FIELD EVALUATION FOR LEAF RUST DISEASE AND RAPD ANALYSIS FOR SOMACLONAL VARIANT LINES IN WHEAT

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ABSTRACT

Somaclones, derived from five local commercial wheat cultivars, were evaluated for resistance to leaf rust under greenhouse conditions. The results indicated that the ratio of the resistance to susceptibility varied, based on the somaclones, as well as virulence of the pathotypes. The analysis of variance for the degrees of rust severity (%) in leaf rust revealed significant differences among the two wheat cultivars (Giza-164 and Sakha-69) and their somaclones. However, the differences between the other two cultivars (Gemmiza-1 and Giza-167) and their somaclones were not significant.

As for field evaluation, statistical analysis showed that the number of grains per spike and grain yield per plant recorded significant differrences among wheat cultivars and their somaclones, except for the cultivar,Gemmiza-1. It, also, revealed that there were differences among Gemmiza-1 and their somaclones, as well as Giza-167 and their somaclones in flowering date, but the mean number of spikes per plant revealed significant differences only among Sakha-69 and their somaclones.

The results obtained from RAPD analysis for wheat cultivars and their somaclones revealed that six primers were used to amplify DNA segments from the genomic DNA of the four wheat cultivars, Giza-164, Sakha-69, Gemmiza-1 and Giza-167, and their somaclones. Forty-nine amplification products were obtained, all of these amplified fragments were polymorphic .The RAPD markers, produced by six primers, were used to construct a similarity matrix. The genetic similarity among the fourteen genotypes ranged from 0.05 to 0.89. The wheat cultivars and their somaclones were classified into seven clusters.

INTRODUCTION

Rust diseases, which include leaf rust(*Puccinia recondita*), stem rust (*Puccinia graminis tritici*) and strip rust (*Puccinia striformis*), are, from an economic point of view, the most important group of diseases on a world scale at the present time. In Egypt, however, leaf rust of wheat, caused by *P. recondita* is still the main wheat disease due to the favorable climatic conditions, the susceptibility of the old wheat cultivars (Nazim *et al.*, 1976 and 1983) and the dynamic state of the fungus virulence (Sherif *et al.*, 1992).

One of the main objectives of wheat improvement program is to generate genetically diverse germplasm that has high yield potential, wide adaptation and durable resistance to important diseases, such as rusts. Conventional breeding would probably be more efficient if aided by modern tools, such as somaclonal variation and molecular markers.

In the present investigation, field evaluation for leaf rust disease and RAPD analysis for somaclonal variant lines in wheat were attempted.

MATERIALS AND METHODS

Virulence survey under greenhouse conditions :

Somaclones derived from five comercial wheat cultivars (Giza 164, Sakha 69, Gemmiza 1, Giza 167 and Giza 160)(Barakat,2002) were evaluated during season 2002 for resistance to leaf rust under greenhouse conditions as follows :

Freshly uredospores of two pathotypes;i.e., 52 and 126 of *P. recondita* were collected and mixed with talcum powder (1:20 v/v) and dusted on primary leaves of 7 - day old seedlings of the somaclones by using a baby cyclone method (Tevet and Cassell, 1951).

Inoculated seedlings were incubated in a dark dew chamber for 24 hrs. Plants were transferred into greenhouse benches, having an ambient temperature of 20 ± 4°C . Infection types were recorded, fifteen days after inoculation when uredia on the plants appeared fully developing, as follows:

0.0 = Nso uredia of flecks visible.
0 = Very faint hypersensitive flecks.
1 = Small uredia surrounded by necrosis.
2 = Small uredia surrounded by chlorosis.
3 = Moderate size uredia without chlorosis.
4 = Large uredia without chlorosis
(Johnson and Browder,1966).
Infection types 0-2 were considered as resistant (R), while types 3 and 4 as susceptible (S).

Field evaluation :

The selected resistant somaclones, which were obtained from virulence survey of leaf and strip rusts under greenhouse conditions, as well as the parental line for each cultivar, were evaluated under optimum field conditions at the farm of Nubaria Research Station during season 2003. The experiment was set up in a randomized complete block design with two replicates. The experimental plot consisted of one row; 3m long and 20 cm apart. The plants were evaluated for the following characters :

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(1) Degree of rust severity (%) for leaf.
(2) Flowering date (days).
(3) Plant height (cm).
(4) Number of spikes *per plants*.
(5) Number of grains per spike.
(6) 100 - grain weight (g).
(7) Grain yield per plant (g).

