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RAPD-PCR BASED TECHNIQUE FOR DETECTING THE CONFERRED STRIPE RUST RESISTANT GENE YR15 TO THE EGYPTIAN COMMERCIAL WHEAT CULTIVARS

BY

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ABSTRACT

F_1 wheat crosses having Yr15 gene from the monogenic line (*Triticum dicoccoides*) were resistant to stripe rust under greenhouse and field conditions at both seedling and adult stages. While, F_2 populations segregated at ratio of 3 resistant: 1 susceptible which suggest that resistance to stripe rust is controlled by a single dominant gene. The successful confer of Yr15 gene was emphasized by using the random amplified DNA polymorphism technique and the specific primer OPB-13. Among F_2 populations of Yr15xSakha-69 (70 plants) and Yr15xGiza-163 (40 plants), the Yr15 gene was detected in 52 and 29 plants, respectively with the ratio 3:1. Yr15 gene was not detected in the tested wheat parents (Sakha-69 and Giza-163) nor in their susceptible F_2 segregates.

Key words: stripe rust, Yr15 gene, RAPD-PCR and DNA polymorphism.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the most important dynamic sector in world grain production. Under Egyptian conditions, wheat cultivars are attacked with several rust diseases like stem, leaf and stripe caused by *Puccinia graminis tritici*, *P. recondita* and *P. striiformis*, respectively. Recently, stripe rust is one of the major problems affecting wheat production in Egypt. In this respect, the first stripe rust epidemic was recorded in 1967 and then three major epidemics were recorded in 1995, 1997 and 1999 seasons where the stripe rust destroyed most of the wheat cultivars in Northern and Southern Delta areas (Abdel-Hak *et al.* 1972, El-Daoudi *et al.* 1996 and Abu El-Naga *et al.* 1999).

Production of the resistant cultivars is an effective approach to eliminate the use of fungicides and minimize crop losses due to this disease. However, most of the described major genes for resistance to stripe rust in cultivated wheats have become ineffective to one or more of the known pathogenic races when acting singly (Stubbs 1985). *T. dicoccoides* G-25 was shown to be highly resistant to more than 20 stripe rust races from six countries (Gerechter and Stubbs, 1970).

Further studies showed that this resistance was conferred by one dominant gene, designated as Yr15 (Gerechter and Adriana 1974). Adriana *et al.*, (1984) reported that the wheat lines conferred Yr15 had good baking quality, high yield, yellow rust resistance, high protein content, good agronomic characters, and resistant to stem and leaf rusts. Gerechter *et al.*, (1989) investigated 3 crosses between *Triticum spelta var. album* (Yr5) and *Triticum dicoccoids* sel G.25 (Yr15) and found that the segregation ratio of the F_2 seedlings (inoculated with *Puccinia striiformis*) had a good fit for a 15 resistant: 1 susceptible ratio. Luthra *et al.*, (1989) reported that *Triticum. spelta var. album* has three dominant genes and one recessive gene for resistance to Indian races. Based on cytogenetic analysis, McIntosh *et al.* (1996) revealed that the Yr15 gene resides in the short arm of chromosome 1B of wheat. Sun *et al.*, (1997) recorded that the F_2 population (123 plants) derived from crosses between stripe rust susceptible *Triticum durum* wheat cultivar D447 and resistance line (Yr15) were segregated at ratio of 95 resistant: 28 susceptible. They concluded that this resistance was conferred by one dominant gene Yr15. The single dominant Yr genes conferring stripe rust resistance was reported by several investigators (Peng *et al.*, 1999, Robert *et al.*, 1999, McIntosh and Lagudah, 2000 and Johnson *et al.* 2000).

The molecular markers (primers) showed polymorphism with resistant genes (using the RAPD, AFLP and some other new techniques) provided great help in breeding for plant disease resistance. Among hundreds of the RAPD primers tested fewer numbers could link to the stripe rust resistance genes (Sun *et al.*, 1997). In a similar way, the RAPD primers were used also for detecting markers linked with the yellow rust resistance gene *Yr17* (Robert *et al.*, 1999). Smith, (2002) developed a sequence-tagged-site (STS) marker for a yellow rust resistance gene designated YrMoro in the wheat cultivar Moro. The single YrMoro gene was mapped to the group 1 chromosomes. Wang LanFen *et al.*, (2002) mentioned that a microsatellite marker, *Xpsp3000*, located on the end of chromosome 1BS was linked with the yellow rust resistant gene Yr10.

