

Original Research Article

Studies on the biotic Stress Resistance in Rice through Molecular Markers and Six Population Analysis

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ABSTRACT

Keywords

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RAPD,
PCR analysis

The present investigation was carried out in the farm of (El-Mansoura City) and National Research Center, Dokki, Cairo, Egypt from 2011 to 2013 seasons using six population analysis through three promising lines of rice namely, L1 (GZ 1368-S-5-4), L2 (Nabatat Asmar) and L3 (IR 28), which different reaction for the biotic stress resistance " in three replications for each genotype to study the genetic mode of action responsible for resistance of the biotic stress through some physiological, morphological and biochemical traits including plant height, 1000-grain weight, grain yield per plant, Chlorophyll content, flag leaf area, Blast reaction, Stem borer % and White-tip Nematode (%), traits in addition to study SDS-Protein electrophoresis (water soluble protein), Antioxidant Enzyme, RAPD-PCR-markers using four primers namely, PRM-7, PRM-11, PRM-12, PRM-14, Dendrogram analysis, respectively. The results revealed that the crosses, GZ1368-S-5-4 X IR28 and Nabatat Asmar X IR28 were highly resistance for the biotic stress and recorded high data using physiological, morphological and biochemical traits.

Introduction

Rice (*Oryza sativa* L.) is one of the world's most important crops, providing a stable food for nearly half of the global population (FAO 2004). Almost 90% of the rice is growing and consumed in Asia. The striped stem borer (*Chilo suppressalis* Walker) is one of the most important rice pests in Asia. Rice stem borers cause damage to the crop at the larval stages.

Larvae borer in to the plant stems and feed on plant nutrients causing, in many cases, severe crop loss. Rice plants are most prone to stem borer infestation at the tillering and flowering stages. In a transplanted crop, stem borer larvae cut off the growing points of tillers causing them to die, a condition commonly known as dead heart. When the plants are attacked later, during the

flowering stage, larvae feed on the meristem and empty, whitish-looking panicles called white heads appear. In the infested fields these white heads stand erect and contain empty and unfilled glumes. The most commonly cited crop loss figures from rice are those of Cramer (1967), who estimated worldwide losses in rice production due to insect damage to be 34.4 percent. Chemical methods such as insecticide to control the insect have been negative efficiently upon quality of maize. Therefore, it is important to develop rice cultivars with adequate levels of resistance to the striped stem borer. Resistant cultivars provide insect control at no additional cost to the farmer and are compatible with other control methods in an integrated pest control programme. Resistance to stem borers appears to be under polygenic control. Many morphological, anatomical, physiological and biochemical factors have been reported to be associated with resistance, each controlled by different sets of genes.

The extensive variability of the blast pathogen means that breeding for greater cultivar diversity must induce changes in the genetic background of promising lines through the introduction of a new resistance gene. White – tip disease of rice leaves caused by the leaf nematode (*Aphelenchoides besseyi*) Christie, is widespread and present in nearly all rice ecosystems worldwide. And has recently been found in Egypt where the response of rice cultivars has been significantly different in terms of infection level and yield losses. The aim of this research is to evaluate and study the genetic mode of action responsible for resistance of Blast reaction, stem borer % and white-tip nematode % and their related physiological, morphological and biochemical traits in rice cultivars through water-soluble protein and marker-assisted selection.

Materials and Methods

The present investigation was carried out in the farm of (El-Mansoura City) and National Research Center, Dokki, Cairo, Egypt from 2011 to 2013 seasons using six population analysis through three promising lines of rice namely, L1 (GZ 1368-S-5-4), L2 (Nabatat Asmar) and L3 (IR 28) and all possible combinations were made excluding reciprocal to produce F1 seeds in 2011 season. The parents and their F1 hybrids were growing in a randomized complete block design with three replications for each genotype in 2012 season to produce F2 – generation and make back crosses hybridization for the three crosses of rice using the three parents. In 2013 season, The Parents, F1, F2 and back crosses were grown in a randomized complete block design in the farm of El-Mansoura city" in three replications for each genotype to study the genetic mode of action responsible for resistance of the biotic stress through some physiological, morphological and biochemical traits including plant height, 1000-grain weight, grain yield per plant, Chlorophyll content, flag leaf area, Blast reaction, Stem borer % and White-tip Nematode (%), in addition to study SDS-Protein electrophoresis (water soluble protein), Antioxidant Enzyme, RAPD-PCR-markers using four primers namely, PRM-7, PRM-11, PRM-12 and PRM-14. All recommended agriculture practices were applied.

Methodology

1. The three promising lines of rice were different reaction for the biotic stress.
2. F1 seeds were three crosses, (GZ1368-S-5-4 x Nabatat Asmar), (GZ1368-S-5-4 x IR 28) and (Nabatat Asmar x IR 28), respectively.

A.Traits Studied

1. **Plant height (cm):**Length of the main culm was measured from the soil surface to the tip of the main panicle at maturity.
2. **1000-grain weight (g):**Was recorded as the weight of 1000 random.
3. **Grain yield per plant (g):**Was recorded as the weight of grain yield of each individual plant and adjusted to 14% moisture content.
4. **chlorophyll content:** Total chlorophyll content in the flag-leaf was recorded at heading stage using a chlorophyll meter (5 SPAD-502, Minolta Camera Co. Ltd, Japan).
5. **Flag leaf area:**The flag leaf area was estimated at maximum tillering stage at (days from transplanting) following, the formula reported by Yoshida et al.(1976) as follows:
$$\text{Leaf area (cm}^2\text{)} = K \times \text{length(cm)} \times \text{width (cm)}$$
where k(0.75) a correction factor can be used for all growth stages except the seedling and maturity stages.
6. **Blast reaction was recorded:-** according to the standard evaluation system using a scale of 0–9 (IRRI, 1996).
7. **White-tip nematode infection**The resistance of different varieties was evaluated according to infection percentage:
<1% resistant
1–30% moderately susceptible
>30–60% susceptible
>60% highly susceptible.
To estimate the resistance of rice varieties, all rice hills were examined to record the infected hills and calculate the infection percentage using the following formula:
$$\text{Percentage of infection} = \frac{\text{No. infected hills}}{\text{Total no. rice hills}} \times 100$$
8. **Stem borer evaluation:** Parents and F1 offspring were evaluated for stem borer infestation. The reaction of evaluated genotypes was classified into five

categories according to the standard evaluation of the Rice Research and Training Center (RRTC, 2006), Sakha, Egypt.

< 3% whiteheads (WH) resistant (R)

3–6% WH moderately resistant (MR)

6–9% WH moderately susceptible (MS)

9–12% WH susceptible (S)

> 12% WH highly susceptible (HS).

A.1.Statistical analysis

A.2.Six populations analysis

A.2.1.Heterosis

It was determined according to Mather and Jinks (1971).

A.3.Estimation of gene effects and genetic components of variations

The present data were predicted to biometrical model suggested by Mather (1949) and Hayman (1958) in order to estimate the gene action and to separate the genetic components of variations controlling the studied characters.

A.3.1. Scaling test

The scaling test of inquest is important because in most of the cases, estimation of additive and dominance components of variances are made immoderation of the gene interaction. Individual scaling test was applied to the six populations data of each cross as outlined by Mather (1949).

A.3.2. Components of generation means

The means of the six populations in each cross were used to estimate various genetic components using the methos of Hayman (1958).

A.4.Estimation of inbreeding depression

The inbreeding depression was determined

for each characters as follows:

$$\text{Inbreeding depression \%} = \frac{\bar{F}_1 - \bar{F}_2}{\bar{F}_1} \times 100$$

Where:

F_1 = Mean value of the first generation.

F_2 = Mean value of the second generation.

A.5. Estimation of genetic advance

Genetic advance under selection was (Δg) calculated as given by Johanson *et al.* (1955).