RAPD analysis :

Plant material :

PCR analyses were carried out, using genomic DNA from the wheat cultivars, Giza-164 , Sakha-69 , Gemmiza-1 , Giza-167 and their selected somaclones. The selected somaclones for the cultivar,Giza-164, were designated as Soma164-3 (susceptible line)and Soma164-4 and Soma164-12 (resistant lines). The selected somaclones for the cultivar, Sakha-69 , were designated as Soma 69-11 (susceptible line) and Soma 69-1 and Soma 69-9 (resistant lines) .The selected somaclones for the cultivar, Gemmiza-1,were designated as Soma1-1 and Soma 1-2 (susceptible lines). The selected somaclones for the cultivar, Giza-167, were designated as Soma 167-1 and Soma 167-2 (susceptible lines) .

DNA extraction:

Genomic DNA was extracted from fresh leaves of the four cultivars and their selected somaclones, using CTAB(Soghai - Maroof *et al.*, 1984). RNA was removed from the DNA preparation by adding 10µl of RNAase (10mg /ml) and, then, incubating for 30 min. at 37°C. DNA sample concentration was quantified by using a spectrophotometer (Beckman Du-65).

RAPD analysis:

Six primers (Table 1) were used in this experiment to amplify the templated DNA. Each amplification reactions was performed in a 25-µl vol., containing 50 ng of genomic DNA, 1x pcr buffer Mg Cl2 (60 mM Kcl, 10mM Tris- Hcl (pH 9.0), 2mM Mgcl2 and 1% Triton x-100), 200 mM each of dATP, dCTP, dGTP and dTTP (promega), 50 pM primer and 1.5 U of Taq DNA polymerase. Amplifications were carried out in an MJ Research PTC-100 thermal cycler with amplification conditions adopted from Williams *et al.* (1990): DNA de- maturation at 94°C for three minutes and 45 cycles of melting at 94°C for one min., annealing at 36°C for one min. and extending at 72°C for two min. This was followed by a seven min. final extension step 72°C, then, the reactions were kept at 10°C. RAPD fragments were size- fractionated in a 2% agarose gel in TBE 0.5 x TBE buffer, with a 1-kb ladder moleculer- weight marker. Gels were stained in ethidium bromide solution and,then, photographed.

Table 1 : Primer sequences used to detect polymorphism between somaclones and their parents.

No. of primer	M.W.	Nucleotide sequence(5' to 3')
1	3108	GAAACGGGTG
2	3044	GGGTAAGGCC
3	3037	AGCCAGGGAA
4	3010	GTTGCGATCC
5	3019	GACCGCTTGT
6	2915	TTCCCCCGCT

Data analysis:

Data were scored for computer analysis on the basis of the presence or absence of the amplified products for each primer. If a product was present in a genotype, it was designated as "1", if absent it was designated as "0" after excluding irreproducible bands. Pair-wise comparisons of genotypes, based on the presence or absence of unique and shared polymorphic products, were used to regenerate similarity coefficients,according to Jaccard (1908).The similarity coefficients were,then, used to construct a dendrogram by UPGMA (Unweighted Pair- Group Method with Arithmetical Averages), using NTSYS-PC (Rohlf, 1993).

RESULTS AND DISCUSSION

Virulence survey of leaf rust under greenhouse conditions :

Data presented in Table (2) showed the response of 590 and 606 somaclones to leaf rust pathotype 52 and pathothype 126, respectively. The ratio of the resistant to susceptible response varied, based on the somaclone,as well as virulence of the pasthotypes. The somaclones, derived from the cultivars, Giza 164 and Sakha 69, showed good resistance to the two leaf rust pathotypes at seedling stage.

The resistant somaclones have been planted in the field for R2 seed production before evaluating the progeny under field conditions.

Table (2). Response of somaclones originated from some commercial grown wheat cultivars to *P. recondita* at seedling stage.

Cultivar	No. of somaclone / pathotype					
	Pathotype 52			Pathotype 126		
	Total No.	R.No.	S.No.	Total No.	R.No.	S.No.
Giza 164	135	9	126	122	2	120
Sakha 69	124	6	118	133	3	130
Gemmiza 1	113	2	111	109	0	109
Giza 167	103	1	102	130	0	130
Giza 160	115	0	115	112	0	112

R = Resistant.
S = Susceptible.

Field evaluation :

Giza-164 cultivar :

Analysis of variance in Table(3) indicated either highly significant or significant differences for the degrees of rust severity (%) in leaf rust, number of grains per spike and grain yield per plant among somaclones selected for leaf and stripe rusts resistance, as well as the parental lines. Differences were not significant for flowering date, plant height, number of spikes per plant and 100- grain weight.