This work aimed to investigate the effect of Yr15 resistant gene when conferred to the commercial wheat entries on severity of stripe rust (*Puccinia striiformis*) at seedling and adult stages. Detection of the Yr15 gene in F_2 wheat population using linked molecular markers was also investigated.

MATERIALS & METHODS

Breeding against wheat stripe rust:

During 1999/2000 season, the wheat Yr15 monogenic line *Triticum dicoccoides* resistant to stripe rust infection at seedling and adult stages (Abou-Aly, 2004) was crossed "as male (P_1) parent" with each of the wheat cultivars Sakha-8, Sakha-69, Giza-163 and Sids-7 as female susceptible (P_2) parents. Then, kernels representing the F_1 hybrids Yr15xSakha-8, Yr15xSakha-69, Yr15xGiza-163 and Yr15xSids-7 were obtained. In 2000/2001 growing season, some plants of each of the 4 F_1 hybrids were self-pollinated to produce the F_2 kernels.

Evaluation of resistance to stripe rust infection:

In the season 2001/02, parents and the resultant hybrids were evaluated against artificial stripe rust infection (*P. striiformis*) at the seedling stage (in a conditioned greenhouse) and booting stage at Sakha Agric. Res. Sta. In this respect, individual pustules (*Puccinia striiformis*) were taken from the collected samples and propagated on the susceptible wheat cv. Giza-160. Seedlings of the P1, P2, F1, F2 (7-10 days old grown in 25 ϕ clay pots) were dusted with a mixture containing urediospores of each rust sample plus talcum powder at the rate of 1: 20 (w: w) as suggested by Tervet and Cassel (1951). Pots were kept for 48 hr at 9°C followed by incubation for 18-20 days at 15-18°C as adopted by Stubbs, (1988), then the infection types of stripe rust were estimated using the scale of McNeal *et al.* (1971) as shown in Table (1).

Table (1): The infection type scale (Expressions) of wheat stripe rust as described by McNeal *et al.* (1971).

Infection type	Expression	Descriptions
0	0 = Immune	No visible infection
1	VR = Very resistant	Necrotic or chlorotic flecks, no sporulation
2	R = Resistant	Necrotic and/or chlorotic stripes, no sporulation
3	MR = Moderately resistant	Necrotic and/or chlorotic stripes, trace sporulation
4	LM = Low moderate	Necrotic and/or chlorotic stripes, light sporulation
5	M = Moderate	Necrotic and/or chlorotic stripes, intermediate sporulation
6	HM = High moderate	Necrotic and/or chlorotic stripes moderate sporulation
7	MS = Moderately susceptible	Necrotic and/or chlorotic stripes, abundant sporulation
8	S = Susceptible	Chlorosis behind sporulating area abundant
9	VS = Very susceptible	No chlorosis or necrosis abundant sporulation

At booting stage, a complete randomized block design with three replicates was conducted to evaluate plant population of each parent and the resultant hybrids against natural stripe rust infection at the adult stage. The seeds of any population were sown in rows 3-m long and 20-cm apart at the rate of 20 seeds/row. Each replicate contained one row for each of P_1, P_2 and F_1 and 6 rows for F_2. A mixture of highly susceptible wheat cultivars (Giza-160, Giza-163 and little clube) was sown around the experiment to disseminate the urediospores of the pathogen *Puccinia striiformis*. Natural infection was achieved at booting stage from the spreader plants around the experiment as mentioned before according to the method of Tervet and Cassel (1951). Disease severity and infection type (IT) of stripe rust were recorded 20 days after inoculation on each individual plant according to the disease severity scale of Peterson *et al.* (1948). According to this scale, plants with disease severity of 0, 5R, 10R, 5MR and 10 MR were described as resistant phenotypes, while, those recording 5MS, 10 MS, 10S, 20S, 40S and

80S were described as susceptible phenotypes, then ratio of resistant to susceptible plants were determined.

Statistical analysis were computed for all populations by determining the expected ratio and confirmed by X^2 analysis according to Steel and Torrie, (1960).

