DETERMINATION OF ALLELOPATHIC ACTIVITY IN RICE BY USING MOLECULAR MARKERS

DORA, S.A.[1], S.A. ABD ALLA[1], A.E. DRAZ[2]AND M.I. ABO YOUSEF[2]

1 Genetic Dept., Fac. of Agric., Kafr El-Sheikh, Tanta Univ.
2 Rice Research and Training Center, Field Crops Research Institute, (ARC) Egypt

Abstract

This study was conducted during 1999 at Rice Research and Training Center, Sakha, Kafr El-Sheikh, Genetic Dept., Fac. of Agric., Kafr El-Sheikh, Tanta Univ., Egypt and DRR, Hyderabad, India to study the utilization of molecular markers as a tool to determine allelopathic activity in rice.

Six rice varieties, i.e. Sakha 101, sakha 102, Kim Rad F 87, Rikuto Norin 22, Shimokita and Dular, as well as a check variety (Giza 176) were tested for allelopathic activity against *Echinochloa. crus-galli* in the lab. and greenhouse to determine weed control percentage in 1999. Isozyme and Molecular marker analysis were used as markers for Marker-Aided Selection (MAS) of allelopathic genes against *E. crus-galli.*

The results showed that the reduction percentage of barnyardgrass root growth ranged 13.4-53.2 in the greenhouse and 19.95-52.42 in the lab. While, the reduction percentage of barnyardgrass shoot growth ranged between 7.6-20.7 in the greenhouse and 4.55-34.85 in the lab. These results showed that the allelopathic varieties strongly inhibit root elongation of barnyardgrass with weak affect on the shoot growth.

Complementary band No. 1 and No. 2 and high expression in peroxidase isozyme may increase in these varieties. While weak band No. 3 and very weak band No. 6 in esterase isozyme may be associated with allelopathic activity in the varieties and mean low expression of their genes.

The allelopathic specific RAPD markers 4.044 Kbp of (OPA 19) and 1.780 Kbp (OBJ 18). These two amplified fragments may carry gene(s) which are responsible for synthesizing activity protein(s) which are tightly
linked to the increase the potentiality of allelopathic activity. While, fragments, 9.057 Kbp of (OPA 19) and 0.583 and 2.090 Kbp of (OBJ 18) amplified bands were absent in strong allelopathic varieties. These results indicate that these fragments may carry gene(s) which express protein(s) are tightly linked to the decrease of the potential of allelopathic activity. Therefore, this can be used as marker for marker-aided selection (MAS) of allelopathic genes against *E. crus-galli.*

INTRODUCTION

Rice is one of the most important food crops in Egypt, contributing over 20% of the cereal consumption. It is cultivated over an area of 1.4 million feddans (Badawi, 1999), covering 22% of the total cultivated area in Egypt and utilizing 18% of the annual water consumption.

To meet the increasing food demand of the fast growing population in Egypt, increasing the grain yield of rice is a national necessity. The increase of grain yield per unit area is an urgent goal. This goal could be achieved through growing rice

cultivars of high yield potentiality possessing desirable agronomic traits including allelopathic activity under appropriate management to get the best results.

Isozyme polymorphism offers a very powerful mean for varieties and traits identification. Moreover, isozyme polymorphism in cultivated rice has been widely surveyed and applied in recent years by many scientists (Second 1982 and 1985). Also, Chu (1967) found that a peroxidase band (4 band) was related to the differentiation of rice ecotypes since this band is existed in most indica, but was absent in japonica rices.

The selection of plant varieties based only on morphological markers is not very reliable because many characters of interest have low heritability and are genetically complex. Molecular markers based on the DNA sequence are more varied and reliable (Raghunathachari *et al.*, 2000).

The present study was designed to investigate the following objectives:

1. Identification of allelopathic activity of some rice varieties.
2. Demonstration of isozyme variation and molecular markers selection for allelopathic effects.

MATERIALS AND METHODS

This investigation was carried out at Rice Research and Training Center (RRTC), Sakha, Kafr El-Sheikh, Egypt, Genetic Department, Faculty of Agriculture, Kafr El-Sheikh, Tanta University, Egypt and Directorate of Rice Research (DRR), Hyderabad, India during 1999 and 2000 seasons.

1. Screening for Allelopathy Under Laboratory and Greenhouse Conditions:

Six rice varieties, sakha 102, Sakha 101, Kim Rad F 87, Rikuto Norin 22, Shimokita and Dular, as well as a check variety Giza 176 were evaluated for allelopathic activity using relay seeding technique and plant box methods. The first one used 20 seeds of each variety in two rows (10 seeds in each row) and 10 seeds of barnyardgrass in one row between them after one week from sowing rice for each petri dish which is provided by bridge of filter paper to absorb the water from the tray under laboratory conditions in three replications. The root and shoot lengths of barnyardgrass were measured after 10 days from sowing, according to Navarez and Olofsdotter (1996).

The second one (plant box method) was conducted in 40x20x80 cm metal box containing 10 kg of the mixed soil (3 loamy : 1 sand) samples under greenhouse conditions in three replicates. The seeding rate was 15 g/m^2 for each variety and 2 g/m^2 for barnyardgrass. Each box had five rows of rice and 4 rows of barnyardgrass

in direct seeded rice, data collected were taken after 40 days to barnyardgrass shoot and root length, according to Fujii (1992).