The ratio of the resistance to susceptible response for leaf rust varied, based on the somaclone and exhibited different degrees of rust severity (%) . The somaclones (Soma 164-4 and Soma 164-12) derived from the cultivar, Giza 164, showed good resistance to leaf rust (Table 4).

The mean number of grains per spike ranged from 80.50 grains per spike for the somaclones Soma 164-1, Soma 164-10 and Soma 164-12 to 45.00 grains per spike for Soma 164-3 (Table 5) .

The somaclones, Soma 164-4 and Soma 164-12, had higher grain yields per plant (27.44 'and 25.56 g / plant, respectively) than the parental line (16.06 g / plant) (Table 4). However, the difference was not significant between Soma 164-4 and Soma 164-12.

Sakha-69 cultivar :

The differences among the somaclones, as well as the parental line, were either significant or highly significant for the degrees of rust severity (%), plant height, number of spikes per plant, number of grains per spike and grain yield per plant, but were not significant for flowering date and 100- grain weight (Table 3).

The somaclone, Soma 69-5, had a significantly higher grain yield (25.92 g / plant) than the parental line (18.80 g / plant) and the other somaclones, except for Soma 69-1, Soma 69-8 and Soma 69-9 (23.57, 24.03 and 23.56 g / plant, respectively), where the differences were not significant (Table 5).

All somaclones had higher number of grains per spike than the parental line and the differences were significant between the somaclones and parental line (Table 5).

The mean number of spikes per plant ranged from 2.00 to 2.64 spikes / plant for the parental line (Sakha-69) and Soma 69-1, respectively (Table 5).

The mean of plant height ranged from 70.0 cm for the somaclone, Soma-8, to 90.0 cm for the somaclones, Soma 69-2, Soma 69-9, Soma 69-10 and Soma 69-11,as well as the parental line, Sakha - 69(Table 5).

Concerning the evaluation of leaf rust, the obtained data revealed that the ratio of the resistance to susceptible response varied on the basis of the somaclone and exhibited different degrees of rust severity (%). The somaclones Soma 69-1, Soma 69-2 and Soma 69-3, derived from the cultivar, Sakha-69, showed good resistance to leaf rust (Table 5).

Gemmiza-1 cultivar :

The analysis of variance for the cultivar, Gemmiza-1, and their somaclones in Table (3) revealed insignificant effects of genotypes on all studied traits, except for flowering date .

All somaclones of Gemmiza-1 were significantly different from the parental line for flowering date (Table 6) .The number of days to heading ranged from 98.5 to 106.5 days for the somaclone, Soma 1-3, and the parental line, (Table 6).

Giza-167 cultivar :

The analysis of variance for the cultivar,Giza-167, and their somaclones in Table (3) revealed significant effect of genotypes on flowering date, number of grains per spike and grain yield per plant, but not significant on the other studied traits .

The mean of grain yield (25.30 g /plant) and number of grains per spike (81.0 grains /spike) of the somaclone Soma, 167-1, was significantly higher than that of the parental line, which were 19.16 g /plant and 57.50 grains / spike, respectively (Table 6) .

The results of this field evaluation showed that significant somaclonal variation could be generated for a number of agronomic characters. Larkin *et al* (1981) reported extensive heritable somaclonal variations in bread wheat. Variations for morphological and biochemical traits were observed among 142 regenerants of a Mexican breeding line "Yaqui 50 E " and their progeny. The variant characters, which included height, awns, tiller number, grain colour, heading date, gliadin protein and α-amylase regulation, were heritable through two seed generations. Based on these studies, Larkin *et al* (1981) suggested that somaclonal variation might encompass two mechanisms, one operating to generate a mutant gene and the other, in some cases, operating to make the mutant homozygous. Chromosome loss or addition was not evident as the primary cause of the variation in wheat.

Somaclonal variation and heritable changes, that resulted from *in vitro* procedures, had encourged plant breeders to consider the importance of such method. The ability to select somaclonal variation can yield a pool of individuals, upon which selection procedure can be applied for isolation of unique forms of standard cultivars. Oberthur *et al* (1993) obtained two wheat somaclones showing increased field resistance to leaf rust (*P. recondita* f. so. Tritici) .The present study revealed that somaclonal variation was found in resistance. Recent achievement in the production of new cultivars in the field crops such as the wheat variety "HE ZU No.8", the maize variety "YIDAN No.6", the flax variety "ANDRO", the rice variety "DAMA" are encouraging in pursing the research on somaclonal variation and in vitro selection for solving specific breeding amis (Jain, 2001) . Jinza No.18 is a new *Sorghum bicolor* variety was resulted from a cross between two somaclones lines of sorghum and it is characterized by strong hard stem, tight panicles and large disease resistance than the other commerical hybrids (Wang *et al*, 2002) .

