FIELD EVALUATION AND SELECTION OF RESISTANCE TO RUST DISEASES (Puccinia Spp.) IN WHEAT SOMACLONAL VARIANT LINES

Milad,* S.I.

ABSTRACT

Forty somaclonal variant lines, produced from Sakha-69 wheat cultivar, using stepwise in vitro selection method, using p-fluorophenylalanine (PFP) as in vitro mutagenic agent for developing mutant lines resistant to rust diseases, were field evaluated, as well as their parent, at Sakha area, for grain yield and leaf rust (LR) severity as average coefficient of infection (ACI). The experiment was set up in a randomized complete block design, with two replicates due to small amount of seeds. There were highly significant differences among genotypes for grain yield, but there were no significant differences for ACI. Seventeen somaclones were selected for their low LR infection and high grain yield.

Two field experiments were conducted, at Sakha and Nubaria locations, to evaluate the seventeen selected somaclones, as well as their parent. They were laid out in a randomized complete block design, with two repliations. Some morphological characters were recorded at the two locations (plant height, 1000-grain weight, number of grains per spike and number of spikes and grain yield per square meter). Two other characters were recorded only at Nubaria area (harvest index and date of maturity).Either highly significant or significant differences were reported for plant height, number of spikes /m² and 1000-grain weight among somaclones, as well as their parent at the two areas, and grain yield / m² at Sakha area and date of maturity at Nubaria location. Meanwhile, differences were not significant for other morphological characters.

Disease severity analysis revealed significant effects of genotypes on the severity of LR(leaf rust) and SR(stem rust) at Nubaria and YR(yellow rust) at Sakha as ACI.

Cluster analysis of morphological characters and rusts severity (as ACI) was conducted to determine the relation between the parent and its somaclones.

The results obtained from the analysis of variane and cluster analysis indicated that there were clear differences among somaclones, as well as their parent.

In conclusion, the present study revealed that, in vitro selection method,was effective for producing valuable variations as somaclonal variant lines. Several of these somaclones were superior to the parent in grain yield and resistance to rust diseases, so that they might be selected and subjected to further evaluation for the production of new lines.

INTRODUCTION

Wheat (*Triticum aestivum L.*) is grown over a wide area in the world than any other major crop. It is the principal food for nearly one-third of the world population (Wiese, 1987). As an important staple food, wheat occupies an important place in crop breeding program in Egypt. Although wheat production in Egypt has been improved during recent years, continuous improvement in wheat productivity is highly desirable because of the increasing demand by the still-growing human population .However ,during the last few years, grain yield improvement in wheat cultivars has not been substantial due to rust diseases ,which include leaf rust*(Puccinia recondita)*, stem rust *(Puccinia graminis tritici)* and stripe rust *(Puccinia striiformis)*, which are, from the 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 fungal virulence (Sherief *et al.*, 1992).During the last three decades , yellow rust disease, caused by the fungal pathogen, *Puccinia striiformis*, was very destructive in different seasons and hit most of the Egyptian wheat cultivars in 1967 ,

1995 and 1997 and was almost epidemic in 1985 and 1998 (EL-Daoudi, 1998, El-Daoudi *et al*, 1996).

One of the main objectives of wheat improvement program is to generate genetically diverse germplasm that has a high grain yield potential, wide adaptation and durable resistance to important diseases, such as the rusts. Conventional breeding methods would probably be more efficient, if aided by modern tools, such as somaclonal variation and molecular markers. Somaclonal variants, heritable changes, that result from *in vitro* procedures, has forced for plant breeders to reconsider importance roles of these methods. The ability to select somaclone variation can yield a pool of individuals, upon which selection procedure may 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 (*Puccinia recondita f.sp.tritici*) .

In the present study, field evaluation for rust diseases for somaclonal variant lines was made to determine the relationship between the somaclones and their parent and select the most resistant and productive ones from them.

*Biotechnology Laboratory, Crop Science Department, Faculty of Agriculture (El-Shatby), Alexandria University, Alexandria, Egypt.

MATERIALS AND METHODS

Plant materials:

The wheat cultivar, Sakha-69, was used to produce somaclonal variant lines, using stepwise *in vitro* selection method. P-fluorophenylalanine (PFP) has been used as *in vitro* mutagenic agent for developing mutant lines resistant to rust diseases (Barakat and Milad, 2006). The calli, derived from mature embryos, were transferred to the medium, containing 100 μM /L PFP.They were monthly subcultured on a fresh medium, with a gradually increasing concentration of PFP by 100 μM until it reached 500 μM. The calli were, then, transferred to the regeneration medium free of PFP. Forty somaclonal variant lines were obtained and grown during 2004/2005 winter season to obtain R_1 seeds.

Field evaluation :

Sakha-69 cultivar (S-69) and the R_1 seeds of its somaclonal variant lines(S_1 to S_{40}) were grown during the season of 2005/2006 at the Farm of Sakha Agricultural Research Station, Agricultural Research Center (ARC),Egypt. The experiment was set up in a randomized complete block design, with two replicates due to small amount of seeds. The experimental plot consisted of one row, 3 m long and 20 cm apart. Grain yield /m^2 and the severity of leaf rust (LR) was recorded for each genotype, but there was no infection with yellow rust (YR) in this season at Sakha area. . Seventeen somaclonal variant lines were selected for their low LR infection and high grain yield.