Detection of conferred Yr15 gene in wheat hybrids using the random amplified polymorphic DNA (RAPD) technique:

For emphasizing successful conferring of Yr15 through the hybridization process, the green leaves of P_1, P_2, F_1 and F_2 plants (Sakha-69 and Giza-163) were collected for RAPD-PCR analysis as following,

DNA extraction:

Samples of green leaves of P_1, P_2, F_1 and F_2 of tested wheat plants (500 mg) were frozen using liquid nitrogen and ground into a fine powder with a mortar and pestle. The powder was transferred into a 1.5-ml microfuge tube and dispersed in 1 ml of extraction buffer (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, and 100mM EDTA), mixed thoroughly and 0.06 ml of 20% sodium dodecyl sulphate (final concentration 1%) was added. The mixture was gently shaken for 1 h at about 20C, mixed with 0.15 ml of 5 M NaCl (final concentration 0.8M) and 0.13 ml of CTAB/NaCl solution (10% CTAB in 0.7 M NaCl, final concentration 1%), and kept at 65 C for 20 min. The mixture was divided into two 1.5-ml microfuge tubes and extracted with chloroform/isoamyl alcohol (24:1). The top aqueous phase was transferred to a clean tube, and about 360 µl of cold isopropanol was added. After 20 min of incubation at 4°C, the solution was centrifuged for 10 min at 10,000 rpm at 20°C to precipitate the nucleic acid. The pellet was rinsed twice with cold 70% ethanol, dried in vacuum, and dissolved in 0.5 ml of TE buffer (10mM Tris-HCl and 1 mM EDTA, pH 8.0). One microliter of ribonuclease from stock 10mg/ml was added (final concentration 20 µg/ml) and kept at 4C overnight to completely digest the RNA. The DNA was reprecipitated, rinsed with cold 70% ethanol, dried, and dissolved in 40 µl of TE (stock DNA), then DNA was quantified by the minigel method (Sambrook *et al.*, 1989). After quantification, the stock DNA was kept at –20°C for later use.

The stock DNA solution was diluted to about 0.1 ng/µl. The solution for amplification reaction consisting 0.2 mM of each dATP, dCTP, dGTP, and TTP (Sigma Chemical Co., St. Louis, MO); 2 mM $MgCl_2$; 0.3 units of Taq DNA polymerase (Promega, Madison, WI); 2µM specific primer; 0.2ng DNA template; 1.25 µl of 10x Taq polymerase buffer (Promega) and sterile water added to reach a final volume 13 µl. Sterile distilled water was used in place of DNA template as a control. To ensure that there was no contamination, the solution was overlaid with mineral oil. The primer OPB-13 (TTCCCCCGCT) which specifically link with Yr15 stripe rust resistance gene (Sun *et al.*, 1997) was obtained from Amersham Pharmacia Biotech UK Limited, England HP79NA. Amplification was carried out in DNA Thermo-cycler "Perkin-Elmer model 480" programmed for 10 min at 94°C for initial denaturation and 45 cycles that consisted of 1 min at 94°C, 2 min at 37°C, and 2.5 min at 72°C, followed by a final 10 min extension at 72°C. After amplification, 5µl of the solution for each sample was

electrophoresed in a 2% Agarose gel in 0.5X TBE buffer (0.089 M Tris-borate, 0.089 M boric acid, and 0.002 M EDTA). A 1-kb DNA ladder (0.15 μg) (Gibco BRL, Bethesda, MD) was used to estimate the size of each amplified DNA fragment. The gel was run for 90 min at 100 volts, stained with ethidium bromide (0.5ug/ml) for 30 min, and photographed under ultraviolet light (Chen *et al.* 1993).

RESULTS

Plant populations of the commercial wheat cultivars; Sakha-8, Sakha-69, Giza-163 and Sids-7 (susceptible to stripe rust), and *Triticum dicoccoides* resistant Yr15 monogenic line and their F_1 and F_2 crosses were evaluated for the stripe rust resistance.

1- At Seedling stage in greenhouse:

Data in Table (2) and Fig (1) reveal that stripe rust resistance was dominant as F_1 population of the four crosses exhibited low infection type ranging between 0; - 1 (resistant). Also, F_2 population of the four crosses exhibited a wide range of infection type (i.e. 0-9). F_2 seedlings of the crosses of Yr15 x Sakha-8, Yr15 x Sakha-69, Yr15 x Giza-163 and Yr15 x Sids-7 have been segregated to resistant and susceptible phenotypes at the ratio of 82:33, 84:36, 95:43 and 90:40, respectively, with expected ratio of 3:1 for all. This 3:1 ratio proves that a single dominant gene pair is controlling stripe rust resistance in wheat.