RAPD analysis for somaclonal variant lines and their parents .

Screening of polymorphic primers among somaclones and their parents :

Six primers (Table 7) were used to amplify the genome of four wheat cultivars (Giza-164, Sakha-69, Gemmiza-1, Giza-167 and their somaclones).The six primers studied amplified a total of 49 DNA fragments. All of these amplified fragments were polymorphic. The number of bands, amplified per primer, ranged from four (primer 6) to eleven (primers 1 and 2) with a mean value of 8.2 bands per primer (Table 7).These values are rather high for RAPD amplification, compared to the average numbers of amplified bands recorded in other crops; namely, three fragments in *Triticum turgidum* (Joshi and Nguyen, 1993), 4.3 fragments in *Solanum tuberosum*. (Masuelli *et al*, 1995) and 6.7 in *Zea mays* (Heun and Helentjaris, 1993).

Figure (1) shows the amplification profiles, generated by primer 2 (5-GGGTAAGGCC-3) across the wheat cultivars and their somaclones. All of the eleven scorable bands were polymorphic across the wheat genotypes and their somaclones.

A high level of polymorphism for DNA markers was reported by Joshi and Nguyen (1993), but not by Devos and Gale (1992). The use of RAPD markers to determine the genetic diversity of diploid wheat genotypes has been reported by Vierling and Nguyen (1992). They reported that electrophoretic analysis of the amplification products revealed a higher incidence of polymorphism in *T. urartu* than *T. monococcum*.

Williams *et al* (1990) reported that polymorphism among individuals could arise through nucleotide change that prevented amplification by introducing either a mismatch at one priming site, detection of a priming site, insertions that rendered priming sites too distant to support amplification and insertions or deletions that changed the size of the amplified product.

The PCR technique has proved to be a powerful tool for the identification of polymorphism in cereals. Using wheat, barley, rye and wheat-barley addition lines, Weinging and Langridge (1991) detected polymorphism, using conserved semi-random and random primers. With different combinations of primers, they were able to detect both inter-and intra-specific diversities.

Two-hundred combinations of a pair of 10-mer primers were tested in wheat species to examine RAPD patterns; 25 combinations of primers produced informative bands (Nagaoka and Ogihara, 1997). They reported that the number of bands produced after the PCR reaction were approximately 220 bands in diploid wheat, 240 in tetraploid wheat and 260 in hexaploid wheat. Recently, Barakat *et al* (2000) used 26 primers of arbitrary nucleotide sequence to amplify DNA segments from the genomic DNA of six wheat genotypes, which had high potentiality for shoot formation. One-hundred and forty-eight amplification products were obtained, out of which 128 showed polymorphism. They, also, reported that the RAPD markers produced by primers were used to construct a similarity matrix.

Cluster Analysis :

One of the goals of the present study was to investigate the efficiency of RAPD markers in determining, accurately, the genetic relationship between wheat somaclones and their parents.

The RAPD markers, produced by six primers, were used to construct a similarity matrix (Table 8). Jaccard coefficients, ranging from 0.05 to 0.89, suggested a broad genetic base for wheat genotypes and their somaclones. Data in (Table 8) indicated the

Table 3 : Mean squares from the analysis of variance for degrees of leaf rust severity, yield and yield components in the selected somaclones for rust resistance, as well as the parental lines of four wheat cultivars .

S.O.V.	D.F.	Leaf rust severity (%)	Flowering date (days)	Plant height (cm)	No. spikes/ plant	No. grains/ spike	100- grain weight (g)	Grain yield/plant (g)
					a- Giza – 164			
Replicates	1	1.889	1.750	175.000	0.151	104.143	2.666	22.286
Genotypes	13	1.701**	0.277	81.593	0.053	313.264*	0.263	24.158*
Error	13	0.213	0.212	44.231	0.040	111.451	0.224	8.778
					b- Sakha – 69			
Replicates	1	0.934	0.667	16.667	0.006	234.375	3.856	33.725
Genotypes	11	2.801**	8.485	103.030*	0.066*	401.769*	0.153	9.194**
Error	11	0.587	8.667	34.848	0.023	151.648	0.223	1.198
					c- Gemmiza – 1			
Replicates	1	1.197	0.000	50.000	0.006	2.000	0.281	0.546
Genotypes	3	2.225	24.333*	66.667	0.023	112.167	0.655	19.049
Error	3	0.743	2.333	50.000	0.006	241.000	0.216	5.341
					d- Giza – 167			
Replicates	1	0.274	4.167	16.667	0.000	54.000	0.045	4.100
Genotypes	2	0.917	2.167*	16.667	0.052	298.167*	0.083	33.780*
Error	2	0.274	0.167	216.667	0.023	6.500	0.080	1.980

*,** Significant at 0.05 and 0.01 probability levels, respectively .