Two field experiments were conducted during the season of 2006/2007 at two location, the first one was the Farm of Sakha Agricultural Research Station, Agricultural Research Center, the second was the Farm of Nubaria Research Station, to evaluate the seventeen selected somaclonal variant lines, as well as their parent. The two experiments were laid out in a randomized complete block design, with two replications. The experimental plot consisted of two rows, 3m long with a spacing of 20 cm among rows.

At harvesting time, in the two locations, ten plants were randomly chosen to measure plant height, 1000-grain weight and the number of grains per spike. Also, number of spikes and grain yield per square meter were recorded. Harvest index and date of maturity were recorded only at Nubaria.

Disease evaluation:

To initiate rusts infection, spreader and rows of susceptible varieties were planted among plots and around the experiments.

In each plot, six plants were randomly selected to collect the data of rust diseases as follows.

(1) The severity was recorded by using the percent leaf area affected (Stubbs *et al.*,1986) for leaf (LR) and yellow rusts(YR), and stem area affected by stem rust(SR). The severity of YR was recorded at Sakha station area, but the

severity of LR and SR were recorded alone at Nubaria area.

(2) The reaction was estimated for each genotype as: (R= resistant;small uredia often surrounded by a necrosis, MR= moderately resistant; small to medium uredia often surrounded by chlorosis or necrosis, X= intermediate; random distribution of variable-sized uredia , MS= moderately susceptible; medium-sized uredia might be associated with chlorosis or rarely necrosis, and S= susceptible; large uredia without chlorosis or necrosis). The scale of reaction had a constant value; i.e., R=0.2, MR=0.4, X=0.6, MS=0.8 and S = 1.0. The constant value was multiplied with severity score of each genotype to obtain average coefficient of infection (ACI) (Stubbs *et al.*, 1986 and Woldeab *et al.*,2007). ACI was calculated. For example: 20MR=20 ×0.4=8.

Data analysis:

Analysis of the morphological data and diseases severity, as ACI, was conducted, using two methods, as follows:

1) Analysis of variance (ANOVA) was applied as a randomized complete block design (RCBD), with two replicates, and each area was separately analyzed. To know the homogeneity of error variances for the studied traits. Hartley test (Winer, 1971) was used. Comparisons among means were made via the least significant difference multiple range test .The data were analyzed using SAS (2007) program.

2) Cluster analysis was conducted, using NTSYS-pc (Numerical taxonomy and multivariate analysis system (Version 2.101) (Rohlf, 2000).The standardized traits mean values (mean of each trait was subtracted from the data values and the results were divided by the standard deviation) were used to regenerate similarity coefficients, according to Jaccards (1908), a dendrogram was generated from the similarity matrix by the unweighted pair group method with arithmatic averages (UPGMA) (Sokal and Michener,1958).

RESULTS AND DISCUSSION

The first season :

The results, presented in Table (1), indicated that there were highly significant differences among the forty somaclonal variant lines, as well as their parent (S-69), for grain yield /m^2 , but there were no significant differences for ACI of LR disease .

The somaclone, S_{13}, had the highest average of grain yield (0.878 kg/m^2), and there was no significant difference between it and the S-69 parent (0.820 kg/m^2) as well as some other somaclones (S_3, S_5, S_{15} and S_{22}). The lowest significant averages were recorded with somaclones, S_{34} and S_{10} (0.268 and 0.413 kg/m^2, respectively) (Table 2).

Seventeen somaclones were selected, according to the average of grain yield and the degree of LR severity as ACI.

The second season
Grain yield and its components:

Using Hartley test (Winer, 1971), it was found that there was no homogeneity of error variances. Hence, each location was separately demonstrated.

Analysis of variance in Table (3) showed either highly significant or significant differences for plant height , number of spikes / m^2 and 1000- grain weight (g) among somaclones selected for rusts resistance, as well as the parent (S-69), at the two location, and grain yield / m^2 (Kg) at Sakha area ,and date of maturity at Nubaria area. Differences were not significant for number of grains / spike at the two areas and grain yield / m^2 and harvest index at Nubaria area.

The somaclone, S_{25}, had the significantly highest plant height at the two areas Nubaria and Sakha (123.9 and 127.5 cm, respectively), but, there were no significant differences between it and S_{29} and S_{24} (122.5 and 123.9 cm , respectively) at Nubaria area . Whereas, the significantly shortest plants were recorded in S_2 (92.9 and 100 cm at the two areas, respectively), but it was not significantly different from S_{13} , S_{28} , S_8 and S_{17} (102.4, 101.6,100.8 and 99.3 cm, respectively) at Nubaria area.