2- At Adult stage in the field:

Data in Table (3) show stripe rust severity among wheat plants of four parent cvs tested and their F_1 and F_2 crosses having Yr15 gene. The obtained results emphasized the high susceptibility of the tested commercial wheat cultivars as well as immunity (0 reaction) of the monogenic line Yr15 plants. All F_1 plants in Yr15xSakha-8, Yr15xSakha-69 and Yr15xGiza-163 were immune while stripe rust severity reaction of the F_1 plants in Yr15xSids-7 hybrid ranged between immune to highly resistant (5R). These results revealed that resistance was dominant over susceptibility in all F_1 progenies. However, plants of the F_2 populations of the four tested crosses having Yr15 exhibited a wide reaction of stripe rust severity ranging from 0–80S. The segregated phenotypes were as follows, 93R:19S, 83R:24S, 77R:20S and 81R:37S for the four crosses respectively, with expected ratio of 3:1. This ratio emphasize that a single dominant gene pair controls resistance to stripe rust in F_1 population.

Detecting stripe rust resistant gene Yr15 in wheat crosses using RAPD-PCR:

This experiment aimed to detect the stripe rust resistant gene Yr15 in F_2 populations. Specific RAPD primer OPB-13 to stripe rust resistant gene Yr15 was used to test DNA of 70 and 40 individual wheat plants (resistant and susceptible) which represent F_2 progenies of the crosses Yr15xSakha-69 and Yr15xGiza-163 as well as to compare with P_1, P_2 and F_1 progenies as shown in Table (4) and Figures (2, 3, 4 & 5)

Table (2): Evaluation the crosses of the four tested wheat cultivars having Yr15 against stripe rust infection at seedling stage.

Crosses & Parents	No. of plants	Infection types (ITs)											Phenotype		Expected ratio	X^2
		0	0;	1	2	3	4	5	6	7	8	9	R	S		
Yr15 x Sakha-8																
P1	50	-	-	50	-	-	-	-	-	-	-	-	50	-	-	-
P2	50	-	-	-	-	-	-	-	-	-	-	50	-	50	-	-
F1	50	-	50	-	-	-	-	-	-	-	-	-	50	-	-	-
F2	115	20	20	18	10	-	14	-	-	14	13	6	82	33	3:1	2.42
Yr15 x Sakha-69																
P1	50	-	-	50	-	-	-	-	-	-	-	-	50	-	-	-
P2	50	-	-	-	-	-	-	-	-	-	-	50	-	50	-	-
F1	50	-	50	-	-	-	-	-	-	-	-	-	50	-	-	-
F2	120	20	14	13	25	-	12	-	6	13	15	2	84	36	3:1	1.6
Yr15 x Giza-163																
P1	50	-	-	50	-	-	-	-	-	-	-	-	50	-	-	-
P2	50	-	-	-	-	-	-	-	-	-	-	50	-	50	-	-
F1	45	-	-	45	-	-	-	-	-	-	-	-	45	-	-	-
F2	138	16	21	23	9	26	-	-	-	19	18	6	95	43	3:1	2.78
Yr15 x Sids-7																
P1	50	-	-	50	-	-	-	-	-	-	-	-	50	-	-	-
P2	50	-	-	-	-	-	-	-	-	-	-	50	-	50	-	-
F1	49	-	-	49	-	-	-	-	-	-	-	-	49	-	-	-
F2	130	21	19	23	10	-	17	-	3	13	16	8	90	40	3:1	2.28

As for the specific primer OPB-13 (Fig. 2), the resulted DNA bands of tested wheat individuals clearly show that Yr15 (lane 4), F_1 of Yr15xSakha-69 (Lane 5) and two resistant individuals of F_2 of Yr15xSakha-69 (lanes 6&7) only linked with the primer OPB-13 through generating a specific clear band at (920 bp). Meanwhile, cv. Sakha-69 (Lane 1), and two susceptible individuals of F_2 of Yr15xSakha-69 (lanes 2&3) did not link with the primer OPB-13 where 920 bp bands were not appeared. Meanwhile, Fig. (3) shows the produced amplified DNA bands in F_2 of Yr15xSakha-69 hybrid which reacted with the primer OPB-13. In this respect, the analysis of this polymorphism revealed that only 52 out of 70 individuals of F_2 have linked with the primer OPB-13. Where, the rest 18 individuals did not link. This result revealed that the resistant: susceptible individuals are 52:18 with expected ratio 3:1 which, verified by X^2. This result confirmed the presence of Yr15 in the segregation's of the resulted cross and verified that a single dominant pair gene controls resistance.

