

# In Vivo and In Vitro Selection of Superior Durum Wheat (*Triticum durum*, Desf.) Genotypes

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## Abstract

Evaluation and selection experiments for thirteen durum wheat genotypes included two local varieties and eleven introduced lines during two successive seasons (2002/2003 and 2003/2004). The tested genotypes showed highly significant differences in growth characters, yield components, callus induction, callus growth rate and plant regeneration in the two growing seasons and their combined analysis. MSWD-2 line followed by MSWD-9 surpassed the other tested genotypes in number of tillers/plant, main spike weight (g), spikes weight/plant (g), 100-grains weight (g), grains yield/plant (g), grains yield ton/feddan, callus induction, callus growth rate and plant regeneration. However, MSWD-19 genotype is the tallest one, while the NRCWD-32 produced the highest value of main spike length. High genetic advance, as percentage of the mean, was found to be associated with high heritability values in cases of main spike weight, spikes weight/plant, 100-grains weight, grains yield/plant and grains yield ton/fed. Superior genotypes are used in breeding programs to save much efforts and costs to breeder.

**Key words:** Evaluation, growth, yield, genetic advance.

## INTRODUCTION

The production of wheat (*Triticum durum*, Desf.) in Egypt is not enough for human consumption. Meanwhile the cultivated land is limited and wheat occupying small area of this land because of the competition between it and the other winter crops. In Egypt, economicists, agriculturists and successive governments planning agencies aim to develop the new reclaimed lands. Plant breeders had the essential role in this problem upon breeding new varieties and introduced genotypes and selection of new genotypes, as well as, suitability to local climate and conditions. The genetic modification of plants *in vitro* via transformation techniques is largely depend upon the ability of the tissue to regenerate whole plants.

The aim objective of this investigation was to determine the performance and behaviour of introduced lines as well as selection of superior genotypes as much as yield, its components and genetic advance are concerned for wider application in the country.

## MATERIALS AND METHODS

The present investigation was carried out during the two successive seasons of 2002/2003 and 2003/2004 at the Experimental Station of National Research Centre at Shalakan, Kaluebia Governorate and tissue culture laboratory, Botany Department, to evaluate of superior genotypes of durum wheat (*Triticum durum* Desf.).

### Field experiment

Thirteen tested genotypes included two local varieties (Benysweif-2, Sohag-2), as well as eleven introduced lines, MSWD-2, MSWD-9, MSWD-19, MSCWD-20, NRCWD-12, NRCWD-13, NRCWD-14, NRCWD-16, NRCWD21, NRCWD-30 and NRCWD-32. The tested genotypes were

arranged in a randomized complete block design with 3 replicates. The experimental unit consisted of 15 rows, each of 3.5 meter length and 20 cm. apart between rows, where the size of each plot was 10.5 square meter. Seeds of the genotypes were sown on 27<sup>th</sup> November in 2002 and 2003 in the two seasons, respectively. The normal agronomic practices of growing wheat was carried out till harvest as recommended by wheat Research Dept., Field Research Institute, Agriculture Research Center, Cairo, Egypt.

At harvest, ten plants were randomly taken from the middle rows of each plot to determine plant height (cm), number of tillers/plant, main spike length (cm), main spike weight (gm), spikes weight/plant (g) 100-grains weight (g), grains yield per plant (g) and grains yield ton/feddan were measured from the whole area of plant and then converted to yield per feddan.

The data obtained were subjected to the proper statistical analysis according to Snedecor and Cochran [1]. For comparison between means L.S.D. according to Wynne *et al.*<sup>(2)</sup> at 0.05 level was used. Combined analysis was performed for the data of both seasons according to Snedecor and Cochran<sup>(1)</sup>. Wheat sense heritability ( $h^2\%$ ), genetic advance under selection ( $\Delta g$ ) and genetic advance under selection as percentage of the mean ( $\Delta g\%$ ) were calculated for the combined data according to Johnson *et al* [3].

### Tissue culture experiment

Immature embryos (thirteen wheat genotypes), 11-15 days old after anthesis (DAA) were harvested from the florets, surface-sterilized for 5 min. in bleaching solution (commercial chlorox 20% mixed with two drops of tween 20), then washed with sterile distilled water for five times. The agar medium used in this study Murashige and Skoog [4]. (MS), modified by B5-vitamins Gamborg *et al* [5].