The highest significant mean number of spikes / m^2 was that of S_{39} (824.9 spikes/ m^2) at Nubaria area and there were no significant differences between it and S_{25}, S_{28} and S_{29} (769.0, 789.6, and 780.1 spikes/m^2, respectively) . The lowest one was the average of S_{15} (629.5 spikes / m^2) and it had no significant differences with some other averages (Table 4), such as S_{11},S_3 and S_{13}. At Sakha, S_{15} had the highest one (1268.0 spikes / m^2) , but it did not significantly differ from some other means, such as S_{11},S_{14} and S_{36}. The lowest one was that of the parent ,S-69, (643.1 spikes/m^2) and was not significantly different from other five means (Table 4).

Moreover, the highest 1000-grain weight (g) was recorded at Nubaria area for S_2 and S_{17} (50.1 g for both somaclones) and there were no significant differences between them and several other genotypes, such as S_{15}, S_{24} and S_{28}. Two genotypes; S_8 and S_{29}, gave the lowest 1000-grain weight (35.0 and 30.5 g, respectively). At Sakha area, S_2 and S_3 gave the heaviest grains , their averages of 1000-grain weight were 57.5 and 53.0 g, respectively. Also S_{25},S_{29} and S_{33} had the lowest grain weight (38.0,40.5 and 43.0 g, respectively) (Table 4) .

Table (4), also, reveals that the parent ,S-69, and the twelve somaclones had the same mean of maturity date (154 days) and there were significant differences among them and the other five genotypes. The somaclone, S_2, was the earliest one (141.5 days).This trait was recorded only at Nubaria area.

Grain yield/ m^2 was recorded and it was significantly affected by genotypes only at Sakha area. The somaclone, S_{39}, had the highest productivity (1.290 kg/m^2). Also, there were no significant differences between it and other seven somaclones (S_2, S_3 S_{11}, S_{14},S_{15},S_{17},and S_{24}). S_{25}, S_{29} and S_{33} somaclones had the significantly lowest average grain yields (0.345, 0.425 and 0.530 kg/m^2 ,respectively) .

The results of this field evaluation revealed the same results achieved by Barakat *et al* (2005).They performed field evaluations for selected resistant somaclones obtained from virulence survey of leaf and stripe rusts under green- house conditions, as well as their parents, and reported that significant somaclonal variation could be generated for a number of agronomic traits . The occurrence of genetic changes in plants, derived from cell cultures (somaclonal variations) had been previously documented (Larkin and Scowcroft,1981). They, also, reported extensive heritable somaclonal variations in bread wheat. Variations for morphological and biochemical traits were observed among 142 regererants of a Mexico breeding line "Yaqui 50 E " and their progeny . The variant characters, which included the highest values of awns, tiller number , grain colour, heading date, gliadin protein and α- amylase regulation, were heritable through two seed generations . Based on these studies, Larkin and Scowcroft (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 homozygous mutant .Chromosome loss or addition was not evident as the primary case of the variation in wheat .That rapid and easily accessible source of variation could be used in breeding programs (Karp,1995).

Disease severity analysis:
Analysis of variance in Table (5) revealed significant effects of genotypes on the severity of LR and SR at Nubaria area and YR at Sakha area as ACI (Average coefficient of infection).

1. Leaf rust (LR) :
Data in Table (6) indicated that S_2 and S_{24} were the highest susceptible genotypes (100 and 80 ACI) and S_{39} was more resistant than the other genotypes (1 ACI) and there were no significant differences between it and ten other genotypes.

2. Stem rust (SR) :
The parent, S-69, and its S_4 ,S_8, S_9, S_{13}, S_{14}, S_{28} and S_{39} somaclones were the most resistant genotypes for stem rust at Nubaria area and they had the same ACI (0.2). In addition, there were no significant differences among them and S_{24} ,S_{25} ,S_{36} ,S_{33} and S_{29}. On the other hand, S_{11} was the highest susceptible somaclone (75 ACI) (Table 6).

3. Yellow rust (YR):
Data in Table (6) revealed that the genotypes that had the highest resistance to YR were S_2 , S_{28}, and

S_{13}. They recorded the lowest ACI (0.5,10,and 12, respectively) . Also,S_{36} was not significantly different from S_{28} and S_{13} and had an ACI of 20 .The somaclones, S_{29},S_{33} and S_{25}, recorded the highest severity as ACI (100,100,and 95).

The previous data revealed that there were some somaclones , which were resistant to one rust (S_2 resistant to YR, S_8 resistant to SR and S_3 and S_{11} resistant to LR). While, others were resistant to two rusts together, but, they were susceptible to the third one (S_9,and S_{14} were resistant to LR and SR). The third group was resistant to two rusts, but, moderately resistant to the other one (S_4, S_{28},S_{36} and S_{39}), but, there was only one somaclone (S_{13}) that had a low ACI value for the three rust diseases .

Barakat and Milad (2006) reported that some somaclones, derived from the PFP treatment were found to have a mutated resistance to yellow rust disease of wheat. Previously, similar results, with the some results of the present investigatean, had been reported (Chauhan and Singh, 1995). They observed somaclonal variation of some morphological traits, as well as leaf rust reaction, among wheat variants.