On the other hand, the produced random amplified DNA polymorphism of tested wheat individuals show that Yr15 (lane 2), F_1 of Yr15xGiza-163 (Lane

3) and three resistant individuals of F_2 of Yr15xGiza-163 (lane5, 6&7) only linked with the primer OPB-13 and generated specific clear band of 700 bp. Meanwhile, cv. Giza-163 (Lane 1), and one susceptible individual of F_2 of Yr15xGiza-163 (lane 4) did not link with the primer OPB-13 where no specific bands were detected (Fig. 4). The reaction of F_2 individuals with the primer OPB-13 appeared in Fig. 5. In this respect, out of 40 F_2 individuals, specific DNA segments of 29 individuals of F_2 have linked with the primer OPB-13 at 700bp band. The rest 11 individuals did not link with the same primer. This result revealed that the resistant: susceptible individuals were 29:11 with expected ratio 3: 1 which, verified by X^2. This result confirmed the presence Yr15 in the F_2 segregations of resulted cross and verified that a single dominant pair gene controls resistance

Table (3): Evaluation the crosses of four tested wheat cultivars having Yr15 against stripe rust infection at adult stage.

Crosses &Parents	No. of plants	Stripe rust severity classes											henotype		Expected ratio	X2
		0	5R	10R	M	10MR	5MS	0M	10S	20S	40S	80S	R	S		
Yr15 x Sakha-8																
P1	60	60	-	-	-	-	-	-	-	-	-	-	60	-	-	-
P2	50	-	-	-	-	-	-	-	-	-	-	50	-	50	-	-
F1	60	60	-	-	-	-	-	-	-	-	-	-	60	-	-	-
F2	112	18	28	36	9	-	-	11	8				93	19	3:1	0.44
Yr15 x Sakha-69																
P1	60	60	-	-	-	-	-	-	-	-	-	-	60	-	-	-
P2	55	-	-	-	-	-	-	-	-	-	-	55	-	55	-	-
F1	50	50	-	-	-	-	-	-	-	-	-	-	50	-	-	-
F2	107	26	12	16	29	-	14	6	4				83	24	3:1	1.2
Yr15 x Giza-163																
P1	60	60											60	-		
P2	55											55		55		
F1	48	48											48	-		
F2	97	24	27	26	-	-	-	-	-	4	3	13	77	20	3:1	1.0
Yr15 x Sids-7																
P1	60	60											60	-		
P2	60											60	-	60		
F1	51	20	31										50	-		
F2	118	19	22	18	8	14	-	-	-	16	13	8	81	37	3:1	0.96

Table (4): RAPD markers linked with Yr15 loci in resistant wheat crosses segregations of F_2.

RAPD Markers	Tested crosses	Total individuals	Phenotypes		Expected ratio	X^2
			R	S		
OPB-13 (920 bp)	Yr15x Sakha-69	70	52	18	3: 1	0.03
OPB-13 (700 bp)	Yr15xGiza-163	40	29	11	3: 1	0.43

