DEVELOPMENT OF RAPD MARKERS ASSOCIATED WITH DROUGHT TOLERANCE IN BREAD WHEAT (*Triticum aestivum*)

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Wheat (*Triticum aestivum*) is the most important strategic cereal crop for the majority of the world populations. It is the most important staple food for about two billion people (36% of the world population). It exceeds in acreage and production than other grain crops (including rice, maize, etc.).

Wheat is an edible grain, one of the oldest and most important of the cereal crops in Egypt. The annual consumption of wheat grains in Egypt is about 12.4 million tons, while the annual local production is about 8.52 million tons / 1.32 million hectare in 2008/2009 (The Agricultural Economics and Statistics Department, Ministry of Agriculture, Egypt (2009)). Though grown under a wide range of climates and soils, wheat is the best adapted crop to regions with rainfall between 300 and 900 mm.

World food production is primarily limited by environmental stresses. It is very difficult to find 'stress free' areas where crops may approach their potential yield. Abiotic environmental factors are considered to be the main source (71%) of yields reductions (Boyer, 1982). Drought is one of the most common environmental stresses that affects growth and development of plants through alterations in metabolism and gene expression (Leopold, 1990). Wheat production suffers from variability in yield from one year to another and from location to another. Plant species vary in their sensitivity and response to the decrease in water potential caused by drought, low temperature or high salinity. It could be assumed that all plants have encoded capability gene(s) for stress perception, signaling and response (Bohnert *et al.*, 1995). Drought stress may occur early in the season or terminally at grain filling and development. Productivity improvement of wheat cultivars under drought conditions becomes one of the important objectives in wheat breeding program. Breeding for drought tolerance of wheat cultivars is a major objective in arid and semi-arid regions of the world. due to inadequate precipitation, shortage of water irrigation and high water demand due to crop evapotranspiration in such climates.

Most of the Egyptian newly reclaimed lands (West and East of the Delta and West of the Nile Valley in Upper Egypt) suffers from drought and

salinity stresses. Therefore, there is a major need to increase drought tolerance for the Egyptian wheat cultivars to increase the Egyptian wheat production, especially in the new lands, to meet the increasing consumption due to the increasing number of population.

Traditional methods of plant breeding have made a significant contribution to crop improvement such as: targeting complex traits like grain yield, grain quality and abiotic stress. Genetic modifications of crops can be carried out by new techniques such as somaclonal variation, protoplast culture, genetic engineering, *in vitro* pollination or hybridization, and double haploid production. The evaluation of genetic variations in wheat has been carried out using molecular markers based on RFLPs and RAPDs (Plaschke *et al*., 1995). Moreover, molecular markers are useful tools to study the genetic variations, since the genetic variability among wheat varieties is narrow as in all self-pollinated crops (Röder *et al*., 2002). The applications of molecular markers in plant breeding programs facilitate the improvement of many crop species (Williams *et al.,* 1990). The detection of RAPD markers on the genomic map of different field crops is beneficial to improve breeding programs for these crops. It offers the simplest and fastest method for detecting a great number of genomic markers in less period of time (Edwards *et al.,* 1992). Michelmore *et al.* (1991) developed the F_2 plants population to the highest and the lowest extremes for the development of RAPD markers needed for marker-assisted selection. Marker-assisted selection program was progressed by RAPD markers in several crop plants such as rice (Naqvi *et al.,* 1995), wheat (Penner *et al.,* 1996), durum wheat (Wang *et al.,* 1995), rapeseed (Jourdren *et al.,* 1996) and maize (Abdel-Tawab *et al.,* 1998).

The objectives of this study are to screen the responses of twenty bread wheat varieties under drought condition with respect to their performances for some drought-related traits, to select the most tolerant and the most sensitive varieties. Test drought stress on these two contrasting parents and their F_1 and F_2 plants by recording the previous drought–related traits. Detect some RAPD markers associated with drought stress to be used in marker-assisted selection (MAS) programs.