A.6. Heritability estimates

The heritability in both broad and narrow sense were determined by Powers *et al.* (1950) and Warner (1952).

B. Molecular Markers

B.1. Electrophoretic analysis of protein by SDS-PAGE

Water soluble protein of the leaves of the three lines of rice and their F1 crosses were performed according to the method of Laemmli (1970) and modified by Studier (1973).

B.2. Isozymes electrophoresis

Native-polyacrylamide gel electrophoresis (Native-PAGE) was conducted according to (Stegemann *et al.*, 1985) to identify isozymes variations in the three parents of rice and their F1 crosses using two isozymes systems; peroxidase and polyphenol oxidase, respectively. After electrophoresis, gels were stained according to their enzyme systems with the appropriate substrate, chemical solutions and then incubated at room temperature in dark for complete

staining. For peroxidase, benzidine – dihydrochloride HCl of 0.125gm and 2mL glacial acetic acid and was completed with distilled water up to 50mL. Gel was placed into this solution and five drops of hydrogen peroxidase was added. The gel was incubated at room temperature until bands appear (Brown, 1978). For polyphenol oxidase, 100mL of sodium phosphate buffer 0.1M at pH 6.8 15mg catechol and 50mg sulfanilic acid were used. The gel was placed into this solution and incubated at 30°C for 30mins. until bands appeared.

B.3. RAPD-PCR –Markers

DNA was extracted from the leaves of the selected plants of three lines of rice and three F1 crosses which different reaction of The biotic stress using four RAPD-PCR-primers to comparison between these lines " according to the method of Williams *et al.* (1990), Graham *et al.* (1997) and Sharma *et al.* (2003).

B.3.1. DNA extraction

Young leaves of six rice genotypes were collected and soaked in liquid nitrogen for DNA extraction using the 2% CTAB method modified by Agrawal *et al.*, (1992).

B.3.2. Random amplified polymorphism DNA-polymerase chain reaction (RAPD-PCR) analysis

A total of four primers were used to amplify DNA (manufactured by Bioneer, New technology certification from ATS Korea). The total reaction mixture was 15 µl contained 10× PCR buffer, 2 mM MgCl₂, 0.2 mM dNTP mixed, 10 pmol primer, 1.25 U *Taq* polymerase and about 150 ng genomic DNA. DNA amplification was obtained through 35 cycles in a DNA

thermal cycler. The temperature profile was as follow: denature temperature 94°C for 30 sec.; annealing temperature 45°C for 1 min; and extension temperature 72 for 1 min, final extension at 72°C for 5 min.

B.3.3 Amplification product analysis:

The amplified DNA (15 µl) for all samples was electrophoresed on 1% agarose containing ethidium bromide (0.5 µg/ml) in 1X TBE buffer (89 mM Tris-HCl, 89 mM Boric acid, 2.5 mM EDTA, pH 8.3) at 75 constant volt, and determine with UV transilluminator. The size of each fragment was estimated with reference to a size marker of 10 Kb DNA ladder (BioRoN, Germany).

B.3.4 Gel analysis:

Gels were analyzed by UVI Geltec version 12.4, 1999-2005 (USA).

B.3.5 Data analysis:

A matrix for SDS-PAGE, RAPD-PCR combined was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the lines. Genetic similarity coefficients were computed following Nei and Li (1979) as under:

$$\text{similarity} = \frac{2N_{xy}}{N_x + N_y}$$

where 'N_x' and 'N_y' are number of bands present in genotypes 'x' and 'y', respectively; and N_{xy} are the number of bands shared by the cultivar 'x' and 'y'. The data were subsequently used to construct a dendrogram using the unweighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal 1973)

employing sequential, agglomerative hierarchic and non-overlapping clustering (SAHN). All the computations were carried out using the software NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.1 (Rohlf, 2000). Correlations coefficients were calculated using similarity coefficients obtained from combined SDS-PAGE and RAPD-PCR analysis.

B.3.6 The sequences of RAPD-PCR analysis for the four primers used to clarify between the six genotypes of rice as follows:-

1. PRM-7 5`TGCACGCCCA3`
2. PRM-11 5`ATCAGTGTAC3`
3. PRM-12 5`ATCGATGACG3`
4. PRM-14 5`ACTGTTGAAG3`

Results and Discussion

A. Six population analysis:

A.1. Mean performance and standard errors:

The results in table (1) showed that, the mean performance of F1, F2 and tow back crosses were higher than the tow parents in the crosses (I and II) only of 1000-grain weight, grain yield/ plant, chlorophyll content and flag leaf area, while the other traits were less than the two parents. On the other hand, the mean values of F1, F2 and the two back crosses were less than the two parents in the crosses number (I and II) only of plant height, respectively.

Finally, the F1 mean values were higher than the better-parent in the crosses (I and II) only for yield components, chlorophyll content and flag leaf area. While, it was less than the better-parent in the biotic stress

traits, indicating the presence of partial and over-dominance of these traits which were verified by the computed values of heterosis. These results were obtained by (Aidy et al., 2006), (Bindu et al., 2006), and (EL-Mouhamady et al., 2014).

A.2. Scaling test

Results in table (2) showed scaling test for adequacy of additive and dominance model of all traits studied in rice. Data presented in table (2) shows highly significant positive values of A, B, C scaling test for all crosses in all traits studied except the crosses (I & III) for plant height, 1000-grain weight, chlorophyll content and flag leaf area and cross (III) only for grain yield per plant, blast reaction, white-tip nematode infection% and stem borer evaluation%, respectively.

These results indicated that the adequacy of additive-dominance model for crosses; I and III for plant height, 1000-grain weight, chlorophyll and flag leaf area may be affected by non-allelic interaction, while the other traits were significantly and highly significantly positively of scaling test for the crosses; I and II, which indicted that some allelic interactions may be play a role in the inheritance of these traits.

A.3. Genetic components

The results in table (3) showed highly significant positively of additive gene effects, additive x additive, and additive x dominance gene effects in the crosses (I and II), only of all traits studied, while, highly significant negatively was showed in the crosses; (I and III) only of dominance and dominance x, dominance gene effects in all traits studied, respectively.

This suggested that additive gene effect made a significant contribution to the inheritance of these traits in the crosses; (I and II). The magnitude of the dominance gene effects relative to the magnitude of additive gene effects and was large in the cross number (I) only, for all traits studied. With regard to the individual types of digenicepistatic gene effects and the three types of gene interaction were very important in the inheritance of 1000-grain weight, grain yield/ plant, chlorophyll content and flag leaf area, blast reaction, white-tip nematode infection % and stem borer evaluation %, respectively.

These results are in agreement with those reported by (EL-Mowafi et al., 2005) and (Aidy et al., 2006), where they concluded that additive gene action was importance in the inheritance in yield components and some traits of roots in rice under normal and stress of salinity conditions.

A.4. Estimation of heterosis over better-parent, heritability in narrow and broad sense, genetic advance and inbreeding depression

The results in table (4) showed that, highly significant and negatively values of heterosis over better-parents were observed in the crosses; (I and II) for plant height, blast reaction, white-tip nematode infection (%) and stem borer evaluation (%), while, highly significant and positively of heterosis over better-parent were showed in the crosses; (I and II) for the other traits, respectively, which indicated that the importance of non-additive (dominance and dominance x dominance) type of gene action in the inheritance of these traits in rice. These results were in agreement with those reported by (EL-Abd 1995) and (EL-Refae 2002).