2. Biochemical and Molecular Analysis:

2.1. Electrophoresis and isozyme techniques:

2.1.1. Sample extraction:

Samples were extracted for characteristic isozyme system. Two hundreds mg of fresh leaf samples of 21 days old seedling (plumules) for the six parents as well as Giza 176 was used as check variety. Each sample was homogenized in 1.0 ml of an acid cold 50 mM Tris-HCl buffer (pH 6.8) containing 20% (W/V) sucrose and 3mM dithiotritol (DTT). The extracts were then centrifuged at 15000 rpm at 4 °C for five min. and supernatants were pipetted.

2.1.2. Polyacrylamide gel electrophoresis (PAGE):

Isozymes, esterase (Est.) and peroxidase (Pox.) were determined using polyacrylamide gel electrophoresis according to the method of Davis (1964).

2.2. Est. and Pox. Detection on gel :

Esterase bands were detected on the gel as described by Scandalios (1969), by using -naphthyl acetate as substrate and subsequent color development with fast blue RR salts.

3. PCR Based RAPD and Analysis :

DNA was extracted from 1 g of 14 day-old etiolated rice seedlings of six parents and the check variety using the method of Dellaporta *et al.* (1983) and illustrated in 50 ml of TE (pH 8.0).

The DNA was then purified by phenol : chloroform extraction and ethanol precipitation and checked for quality and purity by electrophoresis in a 0.8% (w/v) agarose gel in 1 x TAE buffer (Mahyca Res. Found. And Rockfeller Found., 1999).

The six difdferent 10-mer oligonucleotide RAPD primers used in this study were OPA 19, OPF 8, OPF 10, OPI 10, OPJ 18 and OPH 11 (Table 1).

Table 1. Arbitrary of six 10-mer primers and their nucleotide sequences used in analysis

Primer	sequence
OPA 19	'5 – CAAACGTCGG – 3'
OPF 8	'5 – GGGATATCGG – 3'
OPF 10	'5 – GGAAGCTTGG – 3'
OPI 10	'5 – ACAACGCGAG – 3'
OPJ 18	'5 – TGGTCGCAGA – 3'
OPH 11	'5 – CTTCCGCAGT – 3'

DNA ampliffication was performed in 15 ↘l genomic DNA, 0.6 ↘l dNTP (10 mM), 0.3 ↘l $MgCl_2$ (25 mM), 1.5 l (10 x buffer), 0.6 ▯ l primer, 0.2 ▯ l Taq polymerase (3 U/↘l) and (8.8 ↘l) sterile H_2O. The mixture was gently mixed and centrifuged prior to adding 2 drops of mineral oil. The amplification was performed in a thermocycler. The cycline conditions were 1 cycle of 94 °C for 5 min. followed by 45 cycles of 94 °C for 1 min., 36 °C for 1 min., 72 °C for 1 min. and finally 1 cycle of 72 °C for 5 min. After removing the mineral oil with chloroform, 10 ↘l aliquots of amplification products were loaded in a 1.5% (w/v) agarose gel for electrophoresis in 1 x TAE buffer. Gels were stained with ethidium bromide (0.5 ▯ g/ml for 20 min.) destained with tap water for 20 min., and photographed using a Canon camera with red filter using 11 ford pan 135.36, 400 ASA film.

These photographs were used to score the DNA bands. Bands were screened as "1" if present or "O" if absent. The index of similarity between individuals was calculated using the formula :

$$S_{xy} = 2\, n_{xy} / (n_x + n_y) \qquad \textbf{(Lynch, 1990)}$$

Where :

S = Similarity

n_{xy} = is the number of bands shared by individuals x and y

n_x and n_y = are the number of detected bands scored for each individual

The dendrogram is constructed on Euclidean distance basis. All these computations are performed using SPSS Computer Software (1995). A covariance analysis betweenall parts of these Characters,within each group was estimated using the producer of Sokal and Rohlf (1995).

RESULTS AND DISCUSSION

Laboratory bioassays were used for screening allopathic activity, because they allow detection of individual allopathic effects. Whereas under field condition, it is difficult to distinguish between allelopathic effects and competition. Also the plant box method which was developed by Fujii (1992) requires almost two months to obtain the same results, but it is acceptable.

Table 2. Allelopathic potential of rice varieties tested in greenhouse and laboratory conditions

Varieties	Greenhouse "a"			Lab. "b"		
	Germination I	Root II	Shoot II	Germination I	Root II	Shoot II
Sakha 102	64.26	14.82	7.60	63.20	23.00	8.22
Sakha 101	71.00	13.37	8.18	49.60	19.95	4.55
Kim Rad F 87	61.20	33.22	18.75	39.56	46.47	24.90
Rikuto Norin 22	40.57	53.24	20.70	26.45	51.55	34.85
Shimokita	64.96	39.44	17.98	30.03	34.85	19.27
Dular	48.53	44.18	17.45	24.83	52.42	26.42
Check(Giza 176)	75.00	0.00	0.00	78.00	0.00	0.00
LSD at 5%	10.91	11.97	10.20	10.20	1.84	2.72
LSD at 1%	18.10	19.85	16.92	16.92	3.05	4.51