MATERIALS AND METHODS

1. Materials

This study was carried out in the research farm and greenhouse of the Wheat Research Department, Field Crops Research Institute, Agricultural. Research Center, Giza, Egypt and the laboratories of the department of Genetics, Faculty of Agriculture, Ain Shams University, Shoubra El-Kheima, Cairo, Egypt during the period from 2004 to 2008.

Two bread wheat (*Triticum aestivum*) varieties namely, Sahel 1

(drought tolerant) and Line 13 (drought sensitive), Table (1) were chosen. They obtained from a drought tolerance screening trial which comprising 20 bread wheat varieties according to the Susceptibility Index (Fischer and Maurer, 1978). The grains of these 20 wheat varieties were kindly obtained from Wheat Research Department, Field Crops Research Institute, Agricultural. Research Center, Giza, Egypt

2. Methods

The two selected varieties (Sahel 1 and Line 13) were grown in the field and crossed to obtain the F_1 grains. Some of the F_1 grains were sown in the field and selfed to obtain the F_2 grains.

2.1. Sand culture experiment

Five grains of the two parents, their F_1 and F_2 were sown in a mixture of sand and compost culture (2:1) in plastic pots (25 cm diameter) in the greenhouse in a completely randomized design experiment, which was conducted according to Heakel *et al.*, (1981). The seedlings were irrigated by the tap water supplemented with Hoagland solution (Hoagland and Arnon, 1950) which used as a base nutrient solution. The two parents (Sahel 1) and (Line 13) and their F_1 seedlings were irrigated under two irrigation conditions, control (500 mm) and drought treatment (150 mm). The F_2 grains were sown under drought treatment only (150 mm).

Data were recorded for all plants at the end of the experiment for the following traits related to drought tolerance, Plant height (cm), spike length (cm), biological yield (g), number of grains/plant and grain yield/plant (g).

2.2. Statistical analysis

Then the collected data from the two parents and their F_1 plants were statistically analyzed using analysis of variance (ANOVA) procedure according to Snedecor and Cochran (1969). The differences among means were compared using Duncan's new multiple ranges test (Duncan, 1955).

The F_2 plants which represented by 600 plants were classified into ten groups according to their behavior under drought stress. According to their performances under drought stress, Five plants of the two extreme groups of the F_2 individuals (the most drought tolerant and the most drought sensitive) were chosen for further molecular analysis with their parents and F_1 plants.

2.3. Molecular genetic studies

2.3.1. Genomic DNA extraction

DNeasy™ Plant Mini Kit (Qiagen Inc., cat. No. 69104) was used for DNA isolation as described in the manufacturer manual from plant samples (the two parents, their F_1 and five individual F_2 plants from the two extreme groups)

2.3.2. RAPD-PCR analysis

PCR reactions were performed according to Williams *et al.* (1990) using nine preselected 10-mer primers (Operon Technology, USA) Table (2). The reaction conditions were optimized and mixtures (25 µl total volume) were composed of dNTPs (0.25 mM), 10X buffer with 25 mM $MgCl_2$ (2.5 µl), primer (3 µl), DNA (100 ng) and Taq DNA polymerase (1 unit), H_2O up to 25 µl.

Amplification was carried out in a Primus Thermocycler, programmed for 42 cycles as follows: denaturation, 94°C/5 min (one cycle), annealing, 94°C / 1 min, 37°C/1 min, 72°C/1 min (40 cycles), extension, 72°C/10 min (one cycle), then 4°C until use. Agarose gel (1.2 %) electrophoresis was used for separating the PCR products. The run was performed at 100 volts for about one hour. DNA Marker used in this study was 1.5 kb DNA ladder which consists of eleven different DNA fragments (1500, 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp).

2.3.3. Analysis of gel images

All fragments resulting from RAPD gels were detected on an UV-transilluminator filter. All gels were photographed under UV light with Polaroid film 667 and scanned with Bio-Rad video densitometer Model 620 at a wavelength of 577. Software data analysis for Bio-Rad Model 620 USA densitometer was used.