Table.1 Mean performances and standard errors for all traits studied in the three crosses of rice

Traits	Crosses	Plant height (cm)	1000 - grain weight (g)	Grain yield/ plant (g)	Chlorophyll Content	Flag leaf area (cm ²)	Blast reaction	White-tip nematode infection (%)	Stem borer evaluation (%)
P1	I	108.14 ± 1.70	22.36 ± 1.30	37.14 ± 0.20	42.00 ± 0.70	30.20 ± 0.50	2.00 ± 0.10	2.70 ± 0.30	3.20 ± 1.30
	II	108.14 ± 1.70	22.36 ± 1.30	37.14 ± 0.20	42.00 ± 0.70	30.20 ± 0.50	2.00 ± 0.10	2.70 ± 0.30	3.20 ± 1.30
	III	114.50 ± 0.80	20.04 ± 1.50	33.20 ± 0.10	39.50 ± 0.20	28.03 ± 0.10	2.70 ± 0.30	11.50 ± 0.40	4.50 ± 1.70
P2	I	114.50 ± 0.80	20.04 ± 1.50	33.20 ± 0.10	39.50 ± 0.20	28.03 ± 0.10	2.70 ± 0.30	11.50 ± 0.40	4.50 ± 1.70
	II	95.18 ± 0.60	25.30 ± 0.70	35.12 ± 0.40	46.70 ± 0.40	34.70 ± 0.40	3.20 ± 0.40	1.50 ± 0.70	6.73 ± 0.20
	III	95.18 ± 0.60	25.30 ± 0.70	35.12 ± 0.40	46.70 ± 0.40	34.70 ± 0.20	3.20 ± 0.40	1.50 ± 0.70	6.73 ± 0.20
F1	I	102.63 ± 0.20	26.14 ± 0.80	43.50 ± 0.30	46.70 ± 0.30	34.42 ± 0.30	1.57 ± 0.40	2.30 ± 0.50	2.80 ± 0.40
	II	91.70 ± 0.70	28.57 ± 1.20	39.40 ± 1.00	49.50 ± 0.50	37.00 ± 0.60	2.20 ± 0.60	1.70 ± 0.40	3.00 ± 0.50
	III	116.73 ± 1.30	19.00 ± 1.70	30.20 ± 0.80	34.30 ± 0.20	26.18 ± 0.20	3.18 ± 0.10	14.70 ± 0.20	8.42 ± 0.80
F2	I	109.17 ± 3.50	24.07 ± 2.70	40.05 ± 3.70	44.73 ± 4.50	32.34 ± 3.20	2.50 ± 1.90	14.80 ± 4.60	4.70 ± 1.70
	II	87.37 ± 4.00	26.13 ± 3.40	37.04 ± 3.40	47.00 ± 2.80	35.03 ± 3.90	2.73 ± 3.20	13.60 ± 3.40	4.32 ± 2.43
	III	110.50 ± 2.80	17.00 ± 2.50	27.73 ± 2.95	30.32 ± 3.40	24.00 ± 3.30	3.00 ± 2.80	16.70 ± 2.75	10.14 ± 1.80
BC1	I	118.27 ± 1.40	28.34 ± 0.60	48.00 ± 1.30	49.72 ± 0.50	42.00 ± 0.40	2.60 ± 1.30	2.50 ± 1.30	3.70 ± 1.20
	II	116.12 ± 1.20	29.37 ± 1.20	47.70 ± 1.50	51.00 ± 0.70	45.00 ± 1.70	3.00 ± 1.70	2.80 ± 1.70	3.20 ± 1.80
	III	1.34 ± 1.80	24.13 ± 1.70	34.20 ± 0.20	33.00 ± 0.30	37.50 ± 1.80	3.40 ± 1.20	22.00 ± 8.00	8.00 ± 1.50
BC2	I	117.15 ± 0.40	30.08 ± 0.80	44.19 ± 0.13	55.23 ± 1.40	44.00 ± 0.70	3.20 ± 0.80	3.00 ± 1.80	4.36 ± 0.60
	II	114.20 ± 1.80	27.40 ± 0.70	46.40 ± 0.40	47.14 ± 0.60	47.30 ± 0.30	3.50 ± 0.70	2.70 ± 1.20	3.70 ± 0.80
	III	123.43 ± 0.70	21.07 ± 0.20	40.00 ± 1.20	35.40 ± 1.30	42.63 ± 0.40	2.80 ± 0.20	17.73 ± 0.60	7.52 ± 1.20

I: GZ1368-S-5-4X Nabatat Asmar

II: GZ1368-S-5-4X IR28

III: Nabatat Asmar X IR28

*: Significant at 5%

**: Significant at 1%

Table.2 Estimates of mother's scales (A, B and C) in the three crosses of rice

Mother's scales	Crosses	Plant height (cm)	1000 grain weight (g)	Grain yield/ plant (g)	Chlorophyll content	Flag leaf area (cm ²)	Blast reaction	White-tip nematode infection (%)	Stem borer evaluation (%)
A	I	-2.70 ** ± 0.30	-84.30 ** ± 0.50	120.20 ** ± 1.40	-21.40 ** ± 0.30	-12.70 ** ± 1.70	8.30 ** ± 1.60	64.70 ** ± 1.90	6.14 ** ± 0.30
	II	14.60 ** ± 1.30	37.40 ** ± 1.30	78.00 ** ± 1.20	32.00 ** ± 1.20	127.20 ** ± 1.40	2.80 ** ± 0.20	78.00 ** ± 1.40	8.20 ** ± 1.70
	III	-8.40 ** ± 1.40	-26.30 ** ± 1.70	-7.50 ** ± 0.40	-15.80 ** ± 0.20	-8.20 ** ± 1.30	-1.80 ** ± 1.20	-13.80 ** ± 1.70	-1.70 ** ± 0.20
B	I	-11.30 ** ± 1.20	-17.80 ** ± 1.20	41.70 ** ± 0.70	-14.30 ** ± 1.60	-20.53 ** ± 0.70	24.70 ** ± 1.80	12.80 ** ± 1.50	39.70 ** ± 0.80
	II	34.80 ** ± 1.80	40.16 ** ± 1.60	52.40 ** ± 1.30	7.13 ** ± 1.50	11.80 ** ± 1.40	15.80 ** ± 1.40	29.70 ** ± 0.30	16.87 ** ± 1.20
	III	-4.00 ** ± 1.00	-5.18 ** ± 0.80	-11.40 ** ± 0.60	-37.00 ** ± 1.20	-17.72 ** ± 1.30	-2.30 ** ± 1.50	-5.40 ** ± 1.80	-83.70 ** ± 2.70
C	I	-12.70 ** ± 0.80	-19.43 ** ± 0.30	7.80 ** ± 1.70	-12.60 ** ± 1.40	-71.00 ** ± 1.80	9.40 ** ± 1.30	11.80 ** ± 0.20	15.37 ** ± 1.60
	II	20.00 ** ± 1.60	13.00 ** ± 1.70	31.40 ** ± 0.30	57.40 ** ± 1.20	13.50 ** ± 1.30	14.00 ** ± 0.50	23.70 ** ± 1.40	12.46 ** ± 1.90
	III	-9.70 ** ± 1.20	-8.40 ** ± 1.90	-14.30 ** ± 0.50	-17.80 ** ± 0.80	-17.80 ** ± 1.20	-4.63 ** ± 1.20	-54.70 ** ± 1.50	-40.16 ** ± 0.50

I: GZ1368-S-5-4X Nabatat Asmar

II: GZ1368-S-5-4X IR28

III: Nabatat Asmar X IR28

*: Significant at 5%

**:

Significant at 1%

Table.3 Estimates of Genetic components for all traits studied in the three crosses of rice