I = germination percentage (%) of *E. crus-galli*
II = reduction of *E. crus-galli* root growth (%)
III = reduction of *E. crus-galli* shoot growth (%)
"a" = determined 40 days after sowing
"b" = determined 10 days after incubation

High differences in allelopathic potential were observed among the seven tested rice varieties, the range was between 40.57 and 71.00% in the greenhouse but in the laboratory conditions it ranged between 24.83 and 63.20% as revealed by the germination percentage of barnyardgrass as shown in Table (2). Also the range was between 13.37 and 53.24% in the greenhouse and 19.95 and 53.42% in the lab. Finally, the range was between 7.6 and 20.7% in the greenhouse and 4.55 and 34.85% in the lab. for the reduction of barnyardgrass shoot growth. These results showed that allelopathic varieties strongly inhibit root elongation of barnyadgrass but weakly affect the shoot. These results are similar to those of Navarez and Olofsdotter (1996).They found that some allelopathic cultivars strongly inhibit root elongation of barnyardgrass *{Echinochloa crus-galli* (L.)} but weakly affect the shoot. These results referred to high genetic effect which controlled these characters and the environmental effect is not significant. Also Kim and Shin (1998) found that the range was between 21 and 81% for reduction of barnyadgrass root growth %.

Table 3. Mean squares for some characters of *Echinochloa crus-galli* under two conditions

S.O.V.	d.f	Greenhouse			Laboratory		
		Germination %	Root length	Shoot length	Germination %	Root length	Shoot length
Reps.	2	7.72	24.4	1.03	12.15	1.63	0.89
Genotypes	5	183.80**	306.5**	71.52**	242.30**	246.70**	249.10**
Error	10	23.18	27.9	5.16	20.27	0.66	1.47
H2.b.s. %		87.4	90.89	92.78	91.6	99.7	99.4
C.V. %		39.6	84.40	34.10	52.0	1.7	7.5

Analysis of variance in the two experiments showed highly significant differences in allelopathic potential against *E. crus-galli* of rice varieties, Table (3). To emphasize these results, heritability in broad sense (h^2) was estimated and found to be 87.4, 90.89 and 92.78% in the greenhouse and 91.6, 99.7 and 99.4% in the lab. for germination percentage, reduction of barnyardgrass root and shoot %, respectively.

. These results are in agreement with those of Courtois and Olofsdotter (1998). They found that the hertitability was 0.85 for reduction in barnyardgrass root growth. Also, these results were corresponded to field performance and indicated that these traits are highly under genetic control. The results also suggest that the environmental effect is not significant.

Jensen *et al.* (2001) reported that the variation in root mass is not the reason for the variation in allelopathic activity. These results were confirmed with evaluation under field conditions, relay seeding technique and plant box method. These results agree with those of Lee (1999) which reported that phenol amount of rice straw showed higher content of P-coumaric acid in high potential varieties than non allelopathic varieties.

2. Isozymes Analysis :

The biochemical methods of investigation, especially isozyme studies, have provided valuable tools for rice breeders. Isozymes can serve as unique molecular genetic markers for biochemical characterization of genotypes (Tanksley and Orton, 1983). Moreover, isozymes permit early fast assessment of the nature of a variety (Glaszmann, 1987). In the present study , an attempt was made to assay the variation of number and activity of two isozyme patterns, esterase and peroxidase, in the leaves crude extract of three week old seedlings. Six parents as well as the check cultivar Giza 176 were chosen for isozyme studies. Electrophoresis analysis for both peroxidase and esterase isozymes of the six parents were as follows : two varieties (Sakha 101 and Sakha 102) with poor allelopathic activity, two varieties (Kim Rad F 87 and Shimokita) with intermediate allelopathic activity, and two varieties (Rikuto Norin 22 and Dular) with strong allelopathic activity. Variations in number and activity of bands are shown in Figure (1) and Table (4) for the peroxidase and Figure (2) and Table (4) for the esterase.

In the peroxidase zymograms, results showed that sub-patterns were observed among the different tested genotypes. These sub-patterns have specific bands which were presunred to be associated with allelopathic activity in the present study. Nine bands (distributed in two groups, slow and fast mobility) were detected in the zymograms for Sakha 101, seven bands for the check (Giza 176), Sakha 102 and Dular, six bands for Rikuto Norin 22 and Shimokita and five bands for Kim Rad F 87

(Figure 1 and Table 4). In the same zymogram shown in Fig. (1), two clear bands (No. 1 and No. 2) were very strong in their intensity in the two allelopathic rice cultivars, Rikuto Norin 22 and Dular, while they were strong and intermediate in their intensity in cultivars, Kim Rad F 87 and Shimokita, respectively as moderate to allelopathic. Band No. 1 was absent in the non allelopathic parents, Sakha 102 and Sakha 101 as well as, band No. 2 was absent in the check rice cultivar Giza 176.

These results suggested that the complementary bands No. 1 and No. 2 and the high expression of their genes, may be related to allelopathic activity. Also, these results were confirmed by the lab. experiments which reflected the differences in the genotype of the cultivars. In another study, isozyme analysis of peroxidase, indicated a simple characteristic sub-pattern (one to two specific bands) among different genotypes, related to salt tolerance in rice (Draz *et al.*, 1993).