RESULTS AND DISCUSSION

1. Drought-related traits

1.1. Response of the parents and F_1 plants

The means of drought-related traits of the two parents and their F_1 plants under control and drought conditions are shown in, Table (3). Drought treatment caused a reduction in all traits which was higher in the sensitive parent (line 13) than the tolerant parent (Sahel 1) and their F_1 plants.

Plant height trait showed a reduction that was higher in the sensitive parent (43.04) cm than the tolerant one (57.64) cm. However, the F_1 plants showed higher values under control (63.80) and drought (60.24) conditions than their parents.

With respect to number of grains per plant trait, the tolerant parent (Sahel 1) exhibited a higher number (20) than the sensitive one (15) under drought condition.

Grain yield per plant trait of the tolerant parent (Sahel 1) and the F_1 plants exhibited higher mean values (10.64 and 0.52, respectively) than the sensitive parent, line 13 (0.38). There was a high decrease in the value of the sensitive parent under drought condition (0.16) compared with the control (0.59), which indicated that the tolerant parent and the F_1 plants could relatively tolerate drought stress.

With respect to biological yield trait, the sensitive parent (line 13) and the F_1 plants displayed lower values (1.08 and 1.38, respectively) than the tolerant parent, Sahel 1 (1.84) under drought condition.

For spike length trait, there was a slight difference between the control and drought conditions for the tolerant parent and the F_1 plants. But the value of the sensitive parent (6.58) cm was lower than that of the tolerant parent and the F_1 plants (7.66 and 7.38, respectively) under drought condition.

1.2. Response of F_2 plants

F_2 plants represented by 600 individuals were classified into ten groups according to their performances under drought stress for each trait. Then, each trait was classified according to its range as presented in, Table (4) which shows the minimum, the maximum, the averages and standard error values of the five studied traits.

Plant vigor classified the F_2 plants into ten groups based on visual observation. The first group refers to the best growing F_2 plants and the tenth group refers to the worst ones under drought stress. The F_2 plants were arranged in descending order according to their frequency, so plants with high frequency in group one were chosen as the most tolerant F_2 plants. While, the plants in the last group were taken to represent the most sensitive F_2 plants.

According to these classifications, five F_2 plants were selected to represent the most tolerant F_2 plants and five plants were chosen as the most sensitive ones to drought stress for each trait as shown in, Table (5).

These five F_2 tolerant plants and five F_2 sensitive plants were used as individual plants to obtain RAPDs markers associated with drought stress.

Many authors evaluated two contrasting parents and their segregated F_2 population plants to detect some molecular markers associated with abiotic and biotic stresses as well as yield component and quality traits in these plants. However, their results reflected significant differences between parental genotypes for the studied trait(s) which indicating the variability existed between these parents. Moreover, they classified the segregated F_2 population plants to the highest and the lowest groups based on the studied trait(s) to develop molecular markers using bulked segregant analysis. In this respect, Abdel-Bary *et al.* (2005) tested some salt tolerance-related traits in maize, Rashed *et al.* (2006) evaluated some salt tolerance-related traits in sorghum, Atta *et al.* (2006) recorded some iron deficiency-related traits in maize, Fahmy *et al.* (2007) screened some drought tolerance-related traits in rice and Younis *et al. (2007)* measured some salt tolerance-related traits in grain sorghum.

2. RAPD markers for drought tolerance

DNA isolated from the two contrasting parents, Sahel 1 as a drought tolerant parent and line 13 as a drought sensitive parent, their subsequent F_1 plants, and the F_2 segregating population (the most tolerant five individual plants and the most sensitive five individual plants) were tested against nine preselected primers as shown in (Fig.1) and summarized in, Table (6).

Six primers only gave a polymorphism with the studied genotypes, which four primers out of them developed molecular markers for drought tolerance as shown in, Table (6). A20 and B19 primers exhibited four positive molecular markers with molecular sizes of 800 and 600 bp for A20 primer and 900 and 700 bp for B19 primer, which were found only in the tolerant parent (Sahel 1), the F_1 and the most tolerant F_2 individual plants while they were absent in the sensitive parent (line 13) and most sensitive F_2 individual plants.