Genetic Components	Crosses	Plant height (cm)	1000 grain weight (g)	Grain yield/ plant (g)	Chlorophyll content	Flag leaf area (cm ²)	Blast reaction	White-tip nematode infection (%)	Stem borer evaluation (%)
Additive gene action	I	4.55 **	4.24 **	9.24 **	7.41 **	2.44 **	2.00 **	11.93 **	0.76 **
	II	7.10 **	2.63 **	6.79 **	4.07 **	10.60 **	5.29 **	4.13 **	2.38 **
	III	-3.68 **	-1.87 **	-4.22 **	-7.45 **	-2.54 **	-2.05 **	-4.53 **	-1.29 **
Dominance gene action	I	-3.14 **	-1.07 **	-0.82 **	-3.25 **	-3.63 **	-0.30 **	-4.50 **	-0.78 **
	II	4.03 **	4.32 **	1.32 **	0.37 **	2.60 **	1.94 **	2.36 **	1.51 **
	III	-0.74 **	-0.73 **	-0.75 **	-1.59 **	-0.75 **	-1.98 **	-1.25 **	-0.92 **
Additive x Additive gene action	I	2.11 **	0.55 **	1.06 **	4.16 **	1.19 **	0.28 **	1.72 **	1.00 **
	II	1.57 **	1.26 **	0.44 **	1.32 **	0.26 **	1.15 **	2.07 **	0.85 **
	III	-1.05 **	-1.00 **	-0.39 **	-0.38 **	-0.36 **	-1.34 **	-0.77 **	-0.30 **
Dominance x Dominance gene action	I	-1.37 **	-0.38 **	-0.68 **	-2.77 **	-1.03 **	-0.32 **	-0.82 **	-0.15 NS
	II	2.07 **	0.87 **	1.14 **	0.80 **	0.74 **	0.85 **	0.96 **	0.38 *
	III	-0.82 **	-0.64 **	-1.42 **	-1.14 **	-0.64 **	-0.54 **	-0.24 *	-0.25 *
Additive x Dominance gene action	I	0.48 **	0.77 **	0.38 **	1.54 **	1.76 **	0.28 **	1.18 **	0.11 NS
	II	1.03 **	0.67 **	0.79 **	1.03 **	0.69 **	0.66 **	1.78 **	0.50 **
	III	-1.15 **	-0.54 **	-1.53 **	-0.50 **	-0.70 **	-0.84 **	-0.40 **	-0.36 **

I: GZ1368-S-5-4X Nabatat Asmar II: GZ1368-S-5-4X IR28 III: Nabatat Asmar X IR28

*: Significant at 5%

** : Significant at 1%

NS: Not significant

Table.4 Estimates of Genetic parameters for all traits studied in the three crosses of rice

Genetic Components	Crosses	Plant height (cm)	1000 grain weight (g)	Grain yield/ plant (g)	Chlorophyll content	Flag leaf area (cm ²)	Blast reaction	White-tip nematode infection (%)	Stem borer evaluation (%)
Heterosis over-better parent	I	-5.09 **	16.90 **	17.12 **	11.19 **	13.97 **	-21.50 **	-14.81 **	-12.50 **
	II	-3.65 **	12.92 **	6.08 **	5.99 **	6.62 **	-10.00 **	-13.33 **	-6.25 **
	III	22.64 **	-24.90 **	-14.00 **	-26.55 **	-24.55 **	17.77 **	880.00 **	87.11 **
Heritability in broad sense	I	95.10	96.15	88.97	94.46	98.14	88.08	95.22	96.88
	II	98.75	84.34	90.65	96.81	97.89	96.58	97.75	95.25
	III	97.38	76.48	95.51	95.67	94.32	96.30	95.07	96.29
Heritability in narrow sense	I	37.14	58.16	67.49	36.59	23.82	55.40	56.37	26.29
	II	44.37	22.75	58.73	51.91	69.69	51.66	35.72	40.33
	III	48.16	29.92	48.50	64.44	48.01	26.14	59.90	39.81
Genetic Advance Δg (%)	I	2.44	13.43	12.83	7.58	4.85	86.63	36.09	19.58
	II	4.17	6.09	11.09	6.36	15.97	124.73	16.39	46.53
	III	2.47	9.06	10.59	14.88	9.47	50.25	20.31	14.55
Inbreeding depression	I	-6.37 **	7.91 **	7.93 **	4.21 **	6.04 **	-59.23 **	-543.47 **	-67.85 **
	II	4.72 **	8.54 **	5.98 **	5.05 **	5.32 **	-24.09 **	-700.00 **	-44.00 **
	III	5.33 **	10.52 **	8.17 **	11.60 **	8.32 **	-5.66 **	-13.60 **	-20.42 **

I: GZ1368-S-5-4X Nabatat Asmar

II: GZ1368-S-5-4X IR28

III: Nabatat Asmar X IR28

*: Significant at 5%

**:

Significant at 1%

With regard to heritability, the results in table (4) revealed that, the heritability in broad sense was higher than the heritability in narrow sense for all crosses studied of all traits, while the heritability in narrow sense was low in most of traits, except the crosses; (I, II and III) for grain yield/ plant, the cross (I) for 1000-grain weight, (II) for chlorophyll content and white-tip nematode infection (%) and flag leaf area, respectively with respect to genetic advance under selection of F2-generations, the results cleared that the highest mean values of genetic advance were recorded in the crosses; (I, II, III) for all traits studied except, plant height and crosses (II and III) for 1000-grain weight, which means that narrow sense heritability was found to be greater important for controlling these traits.

Regarding inbreeding depression in table (4), all studied crosses exhibited highly significant positively of inbreeding depression except the three the biotic stress and cross (I) for plant height, respectively. These crosses were found to be the best crosses for heterosis and indicated the greater importance of additive gene action for inheritance and controlling these traits. These results were in agreement with those reported by (Seedek 2006) and (EL-Mouhamady et al., 2014).

Finally, the F1 mean values were higher than the high parent values in the crosses; (I, II) in grain yield per plant, 1000-grain weight, chlorophyll content and flag leaf area indicating the presence of partial and over-dominance for these traits, which were verified by computed heterosis. On the other hand, the F2-population means were lower than the F1 mean for most traits, indicating the existence of highly significant depression in F2-generation for these traits. From the previous results, it could be indicated that, for 1000-grain weight, grain

yield/ plant, chlorophyll content and flag leaf area traits, the expression of heterosis in the F1 might be followed by considerable inbreeding depression of F2 performance, indicating that the non-additive gene effects governed the inheritance of such traits. This logic and expected, since there is a tendency towards homozygosity which is accelerated by 50% for each selfed generation.

Note: This in turn led to the conclusion that these traits could be improved by the traditional methods and selection could be effective mostly in the late generation. This was proved by the entire values of the predicted genetic advance which were low to moderate in most cases.

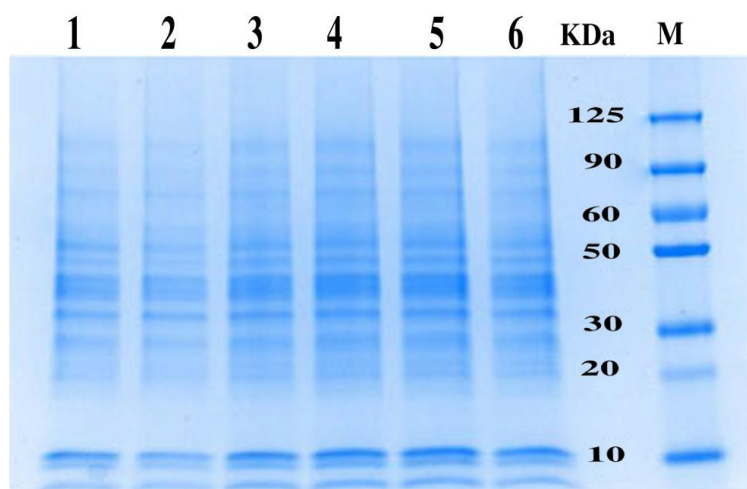
B. Molecular Markers:

B.1. SDS-protein Electrophoresis:

The electrophoretic banding patterns of proteins extracted from the leaves of the six genotypes of rice were showed in (Fig. 1 and Table 5).seven bands ranging from 10 to 125 (KDa), were observed in this study.The bands number (2, 3, 4, 5, 6,7) with molecular weights of (90, 60, 50, 30, 20, 10) KDa, respectively were appeared in all genotypes of rice which means that these bands were commonly bands in these cultivars of rice. On the other hand, the band number (1) with molecular weight of (125) KDa was not appeared in all genotypes, respectively.The appearance of the bands number (2, 3, 4, 5, 6,7) with molecular weights of (90, 60, 50, 30, 20, 10) KDa for all parents and their crosses may be due to manufacture specific protein which responsibility and powering for resistance to the biotic stress in rice,such as blast reaction,white tip-nematode infection % and stem borer %. This increasing of density and intensity of these bands in all genotypes except band number 1 may be due to highly

ability of resistance to the biotic stress traits in rice and this modification of gene expression is due to high conservative genes found in rice. These genes might have a crucial role in the response to different stress as well as the main role of systemic

signals gene rated by the tissue exposed to salinity, drought and the biotic stress resistance in rice. These results reported by El-Fadly et al., (2007) and AL-Wahibi (2010).