Table 4 : Means of leaf rust severity, yield and yield components in the selected somaclones for leaf rust resistance, as well as the parental line of Giza-164.

Somaclone	Leaf rust severity (%)	Flowering date (days)	Plant height (cm)	No. spikes/ plant	No. grains / spike	100- grain weight (g)	Grain yield /plant (g)
Giza-164	2.85 B	101.00 A	77.50 A	2.12 A	63.50 A	4.20 A	16.06 CD
Soma 164-1	2.56 BC	100.50 A	82.50 A	2.23 A	80.50 A	4.68 A	21.86 ABC
Soma 164-2	1.81 CDE	100.50 A	82.50 A	2.23 A	71.50 A	4.49 A	24.66 AB
Soma 164-3	3.10 AB	100.50 A	85.00 A	1.85 A	45.00 A	4.39 A	14.48 D
Soma 164-4	1.55 DEF	101.00 A	85.00 A	2.23 A	74.50 A	4.89 A	27.44 A
Soma 164-5	2.56 BC	100.00 A	75.00 A	2.12 A	78.00 A	4.72 A	20.32 BCD
Soma 164-6	1.55 DEF	100.50 A	80.00 A	2.23 A	47.00 A	3.89 A	21.90 ABC
Soma 164-7	0.91 EF	100.50 A	70.00 A	2.23 A	55.00 A	4.53 A	22.64 AB
Soma 164-8	4.03 A	100.00 A	92.50 A	2.23 A	75.00 A	4.72 A	19.64 BCD
Soma 164-9	3.10 AB	101.00 A	80.00 A	2.12 A	56.00 A	3.62 A	19.32 BCD
Soma 164-10	1.81 CDE	101.00 A	85.00 A	2.34 A	80.50 A	4.42 A	22.58 AB
Soma 164-11	1.81 CDE	101.00 A	90.00 A	2.43 A	73.00 A	4.75 A	22.01 ABC
Soma 164-12	0.64 F	101.00 A	90.00 A	2.55 A	80.50 A	4.31 A	25.56 AB
Soma 164-13	2.19 BCD	101.00 A	90.00 A	2.34 A	74.00 A	4.83 A	23.45 AB

Means with the same letter (s) are not significantly different, at 0.05 level .

Table 5 : Means of leaf rust severity, yield and yield components in the selected somaclones for leaf rust resistance, as well as the parental line of Sakha-69 .

Somaclone	Leaf rust severity (%)	Flowering date (days)	Plant height (cm)	No. spikes/ plant	No. grains / spike	100- grain weight (g)	Grain yield / plant (g)
Sakha-69	4.44 A	97.50 A	90.00 A	2.00 C	37.50 B	3.62 A	18.80 F
Soma 69-1	0.91 E	101.00 A	75.00 BC	2.64 A	88.00 A	4.05 A	23.57 ABC
Soma 69-2	0.91 E	100.50 A	90.00 A	2.23 BC	80.00 A	4.73 A	21.52 CDE
Soma 69-3	0.91 E	103.00 A	80.00 ABC	2.00 C	85.50 A	4.10 A	20.00 EF
Soma 69-4	1.55 DE	104.00 A	85.00 AB	2.23 BC	77.50 A	4.40 A	21.78 BCDE
Soma 69-5	2.72 BCD	100.50 A	85.00 AB	2.35 AB	86.00 A	4.30 A	25.92 A
Soma 69-6	1.81 CDE	100.00 A	80.00 ABC	2.35 AB	79.00 A	4.31 A	22.38 BCDE
Soma 69-7	1.81 CDE	103.00 A	75.00 BC	2.23 BC	86.00 A	4.07 A	23.43 BCD
Soma 69-8	1.81 CDE	100.00 A	70.00 C	2.23 BC	89.50 A	3.92 A	24.03 AB
Soma 69-9	3.38 ABC	103.00 A	90.00 A	2.23 BC	70.00 A	4.31 A	23.56 ABC
Soma 69-10	3.63 AB	103.00 A	90.00 A	2.23 BC	69.00 A	4.06 A	21.06 DEF
Soma 69-11	3.10 ABCD	104.50 A	90.00 A	2.00 C	73.50 A	4.32 A	19.04 F

Means with the same letter (s) are not significantly different, at 0.05 level .