In most disease resistance mechanism, associated host molecules are products of secondary metabolism. Widholm (1987) proposed the selection of variants disturbed, with respect to the appropriate biosynthetic pathway as a novel means of isolating disease –resistant variants. Many classes of resistance compounds were phenolic in nature, synthesized via the shikimic acid pathway. A pivotal step in phenolic biosynthesis was the conversion, catalysed by phenylalanine ammonia-layses, of phenylalanine into f- fluorophenylalanine acid. *In vitro* selection for resistance to the phenylalanine analogue P- fluorophenylalanine isolated cell lines, which over – accumulated phenolics (Palmer and Widholm, 1975; Berlin *et al*, 1982), due to possible overproduction of phenylalanine ammonia-layse. Selection for elicitor-induced phenylalanine analogue P-fluorophenylalanine – resistance may result in localized overproduction of phenolics in response to pathogen challenge. Berlin *et al* (1982) reported that the resistance mechanisms included: (a) overproduction of phenylalanine and (b) increased activity of phenylalanine ammonia-layse, which could detoxify P- fluorophenylalanine by converting it to a secondary metabolite P- fluorocinnamic acid.

In vitro selection of somatic wheat calli, resistant to toxic fusarium metabolites , using double – layer and culture filtrate techniques , were reported by several investigators (Yang *et al.*, 1998; Abdallah *et al*,2002). Inheritance of improved leaf rust resistance in somaclones of wheat had been reported (Oberthur *et al* .,1993).

When the agronomic traits, coupled with rust resistance are considered, some unique genotypes may be selected; namely : 1- The somaclone, S_2 , was the shortest and earliest genotype and it had a high grain yield , grain weight and resistance to YR, but, low severity as ACI for SR; 2- The somaclones, S_{13},S_{14},

S_{28}, S_{36} and S_{39}, had either high or moderate grain yield and high resistance to either two or the three rusts.

Somaclonal variation of some morphological traits and leaf rust reaction among regererants derived from callus of bread wheat cultures , were reported by Chauhan and Singh (1995) .

Cluster analysis :

One of the aims of this study was to determine the relation between the parent, S-69, and its somaclones .

Cluster analysis is a collection of statistical methods, which identifies groups of samples that behave similarity or show similar characteristics.

The standardized mean values of morphological traits and rust severity, as ACI, were used to construct a similarity matrix (Table 7). The similarity coefficients ranged from 0.05 to 0.86, suggesting a broad genetic base for wheat genotypes .Table (7) presents the morphological similarity estimates of the 162 pairwise comparisons among the somaclones and their parent, S-69 .

Figure (1) illustrates the clustering of the somaclones and their parent (S-69). Five clusters were obtained at 0.5 similarity level, the first cluster included the parent (S-69) and the somaclone, S_4. Within the second cluster, two clearly separated clusters could be discerned at 0.6 similarity level, one included the somaclones, S_9,S_{11} and S_{15}, and the other included S_{13} S_{36},S_{14},S_{39},S_{17},S_{24} and S_{28}. It is clear that this group included more of the unique genotypes and they differed from the parent, S-69 . The third cluster included the somaclones, S_8 and S_{33}, and they had the highest similarity level (0.86).The fourth and fifth clusters, each included two somaclones (S_{25} and S_{29}) and (S_2 and S_3) , respectively.

The results obtained from the analysis of variance and cluster analysis indicated that there were clear differences among somaclones, as well as their parent .Jayasankar and Dennis (2003) suggested that the in vitro selection had a significant potential to produce genetically improved plants, compared to their parents.

From the results of the present study, it was concluded that :1- *In vitro* selection method was effective for producing valuable variations in the form of somaclonal variant lines, 2- Several of these somaclones were superior to the parent in grain yield and resistance to rust diseases, so that they might be selected and subjected to further evaluation for the production of new lines.

Table1: Analysis of variance for grain yield and ACI for LR for the selected somaclones for rusts resistance, as well as their wheat cultivar parent (Sakha-69) during 2005/2006 season.

SOV	DF	Grain yield/m^2	ACI
Replicates	1	0.0001	352.44
Genotypes	40	0.0267**	197.80
Error	40	0.0070	297.44

** : Significant at 0.01 probability levels.

Table 2: Means of grain yield /m^2 for the selected somaclones for rusts resistance, as well as their wheat cultivar parent (Sakha-69) during 2005/2006 season.

Genotype	Grain yield/ (kg/m^2)	Genotype	Grain yield/ (kg/ m^2)	Genotype	Grain yield/ (kg/ m^2)
S-69	0.820	S14	0.605	S28	0.608
S_1	0.602	S15	0.808	S29	0.760
S_2	0.538	S16	0.678	S30	0.543
S_3	0.800	S17	0.753	S31	0.518
S_4	0.635	S18	0.613	S32	0.648
S_5	0.800	S19	0.688	S33	0.658
S_6	0.630	S20	0.645	S34	0.268
S_7	0.625	S21	0.678	S35	0.630
S_8	0.685	S22	0.813	S36	0.570
S_9	0.743	S23	0.538	S37	0.668
S_{10}	0.413	S24	0.745	S38	0.683
S_{11}	0.775	S25	0.638	S39	0.605
S_{12}	0.748	S26	0.750	S40	0.710
S_{13}	0.878	S27	0.608		

L.S.D. at 0.05 level= 0.169 kg.