Fig(1):- SDS-PAGE of water soluble protein fraction of the six genotypes of rice.

Table.5 The protein banding patterns of SDS-PAGE of the six genotypes of rice

Bands NO	M.W(KDa)	1	2	3	4	5	6
1	125	-	-	-	-	-	-
2	90	+	+	+	+	+	+
3	60	+	+	++	++	++	++
4	50	+	+	++	++	++	++
5	30	+	+	++	++	++	++
6	20	+	+	+	+	+	+
7	10	++	++	++	++	++	+++
Total of Bands		6	6	6	6	6	6

(+) : very faint 1 : GZ1368-S-5-4 5 : GZ1368-S-5-4 X IR28
 (++) : faint 2 : Nabatat Asmar 6 : Nabatat Asmar X IR28
 (+++) : dark
 (+++ +) : very dark 3 : IR28
 (-) : absence of bands 4 : GZ1368-S-5-4 X Nabatat Asmar

B.2. Isozymes electrophoresis

B.2.1. Peroxidase Isozymes

The electrophoretic patterns of peroxidase isozymes in the six genotypes of rice were showed in table (6) and fig. (2). A total number of four bands were exhibited; all bands were appeared in all genotypes of rice except the band number 1 of the parent number 2, Which indicated that these bands were common bands in these genotypes of rice. The reason for increasing in peroxidase activity of the band number 2 maybe due to manufacture of the protein which control to the biotic stress tolerance in rice. The results showed that the parents number 1 and 3 for the bands number 2 were highly tolerance for the biotic stress, as well as antioxidant enzymes which response to different ability for this resistance and proved the favorable conditions to this protein in order to have their a little activity to neutralize the free radicals which are produced under the biotic stress. Finally, using of peroxidase isozyme as a marker for the biotic stress tolerance in rice and found that the profile of peroxidase enzyme was modified during infection of these conditions, also a new subset of proteins induced by resistance to blast reaction, white tip nematode infection % and stem borer evaluation % compared to normal conditions and this behavior maybe due to its ability to the biotic stress tolerance and which may cause some shift in gene expression (El-baz et al., 2003) and (Roy & Mandal, 2005).

B.2.2. Polyphenol oxidase isozymes

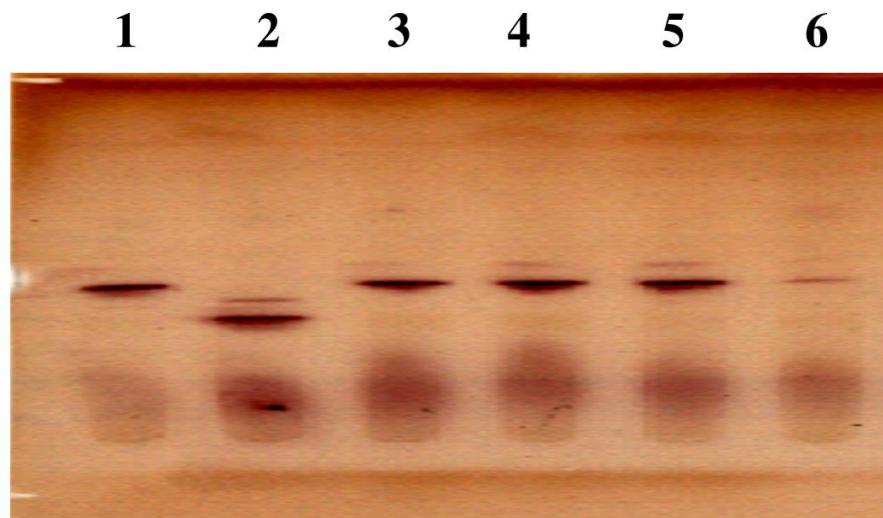
Four bands appeared for polyphenol oxidase in the six genotypes of rice, (Fig. 2 and Table 6). The four bands were commonly bands detected for all genotypes studied except the band number 3 for the cross number 3, respectively. The appearance of

these bands under these conditions was different in densities and intensities especially the bands number 1, 2 and 4 for all genotypes. It is noted that, the variations between all genotypes of rice about the biotic stress resistance maybe go back to the amount of efficiency, enzyme activity of polyphenol oxidase isozymes, its impact and framed by the resistance to the biotic stress. The polyphenol oxidase isozymes activity increased the level of resistance of the biotic stress in the bands number (1, 2, 4) for all genotypes which showed very high activity because the production of the protein which controlled of resistance for blast reaction, white tip nematode infection % and stem borer evaluation %. These results were in agreement with (El-Beltagi *et al.*, 2010) who found that the reason for decreasing in polyphenol oxidase activity after roasting may be due to protein denaturation. Also (Gautam *et al.*, 1998) and (Montavon and Bortlik, 2004) reported that roasting treatments decline polyphenol oxidase activity in mushroom and coffee. The previous results are similar to the results of (Lee *et al.*, 2007) who reported that antioxidant enzymes were upregulated under drought conditions in rice leaves, and also the enzymes related to metabolic pathway were differently accumulated for the ability for the biotic stress tolerance in rice.

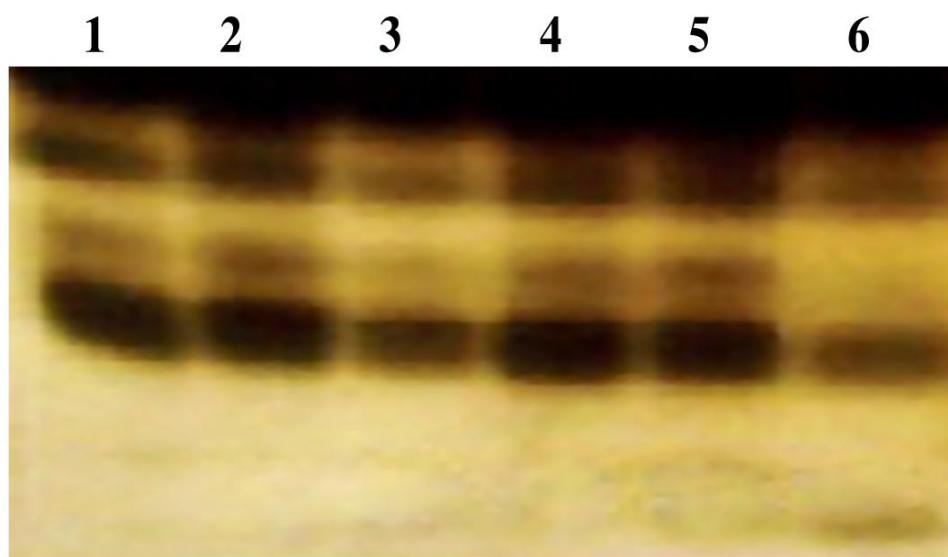
On the other hand, the highest activity from the two isozymes were found in the parents number (1, 3) and crosses number (1, 2) and this is consistent with the results of both (Nagesh and Devaraj, 2008), which concludes that quantitative and qualitative alteration in antioxidant enzyme system are often related to level of resistance to stress, with quantitative changes in the enzyme level alterations were also observed in intensities and number of isozyme bands during applied stress. Like the present results, the decrease in isozyme activity

indicates gradual degradation of these enzymes or their structural modification under increased Free-toxicity levels,

whereas the banding pattern expressing differential intensity shows the varying status of an enzyme affected by the stress.