In respect of the esterase zymograms, a complex of sub-patterns was observed among the different tested genotypes. These sub-patterns have bands associated with allelopathic activity (Table 5 and Figure 2). Description of esterase isozyme patterns of the six parental cultivars and the check cultivar, whereas, the cultivars Giza 176, Sakha 101 and Shimokita exhibited six bands. The cultivars Sakha 102, Kim Rad F 87 and Rikuto Norin 22 exhibited five bands, while Dular cultivar exhibited seven bands. The strong allelopathic activity parents Rikuo Norin 22 and Dular represented weak intensity bands (No. 3, 5 and 6). In contrast, strong intensity to the same bands in poor allelopathic activity parents Sakha 102, Sakha 101 and the check cultivar Giza 176. These results showed that two or three esterase bands may be associated with allelopathic activity in these parents, this means low expression of their genes. This relation may be considered a useful biochemical genetic markers for allelopathic activity to weeds control. Changes in both esterase and peroxidase isozymes patterns seem to be a reflection of some genetic variations which had occurred in new genotypes. Mclaren (1996) mentioned that quantitative trait loci (QTL's) were identified by matching molecular data and phenotypic data and, the development of software allows an easy and fast manipulation of the data. Another study by El-Banna (1998), showed that results of esterase and peroxidase zymogram patterms in some parental cultivars and their AC-derived lines, as well as their F_1 AC-derived lines revealed different trends for either numbers of isozyme bands or their activities.

Table 4. Description of peroxidase patterns of the six parental cultivars and the check cultivar

Cultivars / No. of bands	Giza 176 (check)	Sakha 102	Sakha 101	Kim Rad F87	Rikuto Norin 22	Shimokita	Dular
1	+++	-	-	++++	+++++	+++	+++++
2	-	++++	+++	++++	+++++	+++	+++++
3	++	+++	++	-	-	-	-
4	++	+	+	-	+	++	-
5	++	++	++	+	-	+	+
6	-	-	+	-	+	-	+
7	++	++	++	-	-	-	++
8	+	+	+	-	-	-	-
9	+	+	+	+	+	+	+
10	-	-	+	+	+	+	+
11	-	-	-	-	-	-	-
Total	7	7	9	5	6	6	7

+++++ Very strong ++++ Strong +++ Intermediate

++ Weak + Very weak - Absent

Table 5. Description of esterase isozyme patterns of the six parental cultivars and the check cultivar

Cultivars / No. of bands	Giza 176 (check)	Sakha 102	Sakha 101	Kim Rad F87	Rikuto Norin 22	Shimokita	Dular
1	-	-	-	-	-	-	-
2	-	-	-	-	-	-	++
3	+++	+++	+++	++	+++	+++	++
4	+++	+++	+++	+	++	++	+
5	+++++	+++++	+++++	+++	+++++	+++++	+++
6	+++	+++	++	+	++	++	+
7	-	-	-	-	-	-	-
8	++	-	-	-	+	+	++
9	+++	+++	+++	++	+++	+++	++
Total	6	5	6	5	5	6	7

+++++ Very strong ++++ Strong +++ Intermediate

++ Weak + Very weak - Absent

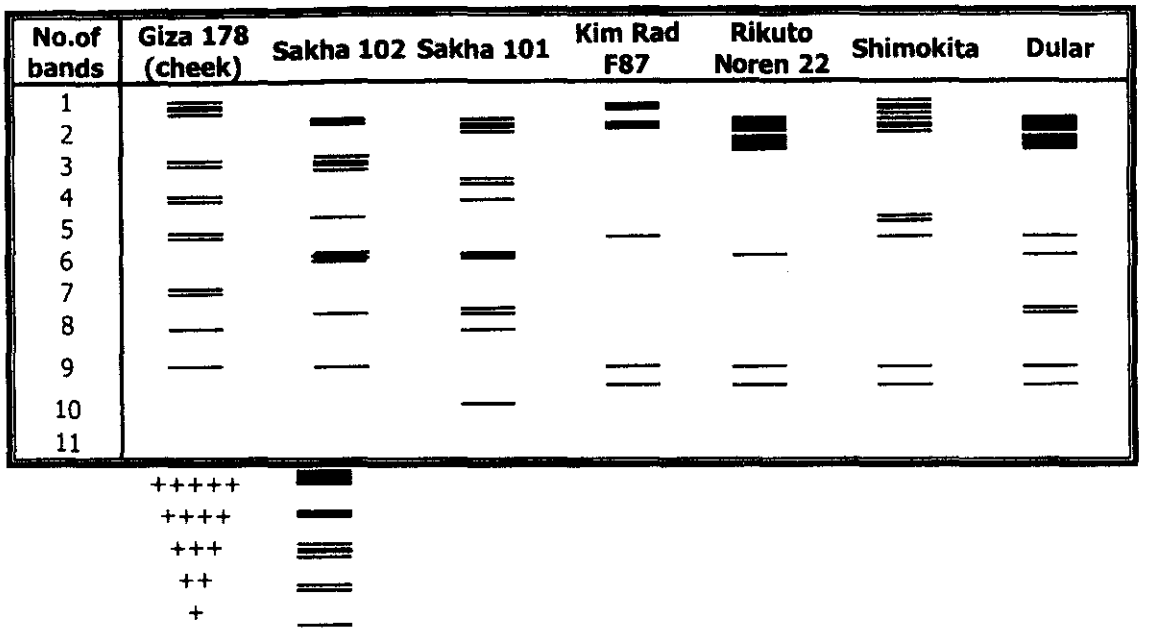

Figure 1. Lsozyme zymogram (a) and diagram (b) of peroxidase for six parents and check cultivar