Table 6 : Means of leaf rust severity, yield and yield components in the selected somaclones for leaf rust resistance, as well as the parental lines of Gemmiza-1 and Giza-167 .

Somaclone	Leaf rust severity (%)	Flowering date (days)	Plant height (cm)	No. spikes/ plant	No. grains / spike	100- grain weight (g)	Grain yield / plant (g)
Gemmiza-1	2.56 A	106.50 A	80.00 A	2.12 A	63.50 A	3.76 A	18.20 A
Soma 1-1	0.00 A	100.00 B	90.00 A	2.35 A	69.50 A	4.56 A	24.34 A
Soma 1-2	1.28 A	101.00 B	90.00 A	2.35 A	81.00 A	3.59 A	23.44 A
Soma 1-3	1.55 A	98.50 B	80.00 A	2.35 A	75.00 A	4.75 A	24.96 A
Giza-167	2.56 A	101.00 A	85.00 A	2.23 A	57.50 B	4.18 A	19.16 B
Soma 167-1	1.28 A	99.00 B	80.00 A	2.55 A	81.00 A	4.59 A	25.30 A
Soma 167-2	0.64 A	99.50 AB	80.00 A	2.45 A	63.50 B	4.36 A	26.96 A

Means with the same letter(s) are not significantly different at 0.05 level.

Table 7 : Number of amplification and polymorphic products, using six primers, in wheat cultivars and their somaclones .

Primer number	Nucleotide sequence 5 ` to 3 `	No. of amplification products (a)	No. of polymorphic products (b)	Polymorphism b/a (%)
1	GAAACGGGTG	11	11	100 %
2	GGTAAGGCC	11	11	100 %
3	AGCCAGGGAA	10	10	100 %
4	GTTGCGATCC	8	8	100 %
5	GACCGCTTGT	5	5	100 %
6	TTCCCCGCT	4	4	100 %

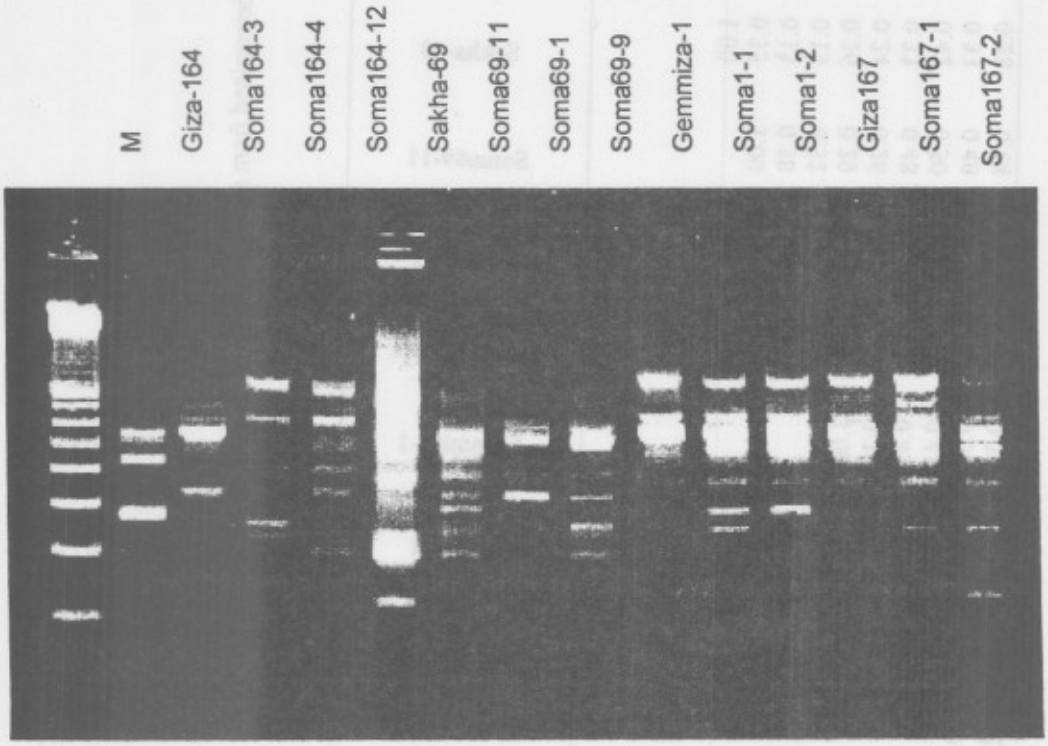

Fig. 1 : RAPD polymorphic in wheat cultivars and their somaclones.
Primer 2 (5 ` - GGGTAAGGCC - 3 `) .