Fig(2):-Electrophoretic patterns In the six genotypes of rice using peroxidase Isozymes .



Fig(3):- Electrophoretic patterns In the six genotypes of rice using polyphenol oxidase Isozymes .

Table.6 Electrophoretic patterns on peroxidase and polyphenoloxidase isozymes of leaves for the six rice genotypes

Band No.	1	2	3	4	5	6
Peroxidase Isozymes						
1	+	-	+	+	+	+
2	+++	+	+++	+++	+++	+++
3	+	+	+	+	+	+
4	+	+	+	+	+	+
Total	4	3	4	4	4	4
Polyphenol oxidase isozymes						
1	++++	++++	++++	++++	++++	++++
2	++++	++++	++	++++	++++	+++
3	++	++	+	++	++	-
4	++++	++++	+++	++++	++++	+++
Total	4	4	4	4	4	3
Total	8	7	8	8	8	7

(+) : very faint 1 : GZ1368-S-5- 4 : GZ1368-S-5-4 X IR28
 (++) : faint 2 : Nabatat Asmar 6 : Nabatat Asmar X IR28
 (++) : dark
 (++++) : very dark 3 : IR28
 (-) : absence of bands 4 : GZ1368-S-5-4 X Nabatat Asmar

C. RAPD-PCR analysis

The densitometric analysis of RAPD-PCR products in the six genotypes of rice using (PRM-7) and (PRM-11) primers are shown in table (7) and figures (4, 5), respectively. The bands number (1, 2, 3, 4) with molecular weights of (1200, 1100, 1000, 780) bp were appeared in all genotypes except the crosses number 2 and 3 for the bands number 1,2,3 only respectively, using (PRM-7) primer in fig.4 which indicated that these bands were primer and common bands for these genotypes of rice, while, the bands number (5,6,7,8,9) with molecular weights of (670, 550, 450,400,350) bp were observed in the genotypes number, (1,2), (1,2,3),(1,3,4), (1,2,4,5) and (1,2), respectively, which means that these bands were commonly bands and marker in these genotypes.

On the other hand, the results in table (7) and Fig. (5) using (PRM-11) primer revealed that, the bands number (1,2,3,4) with molecular weight of (1400 , 1350,1250,1100)bp, respectively, were not appeared in all genotypes of rice using (PRM-11) primer, while, the bands number (5, 6, 7,8,9) with molecular weights of (1000, 700, 550,400,150)bp were observed in all genotypes except the parents number (3) only for the bands number (5,6), respectively, which means that these bands were commonly bands and marker for these genotypes of rice.

The bands number (1, 2, 3, 4, 5, 6, 7, 8, 9,10,11) with molecular weight of (1655, 1450, 1250, 1100, 1000, 750, 600, 350, 200,150,75) bp were showed and appeared in the six genotypes of rice using (PRM-12) primer in table (8) and Fig. (6) respectively, which means that these bands were common

bands and marker for these genotypes of rice, while the bands number (2,4,5,6,7,8,9) with molecular weights of (1700,1240,1000, 750,330,200,100) were observed in the genotypes number, (1,3), (1,2,3,5,6), (2,3,5,6), (1,2,3,4,5,6), (1,2,3,4,5,6),

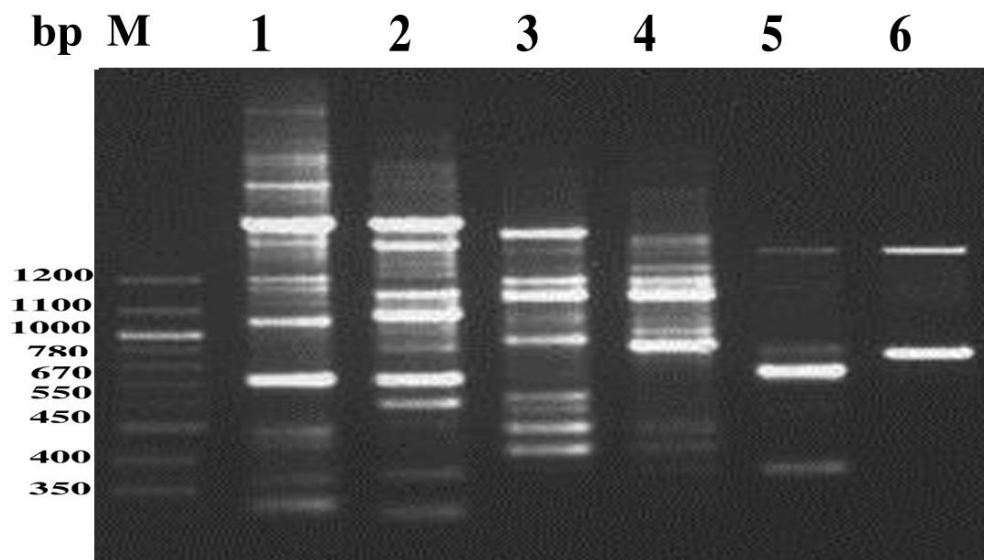
(1,2,5,6) and (5), respectively, which indicated that these bands were marker and common bands in these genotypes using (PRM-14) primer in table (8) and fig(7).

Table.7 The densitometric analysis of RAPD-PCR products of the six genotypes of rice against (PRM-7) and (PRM-11) primers

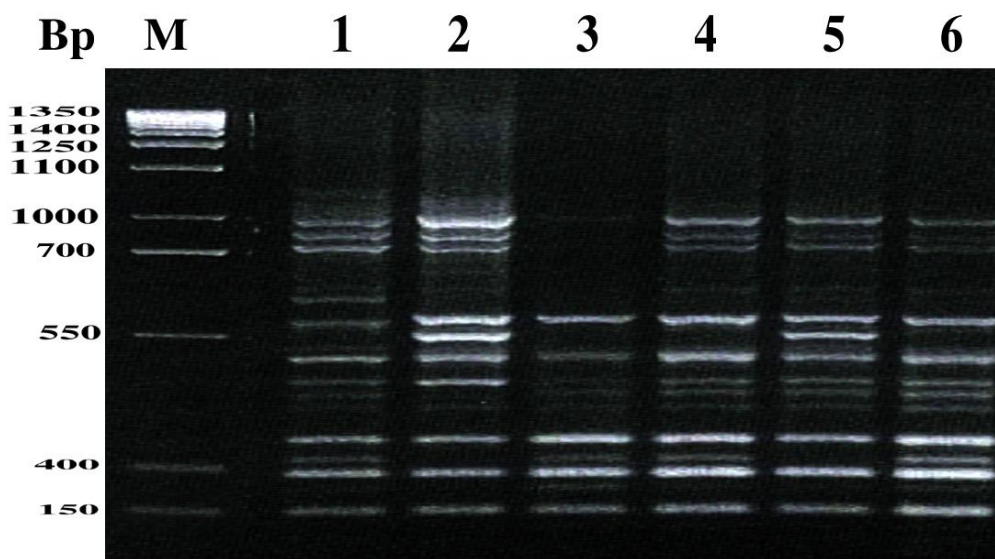
Primer name	Base bairs	1	2	3	4	5	6
(PRM-7)	1200	+	+	+	+	-	-
	1100	+	+	+	+	-	-
	1000	+	+	+	+	-	-
	780	+	+	+	+	+	+
	670	+	+	-	-	-	-
	550	+	+	+	-	-	-
	450	+	-	+	+	-	-
	400	+	+	-	+	+	-
	350	+	+	-	-	-	-
Total Band		9	8	6	6	2	1
Primer name	Base bairs	1	2	3	4	5	6
	1400	-	-	-	-	-	-
	1350	-	-	-	-	-	-
(PRM-11)	1250	-	-	-	-	-	-
	1100	-	-	-	-	-	-
	1000	+	+	-	+	+	+
	700	+	+	-	+	+	+
	550	+	+	+	+	+	+
	400	+	+	+	+	+	+
	150	+	+	+	+	+	+
	Total band	5	5	3	5	5	5

1 : GZ1368-S-5-4
 2 : Nabatat Asmar.
 3 : IR28
 4 : GZ1368-S-5-4 X Nabatat Asmar.