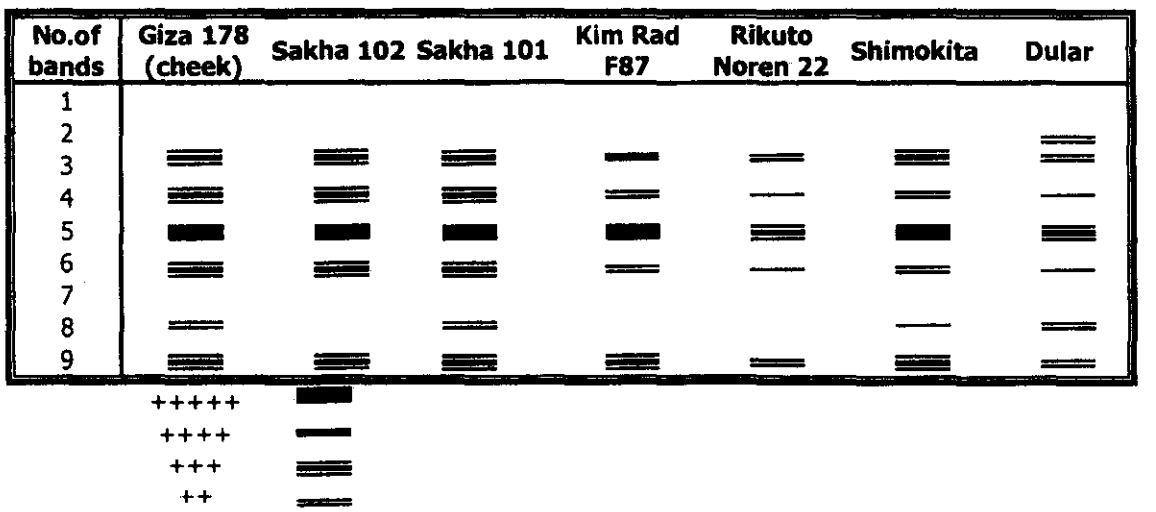

Figure (2). Lsozyme zymogram (a) and diagram (b) of esterase for six parents and check cultivar

From the above mentioned results, it could be concluded that esterase and peroxidase isozymes analysis may be used as a biochemical genetic tool to identify the allelopathic effect in rice varieties with specific characteristics at plumules stage. However, further studies on other isozyme systems and molecular markers such as restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA (RAPD) are needed to substantiate this conclusion.

3. Molecular analysis :

An attempt was conducted to evaluate the molecular genetic effects of six varieties (Sakha 102, Sakha 101, Kim rad F87, Rikuo Norin 22, Shimokita and Dular), as well as the check variety (Giza 176) on the DNA nucleotide sequences using six different 10-mer oligonucleotide RAPD primers. This part was conducted at Biotech. Lab. DRR, Hyderabad, India, using random amplified polymorphic DNA (RAPD) technique to detect the molecular variations among six cultivars compared to the check variety for allelopathic effect. Out of the six primers, two primers, i.e. No. 1 (OPA 19) and No. 5 (OPI 18) showed monomorphic and polymorphic amplified bands. The first primer gave 92 monomorphic and polymorphic bands, but the second amplified 89 monomorphic and polymorphic bands. The percentages between monomorphic and polymorphic bands were 36.8% to (OPA 19) and 35.3% to (OPJ 18).

Figure (4 a,b) depicts photographs of the polymorphic amplified DNA bands which were obtained as a result of using the two primers.

The band sizes were detected against the DNA molecular-weight marker III (□ DNA Eco RI + Hind III). Figure (3 a,b) and Table (5) showed highly amplified DNA in the different genotypes as a result of primer No. 1 (OPA 19). The check cultivar (Giza 176) exhibited 14 bands, Sakha 102, Sakha 101 and Kim Rad F 87 cultivars exhibited 13 bands, Rikuto Norin 22 cultivar exhibited 14 bands, Shimokita exhibited ten bands and Dular cultivar exhibited 15 bands. The bands sizes ranged from 0.250 and 22.119 kbp amplified bands. While, by using primer No. 5 (OPJ 18), the check cultivar (Giza 176) and Sakha 101 exhibited 14 bands, Sakha 102 exhibited 11 bands, Kim Rad F 87 and Rikuto Norin 22 exhibited 13 bands, finally, Shimokita and Dular exhibited 12 bands. The bands sizes ranged from 0.270 to 22.119 kbp amplified bands.