Amersham1 and C13 Primers exhibited two negative molecular markers with molecular size of 600 bp for Amersham1 primer and 200 bp for C13 primer, which were found only in the sensitive parent (line 13), the F_1 and the most sensitive F_2 individual plants, while they were absent in the tolerant parent and the most tolerant F_2 individual plants.

These four positive and two negative RAPD markers could be considered as reliable markers for drought tolerance in wheat. These results agreed with many reports detected RAPD markers for abiotic stresses tolerance. Malik *et al.,* (2000) used RAPD markers to detect DNA polymorphism between two wheat genotypes as a drought-resistant and drought-susceptible. They revealed that RAPD technique has a great potential to find DNA-based polymorphisms between the genotypes of the same species. Abdel-Tawab *et al.,* (2003) detected five positive and negative RAPD markers for drought tolerance in Egyptian bread wheat. Abdel-Bary, *et al.,* (2005) detected eight positive and negative RAPD markers for salinity tolerance in maize.

Moreover, our results were in agreement with those of Nachit *et al.,* (2000) who associated yield-related traits as grain yield, yield components and stress physiological traits with some molecular markers in durum wheat. Several markers showed strong relationships with grain yield, yield components and stress physiological traits, indicating that there are potential markers for use in marker-assisted selection to improve abiotic stresses tolerance by molecular breeding.

SUMMARY

Screening experiment was performed on twenty varieties of bread wheat *(Ttriticum aestivum)* to select the most drought tolerant variety (Sahel 1) and the most sensitive one (line 13) according to drought susceptibility index

(DSI). Crossing was carried out between these two varieties to obtain the F_1 kernels. Some of the F_1 kernels were sown in the field and selfed to obtain the F_2 kernels. The two selected varieties, their F_1 and F_2 plants were evaluated for their response to drought stress by recording some drought–related traits. Five individual plants of the two contrasting F_2 plant groups (the most tolerant and the most sensitive F_2 groups), the two contrasting parents and their F_1 plants were used to develop some molecular genetic markers associated with drought tolerance in wheat by using nine RAPD primers. The results indicated the presence of four positive and two negative RAPD markers that could be considered as reliable markers for drought tolerance in wheat.

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Table (1): Names, pedigree and origin of the two selected parental varieties.

Variety	Pedigree	Origin
Sahel 1 (Drought tolerant parent)	NS732/PIMA//VEERY"S"	ARC
Line 13 (Drought sensitive parent)	CHIL/FINK CP3295-14CF-0Y-0C-4C-0C-0 SY-0AP	CIMMYT/ICARDA

Table (2): List of used primer and their nucleotide sequences.

Primer	Sequence	Primer	Sequence
A01	5' CAG GCC CTT C 3'	C13	5' AAG CCT CGT C 3'
A16	5' AGC CAG CGA A 3'	Amersham1	5' GGT GCG GGA A 3'
A20	5' GTT GCG ATC C 3'	UPC2	5' CCT GGG CTT G 3'
B18	5' CCA CAG CAG T 3'	UPC82	5' GGG CCC GAG G 3'
B19	5' ACC CCC GAA G 3'		

Table (3): Means of the recorded drought-related traits of the two contrasting parents and their F_1 plants at the end of the experiment.

Genotype	condition	Plant height (cm)	Biological yield (g)	Spike length (cm)	No. of grains/plant	Grain yield/plant (g)
Sahel 1	Control	61.28^{b}	2.29^{a}	7.94^{a}	22^{a}	0.81^{a}
	drought	57.64^{c}	1.84^{b}	7.66^{b}	20^{a}	0.64^{b}
Line 13	Control	57.00^{c}	1.94^{ab}	7.12^{bc}	23^{a}	0.59^{bc}
	drought	43.04^{d}	1.08^{c}	6.58^{c}	15^{b}	0.16^{d}
F_1	Control	63.80^{a}	1.96^{ab}	7.81^{a}	24^{a}	0.59^{bc}
	drought	60.24^{b}	1.38^{c}	7.38^{b}	22^{a}	0.52^{c}

Means with the same letter(s) in the column are not significantly different by Duncan's new multiple range test ($P < 0.05$). (Small letters for treatment means and capital letters for each genotype mean.)