5 : GZ1368-S-5-4 X IR28
 6 : Nabatat Asmar X IR28



Fig(4):- The densitometric analysis of RAPD-PCR for the six genotypes of rice using (PRM-7) primer .



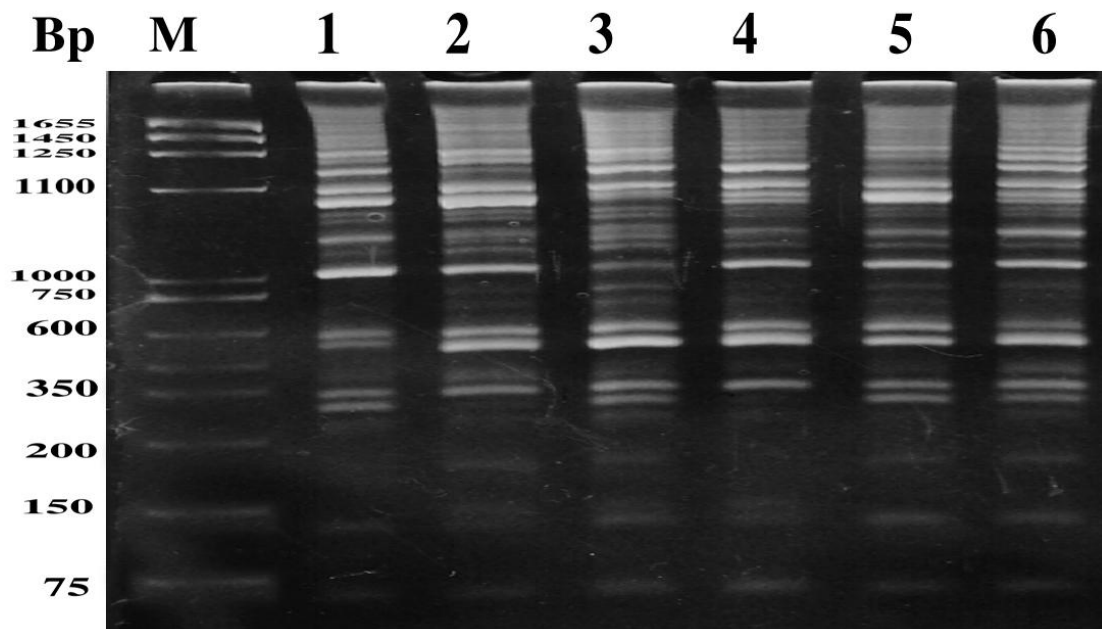
Fig(5):- The densitometric analysis of RAPD-PCR for the six genotypes of rice using (PRM-11) primer .

Table.8 The densitometric analysis of RAPD-PCR products of the six genotypes of rice against (PRM-12) and (PRM-14) primers.

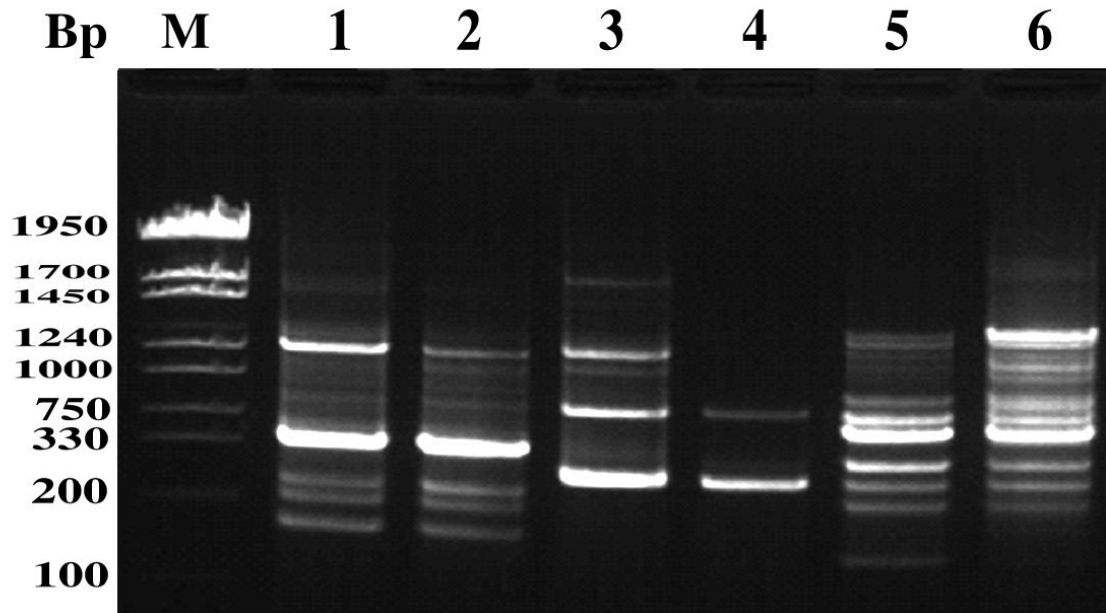
Primer name	Base bairs	1	2	3	4	5	6
(PRM-12)	1655	+	+	+	+	+	+
	1450	+	+	+	+	+	+
	1250	+	+	+	+	+	+
	1100	+	+	+	+	+	+
	1000	+	+	+	+	+	+
	750	+	+	+	+	+	+
	600	+	+	+	+	+	+
	350	+	+	+	+	+	+
	200	+	+	+	+	+	+
	150	+	+	+	+	+	+
	75	+	+	+	+	+	+
Total Band		11	11	11	11	11	11
Primer name	Base bairs	1	2	3	4	5	6
(PRM-14)	1950	-	-	-	-	-	-
	1700	+	-	+	-	-	-
	1450	-	-	-	-	-	-
	1240	+	+	+	-	+	+
	1000	-	+	+	-	+	+
	750	+	+	+	+	+	+
	330	+	+	+	+	+	+
	200	+	+	-	-	+	+
	100	-	-	-	-	+	-
Total Band		5	5	5	2	6	5

1 : GZ1368-S-5-4
2 : Nabatat Asmar
3 : IR28

4 : GZ1368-S-5-4 X Nabatat Asmar
5 : GZ1368-S-5-4 X IR28
6 :NabatatAsmar X IR28.



Fig(6):- The densitometric analysis of RAPD-PCR for the six genotypes of rice using (PRM-12) primer .



Fig(7):- The densitometric analysis of RAPD-PCR for the six genotypes of rice using (PRM-14) primer .

Similar results are in agreement with those reported by Cheria & Fereira, (2010) and El-Mouhamady, A.A. et al (2014). The information gathered here would be helpful in genome mapping studies and for the development of rice genotypes with wider and diverse genetic background to obtain improved crop productivity during the optimum degree of resistance to the biotic stress. The data obtained in this experiment confirmed the efficiency of the RAPD technique for determination and estimation of genetic distance and relatedness among different plant genotypes. The RAPD analysis has been found to a valuable DNA marker system to evaluate genetic diversity.

The information about genetic similarity will be helpful to avoid any chance of elite germplasm becoming genetically uniform, because of the simple experimental procedures, the requirement of minimal amount of plant tissue and the possibility of automation.