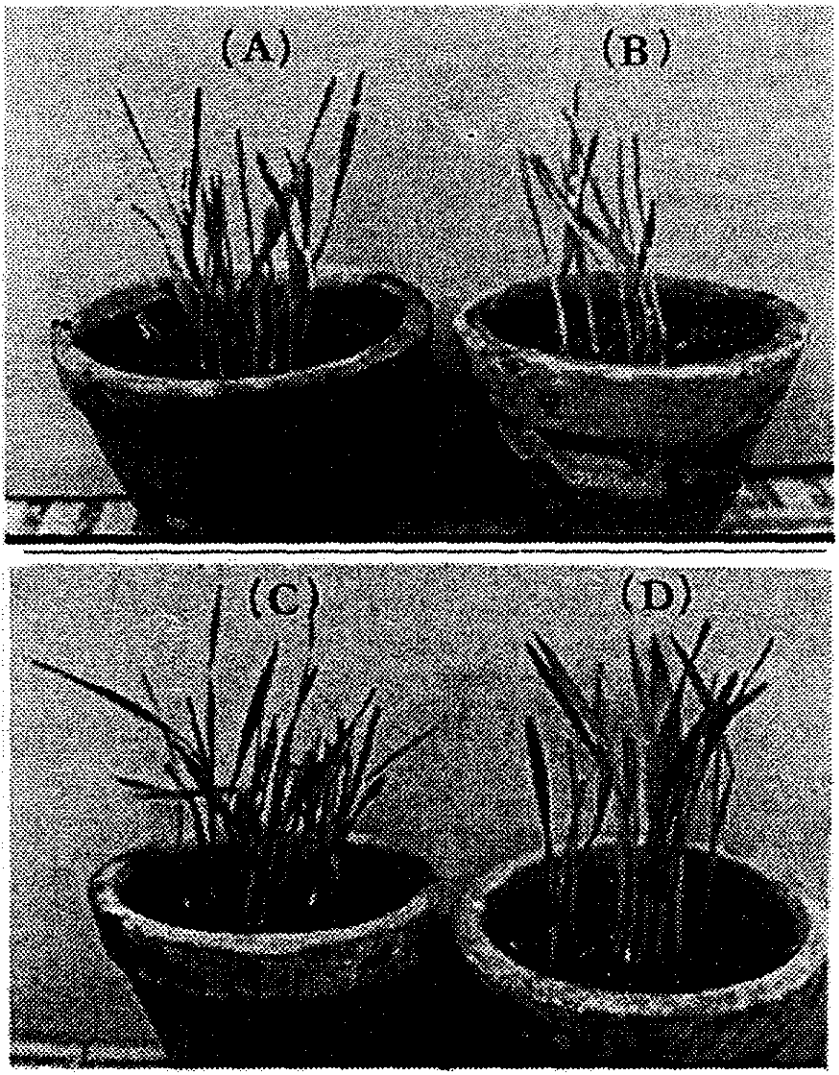

Fig. (1): F_1 population of the four crosses exhibited low infection type ranging between 0; - 1 (resistant), showing role of stripe rust resistance gene Yr15 gene at seedling stage.

A= Yr15 x Sakha-8 B = Yr15 x Sakha-69
C = Yr15 x Giza-163 D = Yr15 x Sids-7

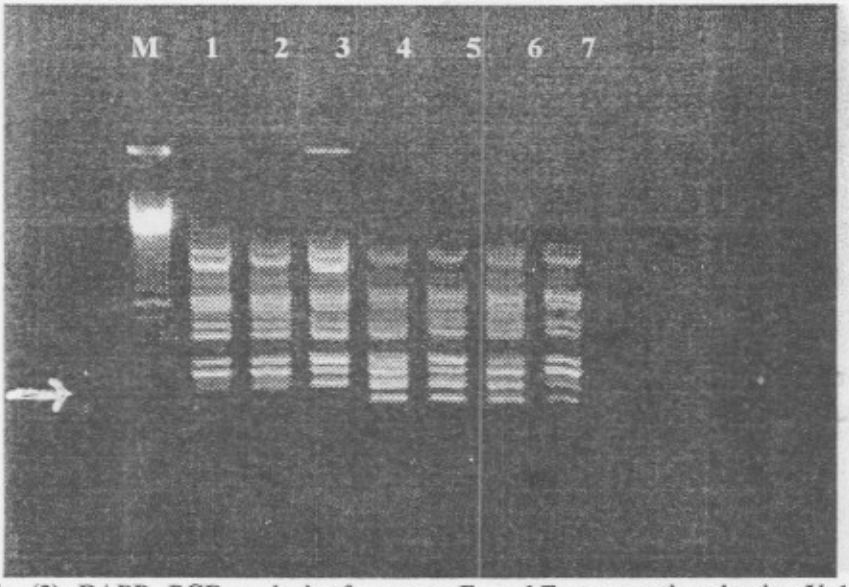

Fig. (2): RAPD- PCR analysis of parents, F_1 and F_2 segregations having Yr15 as a resistance gene and linked with OPB-13 primer.
Sakha 69 (lane 1) F_2 susceptible (Lane 2 and 3), Yr 15 (lane 4), F_1 (Lane 5), resistant F_2 (lanes, 6&7) and marker (M).
The arrowhead indicates to the bands at 920 bp which, differentiating between susceptible and the resistant crosses.