Table (4): The minimum, the maximum and the mean values of the F_2 plants for the recorded drought-related traits.

Trait	minimum value	maximum value	Mean values ± SE
Plant height (cm)	21.00	75.00	49.95 ± 0.301
Biological yield (g)	0.27	2.77	1.18 ± 0.015
Spike length (cm)	3.00	10.00	6.74 ± 0.050
No. of grains/plant	0.00	35.00	15.00 ± 0.299
Grain yield/plant (g)	0.00	0.66	0.17 ± 0.004

Table (5): The performances of the most tolerant and the most sensitive F_2 plants according to the recorded drought-related traits.

	Ser. no	Plant No.	Plant height (cm)	Biological yield(g)	Spike length (cm)	No. of grains/plant	Grain yield/plant (g)	Visual Rank for plant Vigor
The most tolerant	1	164	75	2.72	10.0	35	0.66	1
	2	503	75	2.65	9.5	33	0.46	1
	3	594	72	2.53	9.0	30	0.46	1
	4	588	70	2.47	9.5	30	0.43	1
	5	405	67	2.3	9.0	29	0.43	1
The most sensitive	1	285	25	0.27	4.0	0	0	10
	2	253	30	0.30	3.0	1	0.003	10
	3	398	35	0.38	4.0	3	0.02	10
	4	303	35	0.42	4.5	4	0.03	10
	5	429	37	0.41	4.0	5	0.03	10

Table (6): Survey of the nine tested primer fragments with the two parents, their F_1 plants, the most tolerant, and the most sensitive F_2 plants.

Primer name	MS (bp)	T_1	T_2	T_3	T_4	T_5	P_1	F_1	P_2	S_1	S_2	S_3	S_4	S_5	MT
A20	800	1	1	1	1	1	1	1	0	0	0	0	0	0	P
	600	1	1	1	1	1	1	1	0	0	0	0	0	0	P
	350	1	1	1	1	1	1	1	1	1	1	1	1	1	-
B19	900	1	1	1	1	1	1	1	0	1	0	0	0	0	P
	700	0	1	1	1	0	1	1	0	0	0	0	0	0	P
	600	1	1	1	1	1	1	1	1	1	1	1	1	0	-
	550	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	500	1	1	1	1	1	1	1	1	1	1	1	1	0	-
	400	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	300	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	200	1	1	1	1	1	1	1	1	1	1	1	1	1	-
C13	500	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	300	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	200	0	0	0	0	0	0	1	1	1	1	1	1	1	N
Amersham1	600	0	0	0	1	1	1	1	1	1	1	1	1	1	N
	500	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	400	1	1	1	1	1	1	1	1	1	1	1	1	1	-
A1	800	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	600	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	400	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	300	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	200	1	1	1	1	1	1	1	1	1	1	1	1	1	-
A16	900	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	700	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	500	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	400	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	300	1	1	1	1	1	1	1	1	1	1	1	1	1	-
B18	900	1	1	1	1	1	1	1	1	1	1	1	0	0	-
	700	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	650	0	0	0	0	0	0	0	0	0	0	0	0	1	-
	600	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	400	1	1	1	1	1	1	1	1	1	1	1	0	0	-
UPC2	500	0	1	1	1	1	1	1	1	1	1	0	1	1	-
	400	0	1	1	1	1	1	1	1	1	1	1	1	1	-
	300	1	1	1	1	1	1	1	1	1	1	1	1	1	-
UPC82	500	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	400	1	1	1	1	1	1	1	1	1	1	1	1	1	-
	300	1	1	1	1	1	1	1	1	1	1	1	1	1	-

P_1=Tolerant parent T=Tolerant F_2 plant MT=Marker type P=Positive
P_2=Sensitive parent S=Sensitive F_2 plant MS=Molecular size N=Negative