Table 3: Analysis of variance for grain yield and its components for the selected somaclones for rusts resistance, as well as their wheat cultivar parent (Sakha-69) during 2006/2007 season.

SOV	DF	Plant height (cm)	No. spikes/ m^2	No. grains/ spikes	1000-grain weight(g)	Grain yield / m^2 (kg)	Harvest index %	Date of maturity (days)
				Nubaria				
Replicates	1	28.62	2336	0.16	6.33	0.04	1.69	1.36
Genotypes	17	127.45**	7223 **	0.46	52.99*	0.12	59.43	32.60**
Error	17	21.57	1624	0.41	18.34	0.10	39.6	0.83
				Sakha				
Replicates	1	140.03	2889	0.01	1.00	0.04		
Genotypes	17	58.36**	64973**	0.36	42.29**	0.13**		
Error	17	6.79	20443	0.35	6.35	0.02		

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

Table 4: Means of grain yield and its components in the selected somaclones for rusts resistance, as well as the wheat cultivar parent (Sakha-69) at Nubaria and Sakha areas.

Genotype	Plant height (cm)		Number of spikes / m²		1000- grain weight (g)		Grain yield/ (kg/ m²)	Date of maturity (days)
	Nubaria	Sakha	Nubaria	Sakha	Nubaria	Sakha	Sakha	Nubaria
S-69	109.8	113.0	675.0	643.1	46.0	50.0	0.905	154.0
S₂	92.9	100.0	704.9	854.4	50.1	57.5	1.105	141.5
S₃	106.5	117.5	639.6	651.7	48.0	53.0	1.130	146.0
S₄	106.0	112.5	675.0	842.7	47.0	48.0	0.970	151.5
S₈	100.8	117.5	664.6	1073.2	35.0	43.5	0.830	154.0
S₉	105.2	117.5	645.2	1062.7	44.1	45.0	0.920	154.0
S₁₁	114.1	115.0	630.0	1247.5	47.4	47.0	1.070	146.5
S₁₃	102.4	117.5	640.1	1058.2	43.5	49.5	1.000	154.0
S₁₄	111.9	115.0	730.1	1107.5	44.5	51.0	1.060	154.0
S₁₅	113.8	115.0	629.5	1268.0	49.3	49.0	1.080	154.0
S₁₇	99.3	115.0	649.2	1068.6	50.1	47.5	1.135	154.0
S₂₄	116.4	120.0	675.0	964.7	49.2	49.0	1.090	154.0
S₂₅	123.9	127.5	769.0	892.2	43.8	38.0	0.345	154.0
S₂₈	101.6	120.0	789.6	971.0	48.9	46.0	0.985	154.0
S₂₉	122.5	120.0	780.1	1101.6	30.5	40.5	0.425	145.0
S₃₃	107.1	120.0	680.2	909.7	42.6	43.0	0.530	154.0
S₃₆	108.8	120.0	. 97.0	1102.9	46.3	49.0	0.900	154.0
S₃₉	113.0	117.5	824.9	978.4	47.4	51.5	1.290	154.0
LSD₍₀.₀₅₎	9.8	9.8	85.0	301.7	9.0	5.3	0.260	1.9

Table 5: Analysis of variance for average coefficient of infection (ACI) for leaf, yellow and stem rusts (LR, YR and SR) for the selected somaclones for rusts resistance, as well as their wheat cultivar parent (Sakha-69).

SOV	D.f.	Nubaria		Sakha
		LR	SR	YR
Replicates	1	544.4	527.5	756.3
Genotypes	17	1370.4**	676.3**	1833.6**
Error	17	344.2	48.9	77.8

** : Significant at 0.01 probability levels.

Table 6: Means of average coefficient of infection (ACI) for leaf, yellow and stem rusts, (LR, YR and SR) for the selected somaclones for rusts resistance, as well as their wheat cultivar parent (Sakha-69).