Forty embryos (0.8 to 1.5 mm. in diameter) of each genotypes were planted on the above mentioned MS medium supplemented with 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D) in ten replicates (jars) with a frequency of four embryos per jar for callus induction.

The previous mentioned medium was used for five times subculturing with an interval period of seven days. After two weeks, all calli were transferred to the same medium except the concentration of 2,4-D as decreased to 0.5 mg/l (Shooting medium) and free hormone medium for root formation. All media were adjusted at pH 5.8 and autoclaved for 20 min. The cultures used at the former stages were kept at 26° and 16/8 hours day light and dark, respectively. The percentage of callus induction defined as the embryos forming callus over the total number of embryos. Callus growth rate was calculated according to Bhaskaran *et al* [6]. Percentage of plantlets formation was calculated. The experimental design was complete randomized blocks. Analysis of variance and L.S.D. values were estimated according to Wynne *et al* [2].

## RESULTS

### Varietal performances

Individual and combined analysis of variance for two seasons including all traits studied are presented in Table (1). Mean squares due to genotypes were found to be highly significant for plant height, number of tillers/plant, main spike length, main spike weight, spikes weight/plant, 100-grains weight, grains weight/main spike, grains yield/plant and grains yield/fed. in the two growing seasons as well as when combined.

#### A. Growth characters, yield and yield components:

Mean performances of the thirteen durum wheat genotypes for all studied, based on the combined analysis over the two growing seasons are shown in Tables (2 and 3).

##### 1. Plant height

Results obtained in Tables (2 and 3) reveal highly significant differences among the wheat enterier under study

for plant height ranged from 91 cm for Benysweif-2 to 119 cm for MSWD19, thus the Benysweif-2 genotype is considered as shortest genotypes in the wheat population followed by Sohag-2, NRCWD-30 and NRCWD-32 which had mean values of 91.17, 100.17 and 102.17 cm, respectively. On the other hand, line MSWD-19 is considered the tallest one followed by MSWD-2 and NRCWD-21 which gave mean values of 119.83, 117.30 and 115.33 cm, respectively.

The broad sense heritability ( $h^2$ ) for plant height in the wheat population under the study gave a value of 42.90% which is considered a moderate estimate Table (3). The expected genetic gain upon selecting the tallest 5% of the wheat lines would be 14.77 cm or about 14.10% of the general wheat population mean over the two seasons.

##### 2. Number of tillers per plant

Results presented in Tables (2 and 3) show highly significant differences among the wheat genotypes under study in number of tillers per plant. Thus the best line for number of tillers per plant is MSWD-2 followed by NRCWD-21 and MSWD-19 which gave mean values of 7.44, 6.99 and 6.74 tillers/plant respectively, while the lowest one is NRCWD-12, Sohag-2 and NRCWD-32 with mean values of 5.29, 5.34 and 5.35 tillers/plant respectively. Estimation of the broad sense heritability ( $h^2$ ) for this trait gave a value of 62.81% (Table 3) which is considered a relatively high estimate, indicating that this trait is mainly determined by the genetic factors and partially by the environmental ones.

##### 3. Main spike length

Date presented in Tables (2 and 3), there wee highly significant between NRCWD-32, MSWD-9, MSWD-2, NRCWD-16 and other genotypes under the study this trait, NRCWD-32 was the best main spike length, which gave 10.10 cm, while MSWD-20 the lowest one (6.28 cm) Table (2). As shown in Table (3) the broad sense heritability ( $h^2$ ) for this trait gave an estimate of 29.43%, which is considered as a low value. The expected genetic gain upon selecting the tallest spiked 5% of

**Table 1.** Individual and combined analysis of variance of the growth, yield and yield components of thirteen durum wheat genotypes during 2002/2003 and 2003/2004 season