Table 8 : Similarity matrix based in Jaccard's coefficient determined from analysis, using different primers that amplified 49 distinct product.

	Giza-164	Soma164-3	Soma164-4	Soma164-12	Sakha-69	Soma69-11	Soma69-1	Soma69-9	Gemmiza-1	Soma1-1	Soma1-2	Giza167	Soma167-1	Soma167-2
Giza-164	1.00													
Soma164-3	0.09	1.00												
Soma164-4	0.21	0.50	1.00											
Soma164-12	0.12	0.14	0.19	1.00										
Sakha-69	0.33	0.14	0.21	0.04	1.00									
Soma69-11	0.15	0.17	0.30	0.15	0.35	1.00								
Soma69-1	0.08	0.03	0.12	0.36	0.13	0.38	1.00							
Soma69-9	0.10	0.15	0.16	0.31	0.13	0.32	0.48	1.00						
Gemmiza-1	0.09	0.17	0.23	0.10	0.26	0.29	0.11	0.23	1.00					
Soma1-1	0.05	0.19	0.33	0.12	0.24	0.26	0.08	0.21	0.39	1.00				
Soma1-2	0.12	0.23	0.35	0.20	0.33	0.48	0.26	0.35	0.45	0.61	1.00			
Giza167	0.17	0.24	0.36	0.13	0.42	0.50	0.22	0.28	0.41	0.56	0.84	1.00		
Soma167-1	0.12	0.23	0.35	0.20	0.33	0.48	0.26	0.35	0.53	0.53	0.89	0.75	1.00	
Soma167-2	0.10	0.29	0.37	0.15	0.28	0.44	0.21	0.28	0.35	0.41	0.72	0.67	0.72	1.00

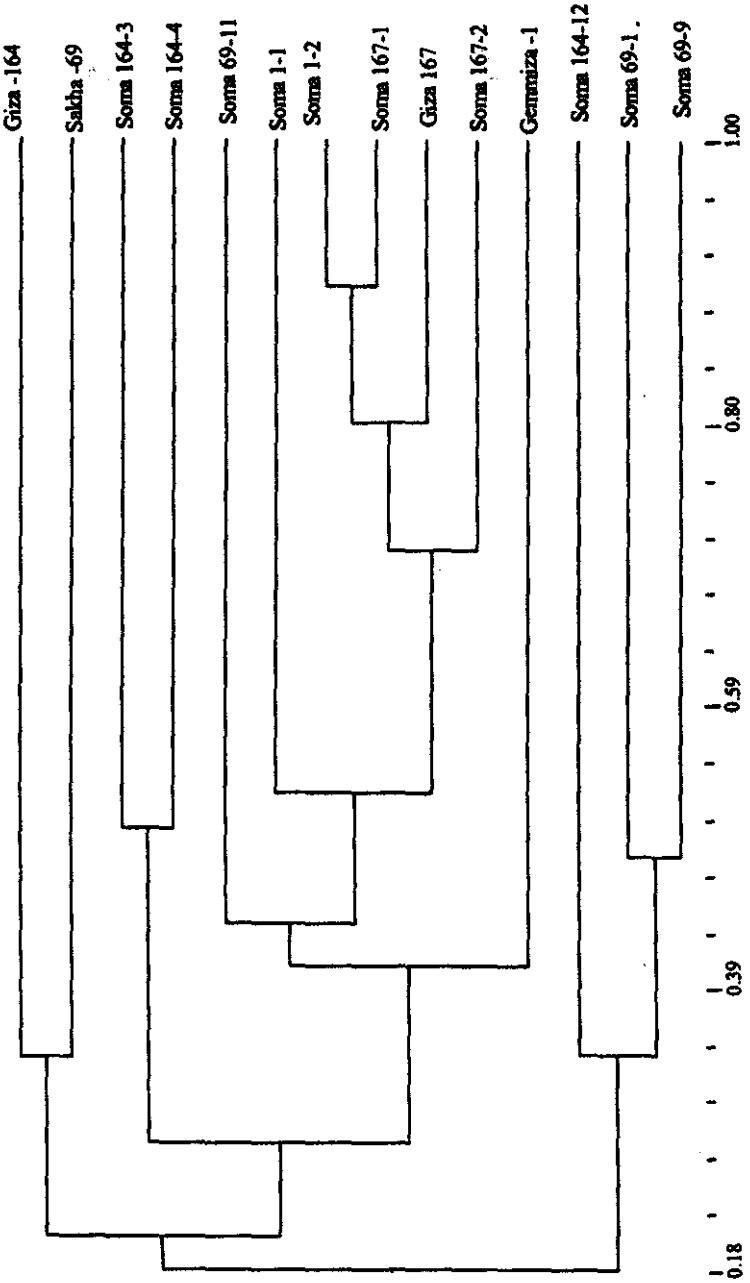

Fig. 2 : Dendrogram for wheat somaclonal variant lines and their parents, based on a cluster analysis (UPGMA) of genetic similarities (Jaccard coefficient) from RAPD data .