Genotype	Nubaria		Sakha
	LR	SR	YR
S-69	33	0.2	65
S_2	100	20	0.5
S_3	25	30	50
S_4	14	0.2	50
S_8	48	0.2	50
S_9	12	0.2	70
S_{11}	25	75	55
S_{13}	10	0.2	12
S_{14}	10	0.2	80
S_{15}	28	15	50
S_{17}	35	20	30
S_{24}	80	3	70
S_{25}	50	3	95
S_{28}	50	0.2	10
S_{29}	55	12.5	100
S_{33}	50	12.5	100
S_{36}	12	4.1	20
S_{39}	1	0.2	50
$LSD_{(0.05)}$	39.15	14.75	18.61

Table 7: Morphological similarity matrix, based on Jaccard's coefficient for Sakha-69 wheat cultivar and its somaclones determined from analysis, using the mean values of grain yield , grain yield components and average coefficient of infection (ACI).

|	S-69	S2	S3	S4	S8	S9	S11	S13	S14	S15	S17	S24	S25	S28	S29	S33	S36	S39
S-69	1.00																	
S2	0.27	1.00																
S3	0.33	0.70	1.00															
S4	0.57	0.46	0.54	1.00														
S8	0.29	0.15	0.23	0.20	1.00													
S9	0.38	0.27	0.33	0.38	0.64	1.00												
S11	0.29	0.36	0.43	0.38	0.38	0.57	1.00											
S13	0.35	0.43	0.50	0.44	0.58	0.53	0.53	1.00										
S14	0.53	0.37	0.42	0.53	0.39	0.61	0.61	0.58	1.00									
S15	0.47	0.38	0.44	0.47	0.50	0.67	0.79	0.73	0.78	1.00								
S17	0.39	0.47	0.44	0.56	0.40	0.56	0.47	0.63	0.68	0.56	1.00							
S24	0.47	0.47	0.53	0.65	0.41	0.56	0.47	0.71	0.75	0.63	0.72	1.00						
S25	0.50	0.05	0.15	0.33	0.33	0.50	0.32	0.32	0.63	0.40	0.42	0.48	1.00					
S28	0.39	0.38	0.44	0.56	0.50	0.67	0.47	0.63	0.60	0.56	0.75	0.82	0.50	1.00				
S29	0.18	0.06	0.12	0.25	0.33	0.33	0.31	0.31	0.42	0.26	0.35	0.42	0.60	0.44	1.00			
S33	0.21	0.08	0.15	0.13	0.86	0.55	0.31	0.50	0.33	0.43	0.33	0.35	0.36	0.43	0.36	1.00		
S36	0.50	0.31	0.38	0.41	0.54	0.50	0.50	0.79	0.63	0.69	0.59	0.67	0.44	0.59	0.38	0.46	1.00	
S39	0.50	0.29	0.33	0.43	0.37	0.43	0.50	0.63	0.76	0.65	0.57	0.71	0.57	0.57	0.45	0.32	0.68	1.00
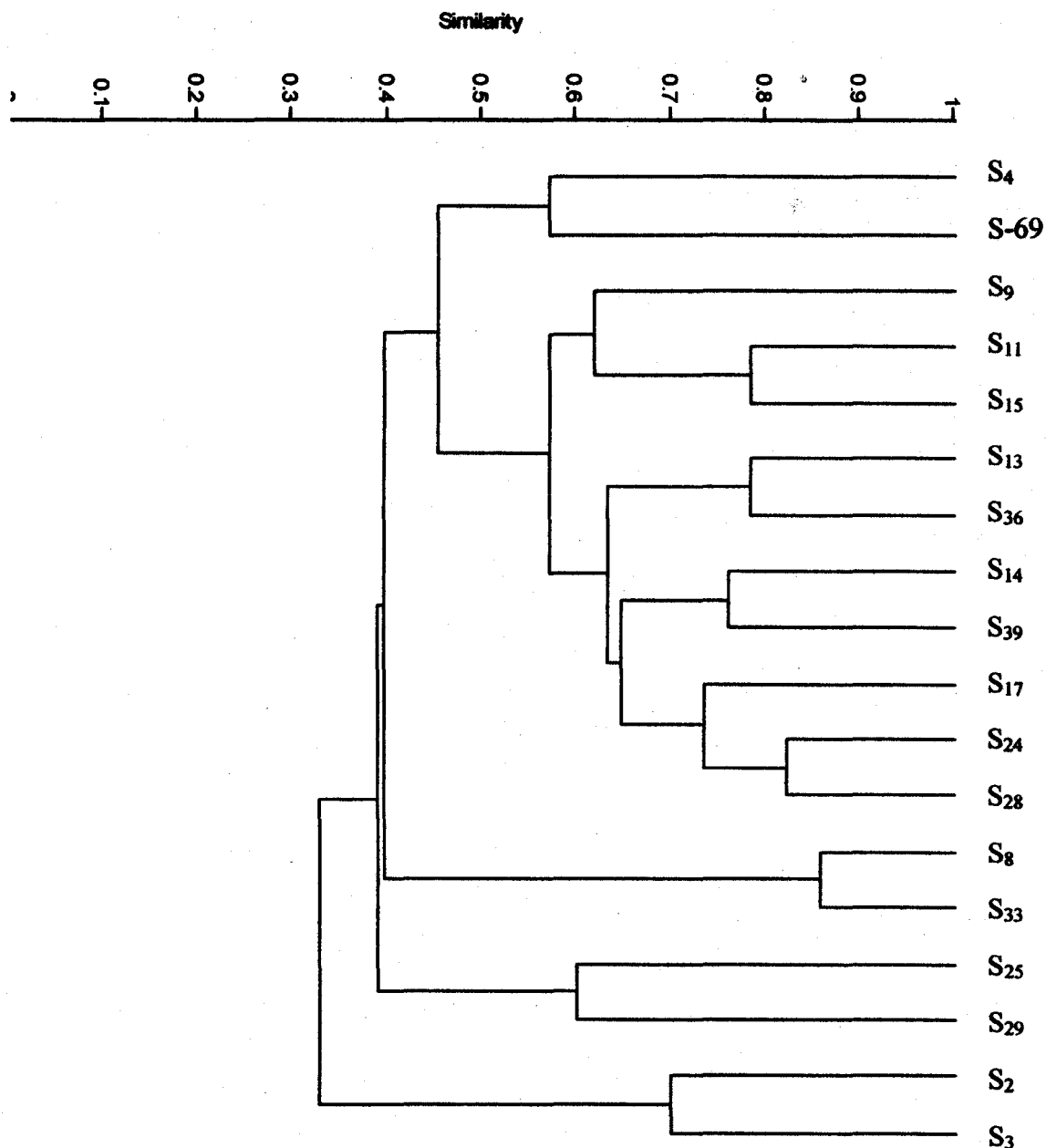
Fig. 1: Dendrogram for Sakha-69 wheat cultivar and its somaclones, based on a cluster analysis (UPGMA) of phenotypic similarities (Jaccard's coefficient) for grain yield, grain yield components and average coefficient of infection (ACI).