Season	S.O.V.	D.F.	Plant height (cm)	No. of tillers/plant	Main spike length (cm)	Main spike weight (g)	Spikes weight/plant (g)	100-grains weight (g)	Grains weight/main spike (g)	Grain yield/plant (g)	Grains yield ton./fed.
2002-2003	Replications	2	56.926*	0.069	0.078	0.179	3.303	0.094	0.017	0.267	3.415
	Genotypes	12	158.559**	1.423**	1.637**	7.147**	148.472**	1.286**	3.376**	75.054**	150.213**
	Error	24	21.893	0.086	0.309	0.185	2.111	0.063	0.066	1.656	2.216
2003-2004	Replications	2	54.674*	0.079	1.377*	0.108	2.238	0.047	0.100	1.359	2.967
	Genotypes	12	176.400**	1.442**	2.218**	7.266**	154.385**	1.489**	3.395**	73.953**	152.632**
	Error	24	28.370	0.146	0.562	0.296	1.666	0.067	0.122	1.091	1.734
Combined	Years	1	0.928	0.001	0.317	0.000	0.769	0.006	0.004	0.726	0.873
	Replications within years	4	55.800	0.074	0.727	0.143	3.271	0.070	0.059	0.813	3.392
	Genotypes	12	332.984**	2.864**	3.746**	14.411**	302.396**	2.763**	6.770**	148.931**	302.613**
	Geno. x years	12	188.127	1.695	2.116	3.603	182.166	0.921	1.693	88.125	175.948
	Error	48	25.131	0.116	0.435	0.240	1.889	0.065	0.094	1.373	1.975

\* and \*\* significant at 0.05 and 0.01 levels, respectively.

the population for main spike length was 1.32 or about 16.50% of the wheat population mean.

#### 4. Main spike weight (g)

Results obtained in Tables (2 and 3) reveal highly significant differences among the wheat lines under the study in main spike weight (g). However, mean values for this trait over the two season ranged from 3.45g for NRCWD-12 to 6.91g for MSWD-2. MSWD-2 is considered the best line in the wheat population under study, followed by MSWD-9 (6.71g), while the lowest one is NRCWD-12 followed by Sohag-2 (3.51g). The broad sense heritability for main spike weight in wheat population Table (3) gave a value 82.17% which is considered a high estimate.

ranged from 18.25 g for NRCWD-12 genotypes to 51.41 g for MSWD-2. Thus, genotype MSWD-2 exhibited the highest spikes in the wheat population under study, followed by MSWD-9 (42.34 g) and MSWD-19 (40.84 g) respectively. The broad sense heritability ( $h^2$ ) for spikes weight per plant gave an estimate of 92.83% which is considered as a high value, indicating this trait is mainly affected by the genetic factors and genotype (Table 3). The expected genetic upon selecting the best 5% for spikes weight per plant would be 20.0 (g) or about 69.66% of the population mean (Table 3).

#### 6. 100-grains weight (g)

Results obtained in Tables (2 and 3) revealed significant differences among the wheat genotypes under the study for

**Table 2.** Means values for growth characters, yield and yield components of thirteen tested durum wheat genotypes (combined analysis for the two growing seasons).

Genotypes	Plant height (cm)	No. of tillers per plant	Main spike length (cm)	Main spike weight (g)	Spikes weight per plant (g)	100-grains weight (g)	Grains yield per plant (g)	Grains yield ton/ fed.
Benysweif-2	91.00	5.52	8.03	3.67	20.26	5.39	10.10	3.11
Sohag-2	91.17	5.34	7.43	3.51	18.74	4.35	9.63	2.78
MSWD-2	117.30	7.44	8.83	6.91	51.41	6.59	20.53	5.54
MSWD-9	109.33	6.31	8.97	6.71	42.34	6.30	16.03	4.99
MSWD-19	119.83	6.74	7.68	6.06	40.84	6.28	15.89	4.96
MSWD-20	106.67	6.20	6.28	5.83	36.15	4.74	14.77	4.92
NRCWD-12	112.33	5.29	8.30	3.45	18.25	4.84	9.33	2.60
NRCWD-13	110.83	6.15	7.48	4.41	25.46	5.80	8.36	3.98
NRCWD-14	103.00	6.44	7.43	5.09	32.78	4.70	13.93	4.82
NRCWD-16	103.67	6.38	8.12	5.16	32.92	5.18	13.97	4.85
NRCWD-21	115.33	6.99	7.82	4.09	28.56	4.91	11.09	4.10
NRCWD-30	100.17	6.06	7.53	4.90	29.69	4.26	13.46	4.34
NRCWD-32	102.17	5.35	10.10	3.55	18.99	5.31	9.95	2.82
Means	106.37	6.17	8.00	4.87	30.49	5.28	12.85	4.14
L.S.D. 5%	11.97	0.45	1.37	0.61	2.90	0.43	1.45	2.96
L.S.D. 1%	19.86	0.73	2.27	1.01	4.81	0.71	2.40	4.91