Results showed that the amplified fragments of 4.044 (OPA 19) and 1.780 kbp (OPJ 18) were found in the two parents which had highly allelopathic activity, Rikuto Norin 22 (as Japonica) and Dular (as Indica type) but were absent in the other varieties which showed intermediate or poor allelopathic activity. These two amplified fragments may carry gene(s) which are responsible for producing active protein(s) which are tightly linked to the high concentration of P-coumaric acid which increase the potential of allelopathic activity. Also, both fragments could be used as marker-aided selection (MAS) for allelopathic gene(s) against *E. crus-galli*. On the other side,

the OPA 19 amplified fragment of 9.057 kbp and OPJ 18 amplified fragments of 0.583 and 2.090 kbp were found in the parents which have intermediate or poor allelopathic activity but were absent in the two parents which have high allelopathic activity, Rikuo Norin 22 and Dular. These results indicate that these fragments may carry gene(s) which express protein(s) which are tightly linked to the high concentration of ascorbic acid which decrease the potential of allelopathic activity.

Moreover, similarity coefficient values of the primer (OPA 19) ranged from 0.34 to 1.0 with an average of 0.78 and similarity coefficient values of primer (OPJ 18) ranged from 0.61 to 0.96 with an average of 0.81. However, when comparing between the strong allelopathic cultivars Rikuto Norin 22 and Dular with the other cultivars, results showed that the highest value of similarity coefficient was between Rikuto Norin 22 (No. 5) and Dular (No. 7) (0.76) by using primer OPA 19, comparing with Dular (No. 7) and the other cultivars (Table 5). Also, the highest value of similarity coefficient was between Rikuto Norin 22 and Dular (0.880) by using primer (OPJ 18), comparing with the other cultivars (Table 6). In contrast, the lowest values of similarity coefficients were among Giza 176 (No. 1) and Dular (No. 7) (0.34) by using primer (OPA 19) and between Sakha 102 (No. 2) and Dular (No. 7) (0.61) by using primer (OJP 18). The dendogram of genetic similarity among the six parental cultivars and the check cultivar across the two primers OPA 19 and OPJ 18 were similar to that obtained from the previous results (Fig 4 a,b). Whereas the primer OPA 19 exhibited two groups which joined in a big cluster, the first group included two subgroup, the first subgroup showed two branches, the first branch was Giza 176 and the second branch was Kim Rad F 87 but the second subgroup was placed on the origin point and joined with the first subgroup in one cluster. The second group included one subgroup which exhibited three branches, the first (Dular) was joined with the second (Rikuto Norin 22) followed by the third (Shimokita). And primer OPJ 18, exhibited two groups which joined in a big cluster, the first one consisted of two subgroups, the first subgroup included two branches Sakha 102 and Shimokita, but the second subgroup included three branches, whereas Sakha 101 was closed with Kim Rad F 87 followed by Giza 176. The second group included two branches, the first branch was Rikuto Norin 22 and the second was Dular. These results indicated that both primers, OPA 19 and OPJ 18 could be used in screening for allelopathy in rice.

Raghunathachari *et al.* (2000) reported that the RAPD analysis offered a rapid and reliable method for the estimation of variability between different accessions which could be utilized by the breeders for further improvement of the scented rice genotypes. Courtois and Olofsdotter (1998) mentioned that polymerase chain reaction (PCR) based markers are easier, cheaper and faster to manipulate. It takes only 2 months to establish a map for many germplasms in screening for allelopathic activity.

Similar results were found in esterase and peroxidase zymograms. Whereas Rikuto Norin 22 and Dular varieties exhibited two very strong bands No. 1 and No. 2 in peroxidase. While the same varieties exhibited weak band No. 3 and very weak band No. 6. These results suggested that the very strong bands No. 1 and No. 2 in peroxidase may increase the allelopathic effect in these parents, while weak and very weak bands No. 3 and No. 6 in esterase may decrease the allelopathic effect.

Table 7. The similarity matrix of primer OPA 10 to six rice cultivars and check cultivar

Cultivar No.	1	2	3	4	5	6	7
1	-	0.88	0.88	0.96	0.79	0.67	0.34
2			1.00	0.92	0.88	0.78	0.71
3				0.92	0.88	0.78	0.71
4					0.81	0.69	0.64
5						0.72	0.76
6							0.72
7							-

Table 8. The similarity matrix of primer OPJ 18 to six rice cultivars and check cultivar

Cultivar No.	1	2	3	4	5	6	7
1	-	0.88	0.93	0.96	0.81	0.92	0.76
2			0.80	0.83	0.66	0.87	0.61
3				0.96	0.81	0.77	0.69
4					0.76	0.88	0.72
5						0.80	0.88
6							0.75
7							-

REFERENCES

1. Badawi., A.T. 1999. Policy of rice varieties in Egypt. Proceed. First Pl. Breed. Conf. December 4, 1999. Giza, Egypt, J. Plant. Breed. 3: 29-51.
2. Chu, Y.E. 1967. Variations in peroxidase isozymes of *Oryza perennis* and *O. sativa*. Japn. J. gent. 42: 233-240.
3. Courtois and Olofsdotter, 1998. Incorporating the allelopathy trait in upland rice breeding programs. Proceedings of the Workshop on Allelopathy in Rice. Chapter 6.
4. Davis. R.J. 1964. Disc electrophoresis 2-method of application to human serum proteins. Ann. N.Y. Acad. Sci. 121: 404-427.
5. Dellaporta, S.L., J. Wood and J.B. Hicks, 1983. A plant DNA mini preparation version II. Plant Mol. Bio. Reporter. 1: 91-21.
6. Draz, A.E., Abd El-Hamid A. Ali and S. Dora, 1993. Genetic evaluation of anther culture derived lines of F1 hybrids of rice. J. Agric. Res. Tanta Univ. 19(4), pp: 876-886.

