBII-F5 وتم استخدامهما فى التهجين بين الأصناف المصرية سخا ١٠١ وسخا ١٠٤ وتم تقييم النسب الانعزالية الناتجة منها بهدف دراسة توارث تلك الجينات وكانت النسب المتوقعة هي ١٥: ١ و ١٣: ٣ وأظهرت قيم احتمالية هي (0.90-.051) و (0.10-0.50) على التوالي.

وللتقيم على المستوى الجزيئي ودراسة التنوع الوراثي لأربع أصناف أرز مصرية تم استخدام تتابع التكرارات البسيطة (SSRs) والوصلات بين مقاطع الجين (ISJ) كمعلمات جزيئية وأدى استخدام تتابعات التكرار البسيطة (SSRs) المرتبطة بمقاومة اللفحة المسماة RM276, RM224 , RM208 إلى ظهور تباين ذات متوسط أليل ٢.٣ أليل للبادئ بحجم جزيئات يتراوح بين 110 kbإلى 270 kb

كما أظهرت نتائج (ISJ) لثلاثة من التتابعات المستخدمة هم ISJ-5 و ISJ-7 و ISJ-9 أن عدد الحزم التي ظهرت للثلاثة كانت ١٥ حزمة وبمتوسط ١١ حزمة تقريبا لكل هذه التتابعات.

كما أوضحت اشتراك الحزمة ٣ و ٤ فى ISJ5 مع الصنف سخا ١٠٣ وهو صنف مقاوم لمرض اللفحة وأيضا الحزمة رقم ١٤ وجودها مع الصنف جيزة ١٧٧ وسخا ١٠٣ لكونهما مقاومين لمرض اللفحة.

وأوضح ISJ9 وجود ١٢ حزمة خمسة منهم اظهروا تنوع واضح وظهرت الحزمة ١ و ٢ مع الصنف سخا ١٠٤ بينما لم تظهر مع باقي الأصناف.

تبعا للدراسة الخاصة بالتنوع الوراثي فقد تم استخدام ستة أزواج من البادئات ثلاثة من SSR وثلاثة من ISJ والتي أنتجت ٣٩ أليل للأربع أصناف وأظهرت الشجرة الوراثية المستخلصة معامل تشابه لمدى ٨٢٠. إلى ٠.٩٢ والذي يشير إلى شدة القرابة بين الأصناف المستخدمة.

أيضا أظهرت الشجرة الوراثية أن الصنف جيزة ١٧٧ وسخا ١٠٣ على نفس فرع القرابة وهذا يرجع للتشابه فى المحتوى الوراثي لهما حيث أن جيزة ١٧٧ هو احد أباء سخا ١٠٣ حيث أن سخا ١٠٣ ناتج من التهجين بين جيزة ١٧٧ مع سيون٣٧٩