The expected genetic gain upon selecting the best 5% for main spike weight would be 4.13 (g) or about 86.76% of the population mean.

#### 5. Spikes weight per plant (g)

Data presented in Table (2 and 3) show that significant differences among the wheat genotypes under the study for spikes weight per plant. Mean values over the two seasons

100-grains weight over the two seasons ranged from 4.26 g for NRCWD-30 to 6.59 g for MSWD-2 giving a difference of about 2.33 g with a general mean value of 5.28 g. (Table 2). Thus MSWD-2 exhibited the lightest kernalled plants in the wheat population under study, followed by MSWD-9 and MSWD-19, which had a mean value of about 6.30 and 6.28 g for 100-grains weight respectively.

**Table 3.** Genetical parameters of yield and its components of thirteen tested durum wheat genotypes (combined analysis for the two growing seasons).

Traits	Genetical parameters		
	Heritability ( $h^2$ %)	Genetic advance	
		( $\Delta$ g)	( $\Delta$ g %)
Plant height (cm.)	42.90	14.77	14.10
No. of tillers/plant	62.81	1.63	27.42
Main spike length (cm.)	29.43	1.32	16.50
Main spike weight (g)	82.17	4.13	86.76
Spikes weight/plant (g)	92.83	20.00	69.66
100-grains weight (g)	75.86	1.74	32.95
Grains yield/plant	89.68	13.81	109.95
Grains yield ton/fed	85.74	28.63	103.73

**Table 4.** Means values for callus induction, callus growth rate and plant regeneration of thirteen durum wheat genotypes.

Genotypes	Callus induction %	Callus growth rate %	Plant regeneration %
Benysweif -2	67.50	170.46	58.26
Sohag-2	64.50	92.64	54.72
MSWD-2	80.27	193.22	82.18
MSWD-9	78.53	189.28	80.31
MSWD-19	77.73	187.63	69.77
MSWD-20	76.73	186.19	68.04
NRCWD-12	63.13	78.24	56.34
NRCWD-13	69.53	173.32	59.76
NRCWD-14	75.77	183.52	65.62
NRCWD-16	76.23	185.63	67.69
NRCWD-21	70.10	174.66	60.12
NRCWD-30	71.57	176.11	61.20
NRCWD-32	65.60	112.88	56.18
Mean	72.09	161.83	64.66
L.S.D. 5%	3.50	5.74	3.76
L.S.D. 1%	4.69	7.69	5.04

As shown in Table (3) the broad sense heritability for this trait gave an estimate of 75.86% which is considered as a high value. The expected genetic gain upon selection the best 5% of the population for 100-grain weight would be 1.74 g or 32.95% of the population mean Table (3).

### 7. Grains yield/plant (g)

Data presented in Tables (2 and 3) reveal highly significant differences among the wheat genotypes under study for grain yield/plant. Mean values of grain yield/plant over the two seasons ranged from 8.36 g for NRCWD-13 to 20.53 g for MSWD-2 giving a difference of about 12.17g with a general mean value of 12.85g. Thus, NRCWD-13 is considered as the lowest grain yielding line in the wheat population under the study followed by NRCWD-12, Sohag-2, and Benysweif-2 which gave mean values 9.33, 9.63 and 10.10 g, respectively. On the other hand, MSWD-2 had the highest yielding capacity in these genotypes wheat studied, followed by MSWD-9, MSWD-19 and MSWD-20 which gave mean values 16.03, 15.89 and 14.77 g respectively (Table 2). As presented in Table (3) the broad sense heritability for grain yield/plant had an estimate of 89.68% which is considered as a high value, indicating that this trait is affected by genetic factors. The expected genetic gain upon selecting the best 5% of the genotypes for grain yield/plant would be 13.81 g or about 109.95% of the population mean.