7. El-Banna, A.N.S. 1998. The use of gametoclonal and somaclonal variation for releasing salt tolerant rice lines. M.Sc. Thesis, Dept. Gent., Fac. Agric. Kafr El-Sheikh, Tanta Univ., Egypt.
8. Fujii, Y. 1992. The potential buiological control of paddy weeds with allelopathy : allelopathic effect of some rice varieties. In: proceedings of the International Symposium on Biological Control and Integrated Management of Paddy and Aquatic Weeds in Asia. Tsukuba (Ibarka, Japan). National Agricultural Research Center. P: 305-320.
9. Glaszmann, J.E. 1987. Isozymes and classification of Asian rice varieties. Theor. App. Genet. 74: 21-30.
10. Jensen, L.B., B. Courtois, L. Shen, Z. Li, M. Olofsdotter and R.P. Mauleon, 2001. Locating genes controlling allelopathic effects against barnyardgrass in upland rice. Agric. J. Vol. 93. P: 21-26.
11. Kim and Shin. 1998. Rice allelopathy research in Korea. Proceedings of the Workshop on Allelopathy in Rice. International Rice Research Institute. P: 39-43.
12. Lee, S.B. 1999. Studies on the allelopathy of rice varieties to *Echinochloa crus-galli* Beauv. Var. Oryzicola Ohwi. Ph.D. Program in Agronomy, Graduate School of Kon Kuk University, Republic of Korea.
13. Mclaren, C.G. 1996. Basic statistical analysis system. BSTAT, IRRI, Philippines.
14. Navarez and Olofsdotter, 1996. Relay seeding technique for screening allelopathic rice (*Oryza sativa*). In: proceedings of the 2nd International Weed Control Congress, Copenhagen, Denmark, p: 1285-1290.
15. Raghunathachari, P., V.K. Khanna, U.S. Singh and N.K. Singh, 2000. RAPD analysis of genetic variability in India scented rice germplasm (*Oryza sativa* L.). Current Science, V. 79, No. 7, p: 994-998.
16. Rimando, A.M., M. Olofsdotter, F.E. Dayman and S.O. Ouke, 2001. Searching for rice allelochemicals: An example of bioassay. Guided Isolation. J. Vol. 93, p: 16-20.
17. Second, G. 1982. Origin of the genic diversity of cultivated rice (*Oryza spp.*). Study of the polymorphism scored at 40 isozyme loci. Jap. J. Genetic. 57: 25-57.
18. Second, G. 1985. Relations evolutives chezle genre oryza et processus de domestication de riz. Collection Etudes et theses. Paris (France): ORSTOM. 189 p.
19. Sokal, R.R. and F.J. Rohlf, 1995. Biometry. Third edit. Freeman and Company, New York, 695-704.
20. Tanksley, S.O. and T.J. Orton, 1983. Isozymes in plant genetics and breeding. Part B. Elsevier, Amsterdam.