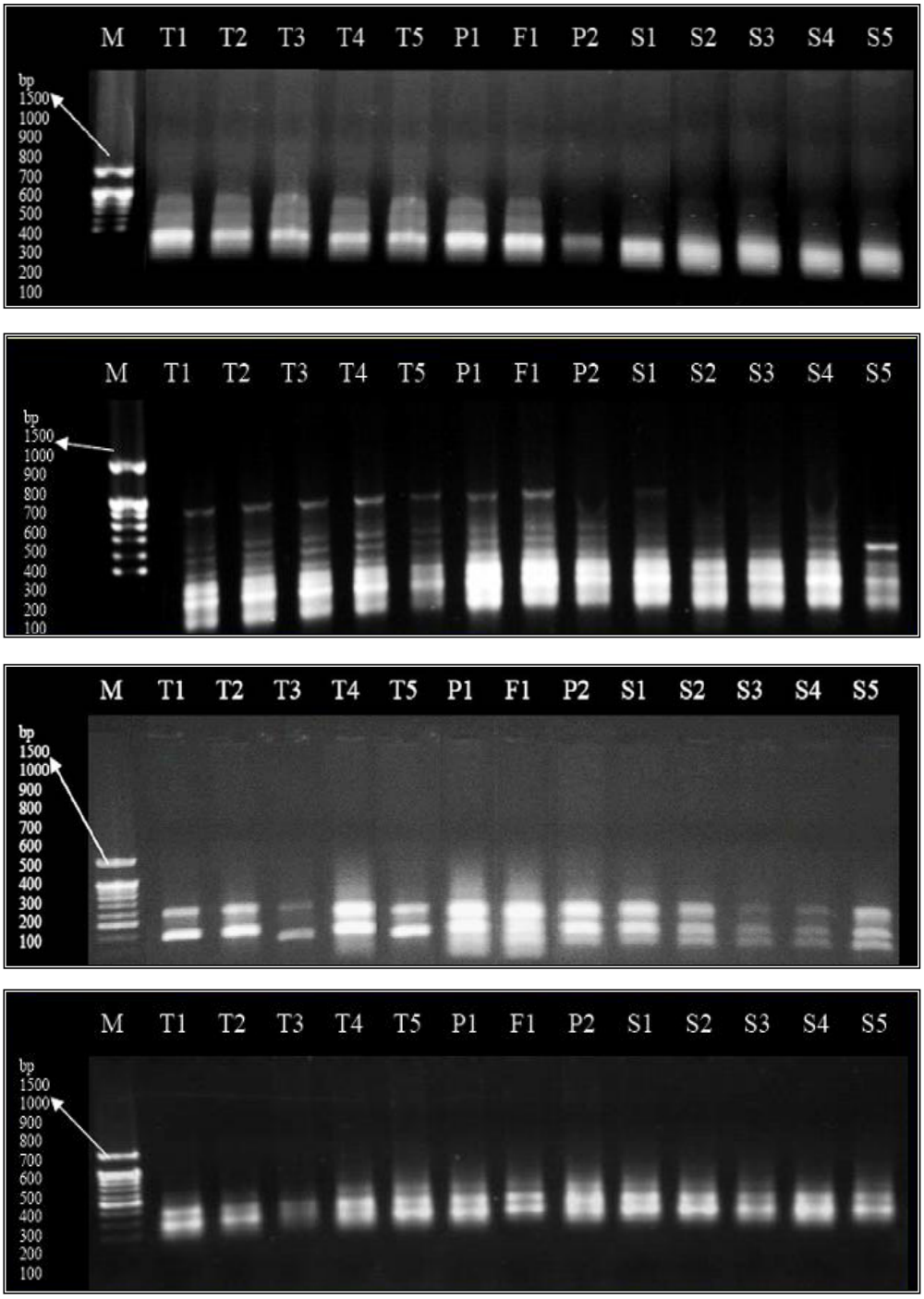

Fig. (1): RAPD-PCR fragments of four primers (A20, B19, C13 and Amersham1) for the most tolerant F_2 plants (T_1-T_5), the tolerant parent (P_1), F_1 plants, the sensitive parent (P_2) and the most sensitive F_2 plants (S_1-S_5).