### 8. Grain yield ton/feddan

Results presented in Tables (2 and 3) show that highly significant differences among wheat genotypes under the study for grain yield ton/fed. Their yield ranged from 2.60 tons/fed for NRCWD-12 to 5.54 tons/fed with a general mean value of 4.14 tons/fed. There are positive correlation between grains yield/plant and grains yield ton/fed. MSWD-2 genotype had the highest yield (5.54 ton/fed) over all genotypes studied, followed by MSWD-9, MSWD-19 and MSWD-20 which gave values 4.99, 4.96 and 4.92 tons/fed, respectively. The broad sense heritability for grains yield/fed had an estimate of 85.74% which is considered as a high value. The expected genetic gain upon selecting the best 5% of the genotypes would be 28.63 tons or about 103.73% of the population mean (Table 3).

## B. Tissue culture studies

### 1. Callus induction

Data presented in Table (4) show that calli friable and embryogenic calli derived from immature embryos were formed on the thirteen tested genotypes. There was highly significant difference between inbred MSWD-2 and two wheat cultivars (Benysweif-2 and Sohag-2) and NRCWD-30, NRCWD-21, NRCWD-13, NRCWD-32 and NRCWD-12 on callus induction, respectively.

### 2. Callus growth rate

There were differences in callus growth rate between the thirteen tested genotypes. There highly significant between two wheat cultivars (Benysweif-2 and Sohag-2). Inbred MSWD-2 scored the highest percentage to callus growth rate (193.22%) over the thirteen tested genotypes, whereas the lowest one was NRCWD-12 (78.24%). There was highly significant differences between MSWD2 and two wheat cultivars (Benysweif-2 and Sohag-2) and NRCWD-13, NRCWD-14, NRCWD-21, NRCWD-30 and NRCWD-32 on callus growth rate, respectively.

### 3. Plant regeneration

The embryogenic calli were transferred to the regeneration medium, where green parts appeared on a proportion of the calli, which developed into plantlets. Taking the mean of the regeneration potential for each genotype on the same medium, there were statistically significant differences between regeneration percentages for the thirteen tested genotypes. Percentage regeneration varied widely with respect to genotypes with values ranging from 54.72% to 82.18% Table (4). Both MSWD-2 and MSWD-9 were highly significantly response on plant regeneration (82.18% and 80.31%) respectively over the tested genotypes. The fact that on the same medium some genotypes produced embryogenic calli and regenerated plants, while others were completely incapable or low regeneration percentage suggests there are genetic components controlling this trait. Several researchers have attempted to discover genes or regions within the wheat genome responsible for favourable tissue culture responses such as high plant regeneration

potential Kaleikau *et al* [26], Langridge *et al* [27]. The better response of some lines, such as MSWD-2, MSWD-9, MSWD-19 and MSWD-20, may therefore be due to the endogenous phytohormonal content and its regulatory influence. Four lines have been selected for initial transformation experiments because they possess high regeneration potentials and also because of their importance to wheat breeding programmes.

## DISCUSSIONS

In the following discussion, individual and combined analysis of variance of the growth, yield and yield components of thirteen durum wheat genotypes during 2002/2003 and 2003/2004 seasons were given in Tables (1, 2 and 3). There is enough genetic variance among the tested genotypes for the main characters. On the other hand, mean squares were found to be insignificant for all studied traits indicating that the tested genotypes carried genes with different additive and additive by additive effects which seem to be consistent from year to year. Genotypes x years interaction mean squares were no significant effect in all studied characters, indicating that there was consistent year effect on the differential responses of genotypes and that testing over seasons is very important such results indicated that the seasonal factors had essential effects on grains yield/plant, though such factors had no effective role on the genetic behaviour of the different genotypes for this trait. Highly significant differences among the wheat entries under study for plant height. Similar results were obtained by Ehdai and Waines [7] as well as El-Seidy and Hamada [8] and Abdo [9].