genetic similarity estimates of the 91 pairwise comparisons among the wheat cultivars and their somaclones, based on the 49 polymorphic bands. Figure (2) represents the clustering of wheat cultivars and their somaclones, generated by UPGMA analysis of the parents; namely, Giza-164, Sakha-69, Gemmiza-1, Giza-167 and their somaclones.

Seven clusters could be observed, the first cluster included Giza-164 and Sakha-69, while the second one included Soma 164-3 and Soma 164-4. The third cluster included only soma 69-11 . The fourth cluster included Soma 1-1 , Soma 1-2, Soma 167-1, Giza 167 and Soma 167-2. The fifth cluster included only Gemmiza-1 . Also, the sixth cluster included only Soma 164-12 . The last one included Soma 69-1 and Soma 69-9 .

These results indicated that RAPD technique could successfully be applied to species with very large genomes, like wheat, to obtain a proper characterization of genetic relationship. Nagaoka and Ogihara (1997) stated that RAPD markers were more easily handled and, thus, were becoming more desirable to estimate genetic relationship among wheat genotypes. Barakat *et al* (2000) reported that wheat genotypes were classified into three clusters :- Giza cultivars and Sakha-69, Gemmiza-1,and Sohag-1. Their results revealed that the closest cultivars to Sakha-69 were Giza-167 and Giza-164. Sohag-1 and Gemmiza-1 were very different from other cultivars and quite distinct from each other.

Recently, RAPD markers have been used to characterize wheat somaclonal variants tolerant to heat stress and to compare them with their parents (Barakat *et al* 2003). They reported that the genetic similarity among the fifteen genotypes ranged from 0.00 to 0.60. They, also, reported that wheat cultivars and their somaclones were classified into six clusters.

This study indicated that the use of RAPD technique to detect genetic variation at the level of DNA, among wheat cultivars and their somaclones, was sensitive and powerful. This would be of particular importance in the future when dealing with *in vitro* selection to stress conditions.

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الملخص العربي

تقييم حقلي لصدأ الأوراق وتحليل RAPD للـ Somaclonal Variant Lines في القمح

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تم تقويم الـ Somaclones الناتجة من خمسة أصناف تجارية محلية من القمح (جيزة ١٦٠ وجيزة ١٦٤ وجيزة ١٦٧ و سخا ٦٩ وجميزة ١) من حيث مقاومتها لصدأ الأوراق وذلك تحت ظروف الصوبة. أظهرت النتائج أن نسبة المقاومة إلى الإصابة اختلفت باختلاف الـ Somaclones وكذلك طراز المسبب المرضي. وأظهر تحليل التباين لدرجة الإصابة كنسبة مئوية اختلافات معنوية بين صنفين من أصناف القمح (جيزة ١٦٤ وسخا ٦٩) والـ Somaclones الناتجة منهما على الرغم من أن الفروق بين الصنفين الآخرين(جميزة١ وسخا ١٦٧) والـ Somaclons الناتجة منهما لم تكن معنوية .

أما بالنسبة للتقويم الحقلي فإن التحليل الإحصائي أظهر فروق معنوية بين الأصناف والـ Somaclones الناتجة منها، فيما عدا صنف" جميزة ١". كما أوضح أيضا أنه توجد فروق بين الصنف" جميزة ١ "والـ Somaclones الناتجة منه وكذلك الصنف "جيزة ١٦٧ " وذلك في ميعاد التزهير. أما صفة متوسط عدد الخلفات بالنبات فقد أظهرت اختلافات معنوية فقط بين الصنف "سخا ٦٩ "والـ Somaclones الناتجة منه. أما تحليل الـ RAPD بالنسبة للأربعة أصناف من القمح والـ Somaclones الناتجة منها باستخدام 6 primers فقد أظهر 49 amplifications وكلها كانت polymorphic . وتم استخدام النتائج المتحصل عليها في عمل similarity matrix . ووجد أن نسبة التشابه الوراثي بين الأربعة عشر تركيبا وراثيا المستخدمة تراوحت ما بين ٠,٠٥ – ٠,٨٩ وصنفت الأصناف والـ Somaclones الناتجة منها إلى سبع مجاميع.