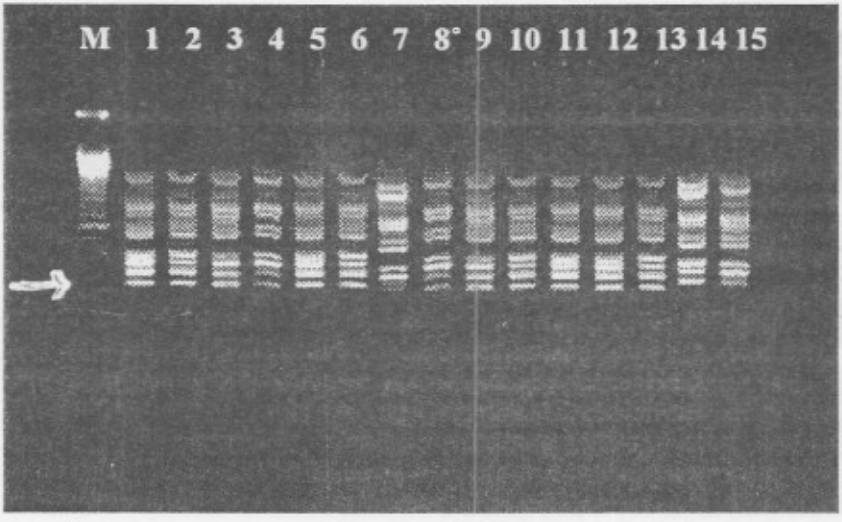

Fig. (3): RAPD-PCR analysis of 15 individuals of F_2 segregations having Yr15 as a resistance gene which, inked with OPB-13 primer.
The arrowhead indicates to the polymorphism bands at 920 bp which differentiating between susceptible and the resistant crosses.

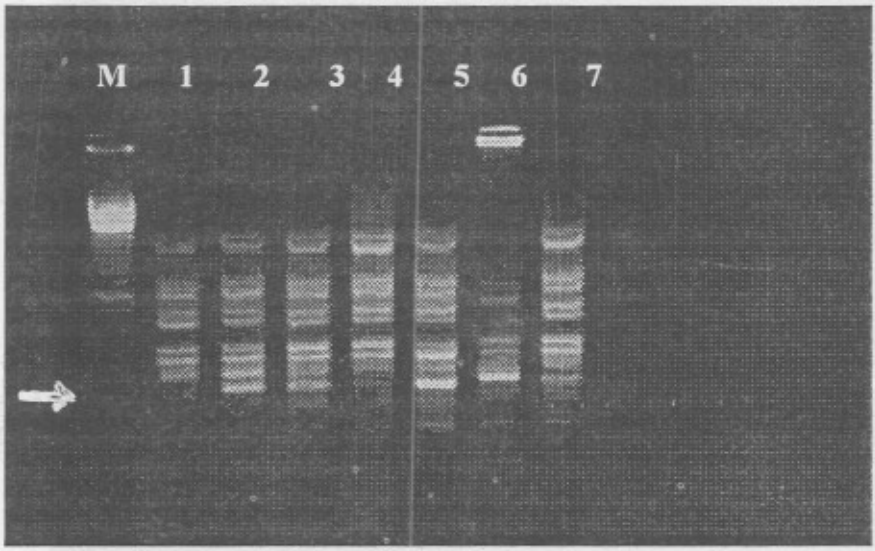

Fig. (4): RAPD-DNA polymorphism of parents, F_1 and F_2 segregations having Yr15 as a resistant gene linked with OPB-13 primer.
Giza-163 (lane 1), Yr15 (lane 2), F_1 (Lane 3), F_2 resistant (lane 5, 6 & 7) and susceptible F_2 (lane 4) and Marker (M).
The arrowhead indicates the bands at 700 bp which, differentiating between susceptible and the resistant crosses.