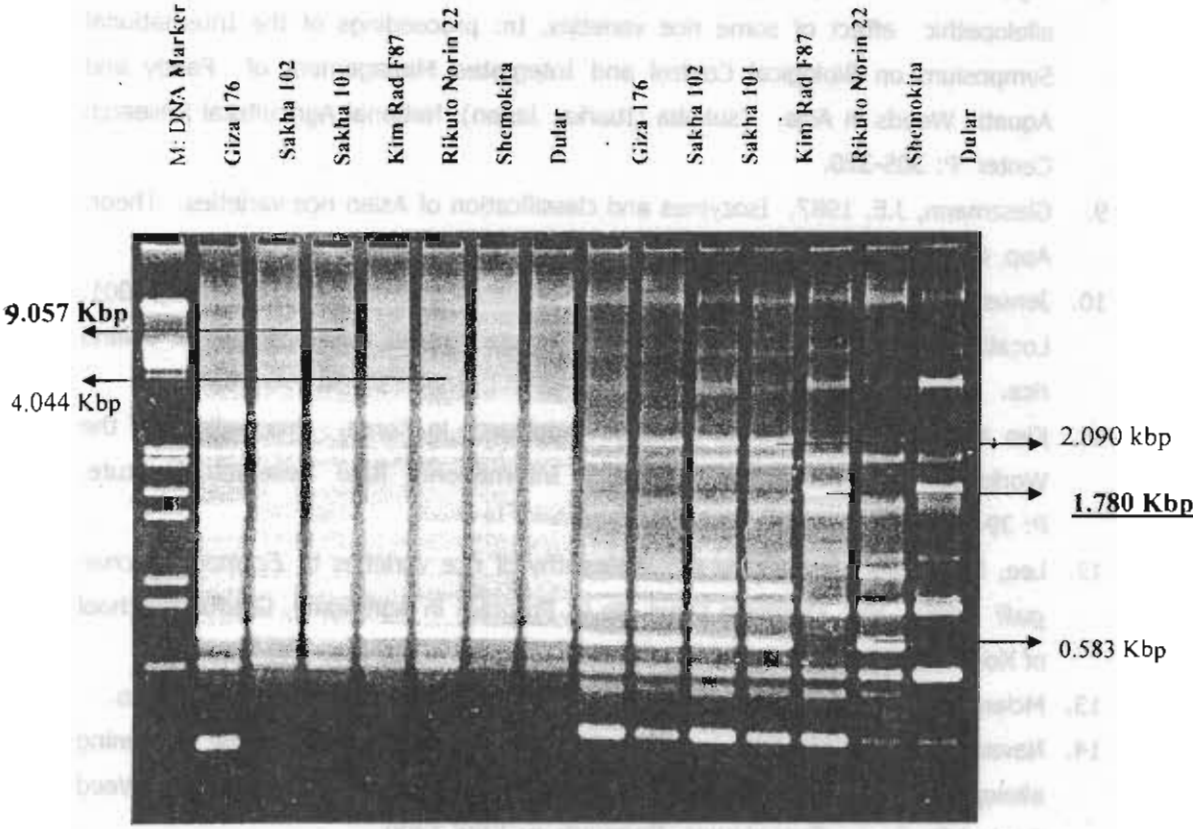

Fig. (3 A and B) Gel Photograph showing RAPD analysis of the six parents and the check variety with two primers
(A) Primer OPA 19 and (B) OPJ 18. M. Molecular marker of (Eco. RI+ Hind III double digest, λ DNA)

تحديد نشاط الأليلوباثى فى الأرز باستخدام المعلمات الجزيئية

سعيد درة [١] ، سالم عبد الكريم عبد الله [١] ، عبد السلام دراز [٢]، محمود أبو يوسف [٢]

١ *قسم الوراثة – كلية الزراعة بكفر الشيخ – جامعة طنطا*

٢ *مركز البحوث و التدريب فى الأرز – معهد بحوث المحاصيل الحقلية – مركز البحوث الزراعية – الجيزة – مصر*

أجريت هذه الدراسة بمركز البحوث والتدريب فى الأرز – سخا – كفر الشيخ ، قسم الوراثة بكلية الزراعة بكفر الشيخ – جامعة طنطا – مصر ومعهد DRR بحيدر أباد بالهند خلال مواسم ١٩٩٩ – ٢٠٠٠ لدراسة الإستفادة من المعلمات الجزيئية كأداة لتحديد نشاط الأليلوباثى فى الأرز .

استخدمت ستة أصناف أرز هى سخا ١٠١ ، سخا ١٠٢ ، Kim Rad F 87, Shimokita Dular, Rikuto Norin 22 بالإضافة إلى صنف المقارنة جيزة ١٧٦ لاختبارنشاط الأليلوباثى ضد حشيشة الدنيبة *E. crus-galli* فى المعمل والصوبة لتحديد نسبة مقاومة الحشائش فى عام ١٩٩٩ .

استخدم تحليل المعلمات الجزيئية والأيزوزيم كعوامل مساعدة فى الإنتخاب لجينات الأليلوباثى ضد حشيشة الدنيبة *E. crus-galli* . وأوضحت النتائج أن نسبة انخفاض نمو جذور حشيشة الدنيبة كانت ١٣,٤ – ٥٣,٢ % فى الصوبة و ٩١,٩٥ – ٥٢,٤٢ % فى المعمل ، بينما نسبة الإنخفاض لنمو جذور حشيشة الدنيبة كانت ٧,٦ – ٢٠,٧ % فى الصوبة و ٤,٥٥ – ٣٤,٨٥ % فى المعمل .

وأوضحت النتائج أيضا أن الأصناف القوية فى نشاط الأليلوباثى ثبطت إستطالة جذور الدنيبة مع تأثير ضعيف على نمو البادرة . تكامل الحزمة رقم ١ ، ٢ والتعبير القوى فى نشاط إنزيم البيروكسيديز ربما يزيد فى هذه الأصناف ، بينما الحزمة الضعيفة رقم ٣ والضعيفة جداً فى الحزمة رقم ٦ فى إنزيم الإستيريز ربما يكون مرتبط بنشاط الأليلوباثى فى هذه الأصناف ، وتعنى التعبير الضعيف ضد جيناتها .

المعلمات الجزيئية المتخصصة لنشاط الأليلوباثى مثل 4.044 kbp من البريمر (op A 19) 1.780 kbp, من البريمر (op j 18) وهذه الشظايا الكروموسومية ربما تحمل جين يكون مسئول عن تخليق بروتين منشط والذى يرتبط بإحكام بزيادة قوة نشاط الأليلوباثى بينما الشظايا 9.057 kbp من البريمر (op A 19) 2.090 kbp من البريمر (op j 18) كانت غير موجودة فى الأصناف قوية الأليلوباثى .

هذه النتائج تشير إلى أن هذه الشظايا ربما تحمل جين والتى تعتبر البروتين يكون مرتبط بإحكام بانخفاض قوة نشاط الأليلوباثى . ومع ذلك يمكن إستخدامها كمعلمات مساعدة للإنتخاب لجينات الأليلوباثى ضد *E. crus-galli* .