REFERENCES

Abdalla , MY , MN Barakat, MI Motawia and AA AL - Rokaibah. 2002. *In vitro* selection for resistance to *Fusarium graminearum* in embryogenic culture wheat and the use of RAPD markers for evaluating. Alex.J.Agric. Res. 47 (1) : 67-75.

Barakat,M.N. and S.I. Milad. 2006. *In vitro* selection and identification of somaclonal variants of wheat resistance to yellow rust . Alex. J. Agric. Res. 51: (2) 87-97.

Barakat,M.N. and S.I. Milad and I.A. Imbaby.2005. Field evaluation for leaf rust disease and RAPD analysis for somaclonal variant lines in wheat. Alex. J. Agric. Res. 50 (3) 11-24.

Berlin ,J. , L. Witte, J. Hammer , K.G. Kuboschke, A. Zimmer and D. Pare. 1982. Metabolism of P-fluorophenylalanine sensitive and resistant cell cultures. Planta 155:244-250.

Chauhan, R.S. and B.M. Singh. 1995. Variation for some morphological traits and leaf rust reaction among regererants of bread wheat, Somalia cultivar. Cereal Research Communications 23(1&2):109-116.

El-Daoudi,Y.H., 1998. Wheat stripe rust management, considering pathotype, dynamics, identified host resistance genes and the econnmic threshold of controlling the disease. Annual Report 97/1998.

El-Daoudi,Y.H., I. Shafik, E.H. Ghanem, S.A. Abu El= Naga, S. Sherief , M.O. Khalifa , R.A. Mitkees and A.A. bassiouni .1996 .stripe rust occurrence in Egypt and assement of grain loss in 1995 . Proc.du symbosium regional sur les maladies des cereals et des legumineuses alimrntaries 11-14 Nov., 1996, Rahat, Morraco pp. 341-351.

Jaccard,P.1908. Nouvelles rechearches sur la distribution lorale. Bull. Soc.Vaud. Sci. Nat. 44:223-270.

Jayasankar, S. and J. G. Dennis.2003. *In vitro* selection for disease resistance in plants as an alternative to genetic engineering.Ag. Biotech.Net . Vol. 5 May , ABN 111.

Karp, A. 1995. Somaclonal variation as a tool for crop improvement . Euphytica 85: 292-302.

Larkin, P.Y. and W.R. Scowcroft. 1981. Somaclonal variation-a novel source of variability from cell culture for plant improvement. Theor. Appl. Genet.60:197-124.

Nazim , Y. , A . Abdou and S. Sherief .1983. Virulence survey and population shifts of *Puccini recondita* in Egypt . Soci. Applied Microbial. Vol. 3 :29-42, Cairo , Egypt.

Nazim ,Y., A. A. EL-Shehedi, S.Z. El-Basiouni and T. M. Abdel-Hak. 1976. The relative effectiveness of wheat leaf rust resistance genes in seedling and adult stages .2nd Congr. Egypt. Phytopathol. Soc. pp. 627-641.

Oberthur.L.E. S.A. Harrison , T.P. Croughan and D.L, Long . 1993. Inheritance of improved leaf rust resistance in somaclones of wheat. Crop Science 33(3): 444-448.

Palmer ,J.E. and J. Widholm. 1975. Characterization of carrot and tobacco cell culture resistant to P- fluorophenylalanine. Plant Physiol. 56:233-238.

Rohlf, F.J. (2000). NTSYS-PC : Numerical taxonomy and multivariate analysis system .Version 2.1.Exerter Software, New York, USA.