Number of tillers per plant had highly significant differences among the wheat genotypes under study. The expected genetic gain upon selecting the best 5% of the genotypes for this character would be 1.63 tillers or about 27.42% of the population mean (Table 3). In this respect, Rady *et al* [10], Mohamed *et al* [11] and Abdo [9]. Spike length is affected by environmental factors more than by the improvement for this character.

Main spike weight affected by the genetic factors and partially by the environmental ones. This relatively high gain percentage supports the conclusion mentioned above for importance of the genetic role in the expression of this trait. Similar results were obtained by El-Banna and El-Ashry [12] and Abdo [9]. Spike weight per plant gave high gain percentage supports the conclusion mentioned above for importance of the genetic role in the expression of this trait.

100-grains weight revealed significant differences among the wheat genotypes under study. It is affected by genetic factors less than the environmental ones. Thus, selection for this trait would be of limited importance for improving such in this population. This result is in agreement with Sherif *et al* [13], Bahy *et al* [14], and El-Marakby *et al* [15] obtained high broad sense heritability estimates which ranged from 77.62 to 99.70% for this trait. In this respect, Dawla [16], and Ehdai and Waines [7] reported expected genetic gain values 20.77 and 20.61% respectively.

Results presented in Tables (2 and 3) show that highly significant differences among wheat genotypes under the study for grain yield ton/fed. It can be generally concluded from these results that the superiority of high grains yield/

plant is mainly due to the increase in grains yield ton/fed. Similar results obtained by Hassanein *et al* [17], Zaki *et al* [18], Abdel-Hady and Ahmed [19] and Ahmed *et al* [20]. It is well known that grain yield/plant in or wheat is a complex quantitative character controlled by several number of genes and highly affected by the environmental conditions such as climatic and soil factors. In order to improve such trait selection would be done through some essential yield components, where such components are less affected by environmental factors and controlled by limited number of genes, in this position. Nevertheless, the estimated heritability and the expected genetic gain values obtained herein for this trait are considered moderate or relatively high estimates, by which the breeder may improve grain yield per plant upon direct selection for the high yielding plants.

There were differences in callus induction between the thirteen genotypes. No significant difference was detected between two wheat cultivars (Benysweif-2 and Sohag-2). Inbred MSWD-2 gave the highest response to callus induction (80.27%) over the thirteen tested genotypes, while the lowest one was Sohag-2 (64.50%). It could be concluded that the response of varietal wheat tissue culture varies according to genotype and good potential can be identified. These variations in callus induction may be due to gene controlling the expression of somatic embryogenesis or the balance of endogenous hormones. Similar results were obtained by Castillo *et al* [21], Abdel-Hady and Hassan [22] and Abdel-Hady *et al* [23], who reported that at the stage of callus induction from immature embryos of wheat, the most important factor regulating the percentage of callus production seemed to be the genotype rather than the media.

The analysis of variance in Table (4) indicated that the relative growth rate, produced from immature embryos of the thirteen tested genotypes. It could be concluded that the tested genotypes differed in their ability to callus growth rate depending on the genetic make up of the cells. Similar results were obtained by El-Hennawy [24], Abdel-Hady [25], and Abdel-Hady and Ahmed [19].

The embryogenic calli were transferred to the regeneration medium, where green parts appeared on a proportion of the calli, which developed into plantlets. Taking the mean of the regeneration potential for each genotype on the same medium, there were statistically significant differences between regeneration percentages for the thirteen tested genotypes (Table 4). The fact that on the same medium some genotypes produced embryogenic calli and regenerated plants, while others were completely incapable or low regeneration percentage suggests there are genetic components controlling this trait. Several researchers have attempted to discover genes or regions within the wheat genome responsible for favourable tissue culture responses such as high plant regeneration potential Kaleikau *et al* [26], Langridge *et al* [27]. The better response of some lines, such as MSWD-2, MSWD-9, MSWD-19 and MSWD-20, may therefore be due to the endogenous phytohormonal content and its regulatory influence. Four lines have been selected for initial transformation experiments because they possess high regeneration potentials and also because of their importance to wheat breeding programmes.

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