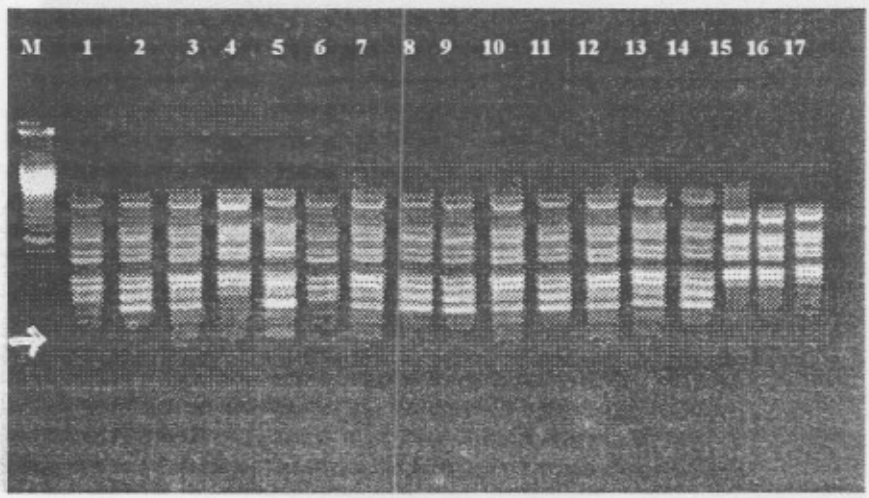

Fig. (5): RAPD-DNA polymorphism of 17 individuals of F_2 segregations having Yr15 as a resistance gene which linked with OPB-13 primer.
The arrowhead indicates to 700 bp band which, differentiating between susceptible and resistant crosses.

DISCUSSION

Breeding against stripe rust under greenhouse and field conditions, F_1 wheat plants of crosses having Yr15 gene were resistant at seedling and adult stages. As well as, F_2 segregation of crosses having Yr15 confirmed the results of F_1 and indicated that resistance was dominant over susceptibility. Similar results were obtained by (Gerechter and Adriana 1974, Gerechter *et al.*, 1989, Luthra *et al.*, 1989, Chen and Line 1987, and Sun *et al.*, (1997) who stated that the resistance in F_2 segregations of crosses between stripe rust susceptible *Triticum durum* wheat cultivar D447 and resistance line Yr15 was conferred by one dominant gene Yr15. These results proved that one dominant gene pair or two interacting gene pairs governed disease severity. Thus, plant breeders should not rely on the host pedigree only, but they should put the pathogen genotype and environment in considerations as two important variables in the pathogen: host: environment systems as indicated by (Shehab El-Din, 1986 and Shehab El-Din *et al.*, 1991).

The development of molecular markers for specific genes allows the detection of these genes independently of the genotype (Rolefs *et al*, 1992). Sun *et al.* (1997) develop molecular marker from the Yr15 DNA sequences which, was very specific for the detection of the Yr15 resistance gene in breeding material of diverse genetic origin. The produced random amplified DNA polymorphism of tested wheat individuals using the primer OPB-13 clearly show that Yr15 gene was successfully introduced in F_1 of Yr15xSakha-69 and Yr15xGiza-163, resistant individuals of F_2 of the same crosses and did not present in susceptible individuals. This result confirmed the presence Yr15 in the segregation of the resulted crosses and proved that a single dominant pair gene controls resistance. The results indicated that the F_2 population of Sakha-69 or Giza-163 showed a good fit with the ratio 3: 1.

In conclusion, the genetic basis of a particular stripe rust resistance in economically important breeding materials is often unknown and therefore, breeding for combinations of different resistance genes can not be reached. Therefore, molecular identification of resistance genes in genetically diverse material is an ideal method in plant breeding. Also, it will allow manipulation of wheat resistance gene in way that the durability of resistance is increased by reducing pathogen adaptation to single or combined resistance genes.

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استخدام تقنية RAPD-PCR لكشف جين المقاومة للصدأ الأصفر Yr15 الممنوح لبعض أصناف القمح التجارية المصرية

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كانت كل هجن القمح الفردية للجيل الأول والممنوحة جين المقاومة Yr15 من سلالة القمح الاحادية الجين تريتيكم ديكوكسيدس مقاومة للصدأ الأصفر فى مرحلتى البادرة والبلوغ تحت ظروف الصوبة والحقل ، بينما كانت أفراد عشيرة الجيل الثانى منعزلة بنسبة ٣ مقاوم : ١ قابل للإصابة وذلك يظهر أن المقاومة للصدأ الأصفر يحكمها جين فردى سائد، وقد تأكد نجاح منح جين المقاومة للصدأ الأصفر Yr15 باستخدام تقنية RAPD-PCR وبادىء متخصص لهذا الجين هو بادىء OPB-13 . وباختبار ٧٠ نبات من عشيرة مهجنة بين Yr15xSakha-69، ٤٠ نبات من عشيرة Yr15xGiza-163 فى الجيل الثانى تبين وجود جين Yr15 فى ٥٢، ٢٩ نبات على التوالى لعشيرتى القمح المختبرتين وبنسبة ٣ مقاوم إلى ١ قابل للإصابة ولم يكتشف جين Yr15 فى أباء القمح المختبرة (سخا-٦٩، جيزة-١٦٣) ولا فى نبات الجيل الثانى المنعزلة القابلة للإصابة.