SAS, (2007) SAS/STAT Guide for personal computers. Version9 edn.SAS end. SAS institute, Cary.N.C

Sherief , S. , Y.H. El-Ghamry and R.A. Rizk. 1992. Pathogenetically association and population shifts of *Puccinia recondita* in Egypt during 1989-1991.Zagazig .J. Agric. Res. 19 (1) :227-241 .

Sokal ,R.R and C.D. Michesner.1958. A statistical method for evaluation systematic relationships. University of Kansas. Science Bulletin 38:1409-1438.

Stubbs , R.W. , J.M. Prescott ,E.E Saari , H.J. Dubin .1986.Cereal disease methodology manual CIMMYT , Mexico, pp. 22-25

Widholm, J. M. 1987. Selection of mutants which accumulate desirable secondary compound. In: F. Constable and I.K. Vasil (ed), Cell Culture and Somatic Cell Genetics of Crops. Vol. 4. Cell Culture in Phytochemistry. Academic Press, Inc.,San Diego U.S.A.pp.125.

Wiese, M.V.1987. Compendium of wheat diseases. American Phytopathology Society, St. Paul, MN., USA.

Winer .1971. Statistical Principles in Experimental Design,2nd Ed. McGraw –Hill . N.Y ,USA

Woldeab, G., C. Finisa, H. Singh, J.Yuen and J.Crossa. 2007. Variation in partial resistance to barley leaf rust (*Puccinia hordeii*) and agronomic characters of Ethiopian landrace lines Euphytica 158:139-151.

Yang,Z.X. Yang and D. Haung. 1998. Studies on somaclonal variants for resistance to scab in bread wheat (*Triticum aestivum L.*) through *in vitro* selection for tolerance to deoxynivalenol . Euphytica 101:213-219.

الملخص العربى

تقويم حقلى وانتخاب للمقاومة للأصداء (.Puccinia spp) فى Somaclonal Variant Lines
فى القمح

د. سناء إبراهيم محمد ميلاد

معمل التقنية الحيوية– قسم المحاصيل– كلية الزراعة (بالشاطبى)– جامعة الإسكندرية–مصر

تم إنتاج أربعين somaclones من صنف القمح"سخا – ٦٩" باستخدام الإنتخـاب المعملـى بطريقـة Stepwise بواسـطة مركـب p-fluorophenylalanine (PFP) كمادة للانتخاب المعملى لانتاج خلايا طافرة مقاومة لمرض الصدأ الأصفر. تم تقـويم هـذه المنتخبـات بالاضافة إلى الأب (صنف القمح سخا–٦٩) حقليا فى منطقة سخا وذلك لمحصول الحبوب ومتوسط معامـل العـدوى لمـرض صـدأ الأوراق (ACI). تم إجراء التجربة فى صورة تصميم قطاعات كاملة العشوائية(فى مكررتين).وقد أثبتت النتائج أن هناك فـروق معنويـة جـداً بـين التراكيب الوراثية فى صفة محصول الحبوب، بينما لم توجد فروق معنوية بينها فى درجة الإصابة (ACI) .

تم إنتخاب سبعة عشر somaclones تبعاً لصفة محصول الحبوب ودرجة الإصابة . وتم تقويم هذه المنتخبات بالأضافة إلـى الأب فـى تجربتين، إحداهما فى منطقة سخا والأخرى فى منطقة النوبارية. أجريت كل من التجربتين فى صورة تصميم قطاعات كاملة العـشوائية(فـى مكررتين). تم قياس بعض الصفات المورفولوجية فى المنطقتين (إرتفاع النبات ووزن ألف حبة و عدد الحبوب بالسنبلة ومحصول الحبـوب بالمتر المربع) ، وصفات اخرى تم تقديرها فى منطقة النوبارية (معامل الحصاد وتاريخ النضج) . وجدت فروق معنوية أو معنوية جداً فـى صفات ارتفاع النبات وعدد السنابل بالمتر المربع ووزن الألف حبة وذلك فى المنطقتين، ومحصول الحبوب بالمتر المربع فـى منطقـة سـخا وتاريخ النضج فى النوبارية . بينما لم تظهر باقى الصفات فروقا معنوية بين التراكيب الوراثية.

وبتحليل شدة الأصابة بالامراض، وجدت فروق معنوية جداً بين التراكيب الوراثية فى الأصابة بالـصدأين (LR و SR) فـى النوباريـة والصدأ (YR) فى سخا وذلك عند تقدير الأصابة فى صورة ACI.

استخدم تحليل Cluster للصفات المورفولوجية وصفات الأصابة بالأصداء لتحديد العلاقة بين الأب والمنتخبـات . وأوضـحت النتـائج المتحصل عليها من تحليل التباين وتحليل Cluster أن هناك فروقا واضحة بين التراكيب الوراثية .

يستنتج من هذه الدراسة أن الأنتخاب المعملى فعال لأنتاج اختلافات، وكثير من هذه الأختلافات كانت متفوقة على الأب فـى محـصول الحبوب ومقاومة لأمراض الأصداء تحت الدراسة ولذلك يمكن انتخابها ولإجراء المزيد من التقويم لأنتاج سلالات جديدة.and was not