RAPD-PCR analysis should be very useful in breeding for rapid and early identification of most diverse individuals in large seedling populations, allowing the detection of true to type genotypes for the improvement of our crop breeding programs. Keeping in view the useful information about the close genetic relationship, it is suggested that mission oriented breeding programs with the help of DNA fingerprinting technology will be helpful to produce distinct genotypes with diverse genetic background and improved productivity.

The results in Table (9) revealed that, four primers RAPD-PCR were used to identify six genotypes of rice and the total number of bands amplified per the four primers were 38 band varied between (PRM-7) and (PRM-14) primers whereas, its were 9 bands for each primer, respectively, in addition to 32 band (Monomorphic) and 6 band were (polymorphic) resulting in a polymorphism of 80%. the size of bands varied between 1200 bp and 100bp.

Table.9 Polymorphism of the RAPD-PCR primers among six rice genotypes

Primer code No.	Sequence (5' → 3')	Size range of the Scorable Bands (bp)	Total bands	No. of monomorphic bands	No. of polymorphic bands	Unique bands	% polymorphism
PRM-7	TGCACGCCCA	1200-350	9	9	0	0	0
PRM-11	ATCAGTGTAC	1400-150	9	5	4	0	80
PRM-12	ATCGATGACG	1655-75	11	11	0	1	0
PRM-14	ACTGTTGAAG	1950-100	9	7	2	1	28.57
Total			38	32	6	2	

The extent of polymorphism per primer ranged from 0% (PRM-7) primer to 80% (MOR-11) primer, on the other hand, these two primers scored zero unique marker sizes 1200 and 150bp, while, the primers (PRM-12) and (PRM-14) scored one unique marker with molecular 1655 and 100bp, respectively.

D. Genetic similarity matrix

The dendrogram resulted from the combination between the banding patterns of protein SDS- PAGE and RAPD-PCR (Table.10 and Fig. 8), revealed that, six rice genotypes can be clustered in three distinct groups. Group A (similarity ranged from "0.77 to 0.97") contains on genotypes, (GZ1368-S-5-4) and (Nabatat Asmar), Group B (Similarity ranged from "0.88 to 0.91") comprised of genotypes (IR28, and GZ1368-S-5-4 X IR28), while Group C included on (GZ1368-S-5-4 X Nabatat Asmar, GZ1368-S-5-4 IR28 and Nabatat Asmar X IR28), respectively and (Similarity

ranged from "0.76 to 0.91), respectively. Molecular markers using RAPD-PCR showed better resemblance compared to biochemical markers using SDS-PAGE and isozymes. Thus, their disadvantages include a low level of polymorphism to have few alleles per locus, especially when the genetic base is narrow.

In addition to, proteins can be affected qualitatively and quantitatively in their expression level by environmental factors and tissue type. On the contrary, molecular markers are not environmentally influenced, which means that the same banding profiles can be expected at all times for the same genotype (Kumar *et al.*, 2009). They indicated that RAPD-PCR technique can be used as a tool for determining the extent of genetic diversity among the six genotypes of rice and the importance of high level of genetic variability in the gene pool to face stress of the biotic stress.

Table.10 Similarity indices among six rice genotypes as estimated using SDS-PAGE and RAPD-PCR markers

No.	1	2	3	4	5	6
1	1.0					
2	0.84	1.0				
3	0.89	0.92	1.0			
4	0.69	0.80	0.90	1.0		
5	0.78	0.88	0.88	0.77	1.0	
6	0.97	0.77	0.92	0.91	0.93	1.0

1 : GZ1368-S-5-4
2 : Nabatat Asmar
3 IR28

4 : GZ1368-S-5-4 X Nabatat Asmar
5 : GZ1368-S-5-4 X IR28
6 : Nabatat Asmar X IR28.

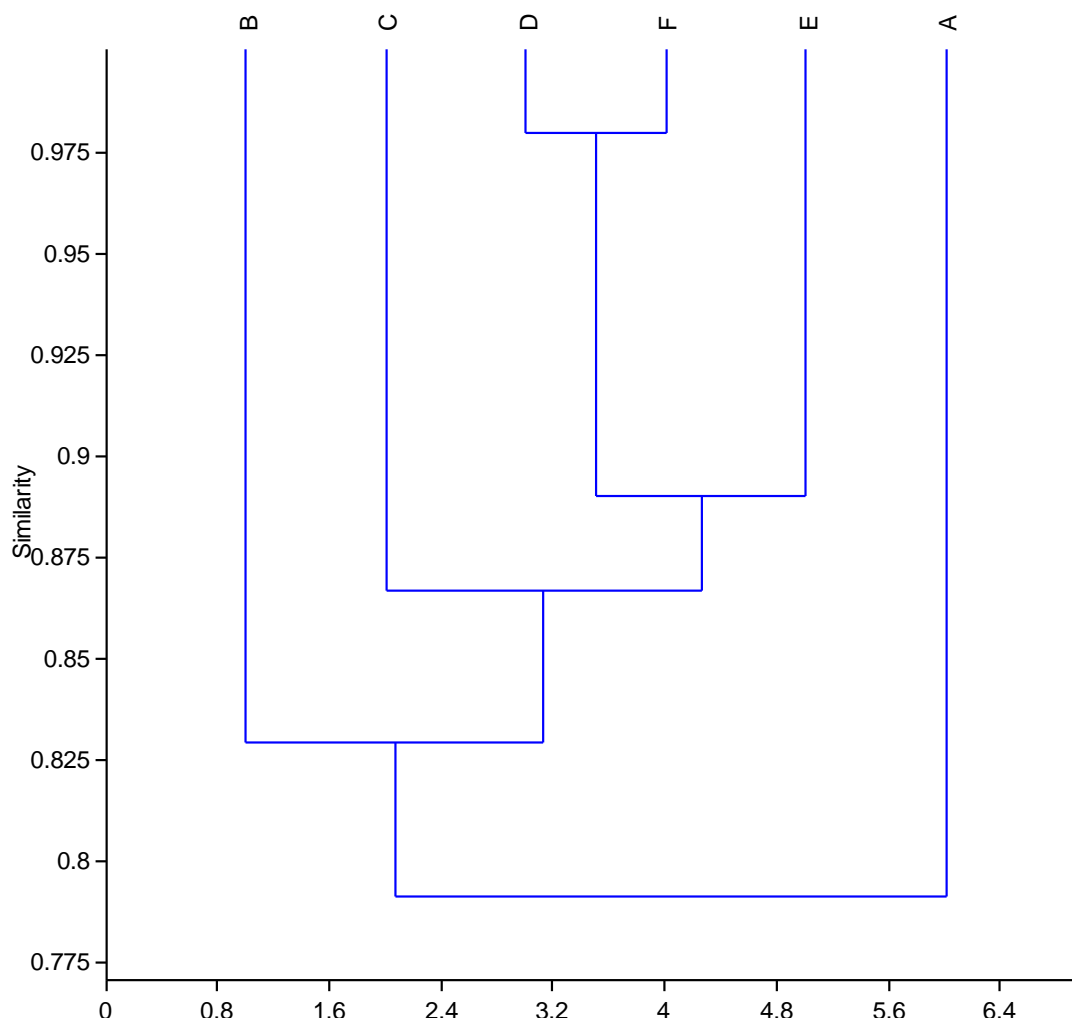


Fig.8: Dendrogram of the six genotypes of Rice namely, A (GZ1368-S-5-4), B(Nabatat Asmar), C(IR28), D(GZ1368-S-5-4 X Nabatat Asmar), E(GZ1368-S-5-4 X IR28) and F(Nabatat Asmar X IR28), respectively, showing genetic distances and relationships among these genotypes based on SDS-PAGE and RAPD-PCR combination by UPGMA algorithm using Jaccard's Similarity coefficients.

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