

## Heterosis and Combining Ability Effects for Callus Growth of Wheat (*Triticum durum*, Desf.) *In Vitro*

M.S.S. Abdel-Hady

Department of Botany, National Research Center, Dokki, Cairo, Egypt.

**Abstract:** This investigation studied the ability of tissue culture technique as early testing for heterosis and combining ability in durum wheat. Genetic determination of callus growth *in vitro* of four genotypes of wheat in a diallel cross. Combining ability analysis showed that mean square due to general (GCA) and specific (SCA) combining ability were highly significant for callus growth and also for grain yield. Data also revealed that additive genetic effects were dominant and played the major role in the inheritance of callus growth. Correlation analysis between callus growth *in vitro* and grain yield of plants *in vivo* showed a strong relationship. It could be concluded that tissue culture technique is a useful tool for early screening for combining ability analysis and for the choice of the best parent and also hybrid in the plant breeding programs.

**Key words:** Durum wheat, tissue culture, heterosis, general combining ability, specific combining ability

### INTRODUCTION

Wheat is the most important crop all over the world, and in Egypt. Wheat production is insufficient to meet the local consumption according to the higher increases of population especially in the recent years.

Plant cell and tissue culture techniques are offering very good tools to the breeder which enable genetic improvement of several crops with less effort and in shorter time compared to the conventional breeding methods.

Heterosis in tissue cultures of various plant species, was reported by Izhar and Power<sup>[1]</sup> and Haggan and El-Hennawy<sup>[9]</sup> and Abdel-Hafez and Hamad<sup>[2]</sup>.

Genotypic and phenotypic correlation indicated the possibility of early prediction of heterosis and combining ability for yield and some agronomic traits of maize by measuring callus growth in tissue Haggag and El-Hennawy<sup>[9]</sup> and Abdel-Hady<sup>[1]</sup>.

The main objective of this study is to evaluate the ability of tissue culture technique to predict heterosis in F<sub>1</sub> hybrids for the choice of parents at early stages in the program to save both money and effort.

### MATERIALS AND METHODS

Four durum wheat (*Triticum durum*, Desf.) genotypes namely Benysweif-2 (local variety) and the introduced lines MSWD-2, MSWD-9, NRCWD-16 were used in this

study. These wheat genotypes were crossed in a diallel combination. This six F<sub>1</sub> hybrids and their parents were grown in a randomized complete blocks design with three replications at the Experimental farm of the National Research Center in Shalakan. Plants were seeded in rows 30 cm apart. Distances between plants were 10 cm apart. Ten guarded plants were harvested at random from each plot. Yield of grains for these harvested individual plants were recorded.

Callus cultures for the parents and F<sub>1</sub> were induced from immature embryos (13-16 days after anthesis) as indicated by Haggag<sup>[8]</sup>. The caryopses were removed from the spike, surface-sterilized for 5 min. in 5% clorex (sodium hypochlorite) mixed with two drops of Tween 20 and washed in sterile distilled water. The excised embryos were placed on a solid agar medium with the scutellar portion of the embryo. Murashige and Skoog<sup>[13]</sup> inorganic components and B5-vitamins<sup>[6]</sup> were used. It was supplemented with 150 mg/l asparagines, 0.5 mg/l thiamine HCl, 30 g/l sucrose, 100 mg/l inositol, 8 g/l agar and 2 mg/l 2,4-dichlorophenoxyacetic acid (2, 4-D). The medium was adjusted to pH 5.8, autoclaved at 15 psi, 121°C for 15 min. and incubated at 22°C with a photoperiod of 16/8 hours day light/dark respectively.

The growth of callus fresh weight was determined from 20 cultures for each genotype (4 parents and 6 hybrids) by weighing callus before and after 30 days. The callus growth rate was calculated according to Bhaskaran *et al*<sup>[4]</sup>.

Heterosis values at both *in vitro* and *in vivo* levels were calculated on both mid parent and better parent. General and specific combining ability variances and effects were analysed according to Griffing<sup>[7]</sup> method 4-model 1. The genotypic and phenotypic correlation between callus growth *in vitro* and grain yield per plant *in vivo* among all population was estimated.

**Statistical analysis:** The experimental design was complete randomized blocks. Analysis of variance and L.S.D. values were estimated according to Wynne *et al*<sup>[14]</sup>.

## RESULTS AND DISCUSSIONS

Analysis of variance showed that highly significant differences among entries for callus growth rate and also for grain yield per plant. Table 1 shows that the parents ranged from 96.46 gm. For Benysweif-2 (P<sub>1</sub>) to 193.22 gm. For MSWD-2 (P<sub>2</sub>), with an average of 165.11 gm. The most performing hybrid was MSWD-2X MSWD-9 (389.44 gm and 36.45 gm.) revealed a highly significant response to callus growth rate and grain yield respectively than the other hybrids under investigation, while the hybrid (Benysweif-2 XNRCWD-16) gave the lowest values.

Results of the present investigation indicated that considerable values for callus growth rate occurred almost in all hybrids. It was also observed that highly significant positive heterosis for grain yield was found in all crosses. These results indicated that heterosis at cellular level may be related to heterotic effects for grain yield.

Many investigators reported also desirable heterosis for callus growth rate and rain yield per plant, in different crops Austin *et al*<sup>[3]</sup>, Haggag and El-Hennawy<sup>[9]</sup>, Haggag and El-Hennawy<sup>[10]</sup> and Abdel-Hady *et al*<sup>[11]</sup>.

**Heterosis in callus growth rate and grain yield:** Estimates of mid and better parent heterosis are shown in (Table 2). Compared to mid-parent values, all hybrids showed highly significant and positive heterosis for callus growth rate and grain yield/plant. The range of mid-parent heterosis was 40.63% and 42.47% to 103.17% and 109.24% with an average 66.25% and 65.05% for callus growth rate and grain yield/plant respectively. The progeny of MSWD-2XMSWD-9 gave the highest mid-parent heterosis 103.17% for callus growth rate and 109.24% for grain yield/plant.

The hybrid (MSWD-2XMSWD-9) followed by (MSWD-2 XNRCWD-16) scored the highest heterosis over all crosses relative to better parent values 101.55%, 76.67 (callus growth rate), 92.35% and 54.41% (grain yield/plant) respectively. These results indicated that heterosis at cellular level may be relative to heterotic

**Table 1:** Mean performance for the four parents and their F<sub>1</sub> hybrids for *in vitro* callus growth rate and *in vivo* grain yield/plant (gm.)

Parents and crosses	<i>In vitro</i> callus growth rate	<i>In vivo</i> grain yield/plant (gm)
Benysweif-2 P <sub>1</sub>	96.46	9.34
MSWD-2 P <sub>2</sub>	193.22	18.95
MSWD-9 P <sub>3</sub>	190.14	15.89
NRCWD-16 P <sub>4</sub>	180.63	13.09
Mean	165.11	14.32
Benysweif-2 XMSWD-2	217.13	90.16
Benysweif-2 XMSWD-9	211.39	18.48
Benysweif-2 XNRCWD-16	194.84	16.50
MSWD-2 XMSWD-9	389.44	36.45
MSWD-2 XNRCWD-16	341.36	29.26
MSWD-9 XNRCWD-16	321.91	23.54
Mean	279.35	24.07
L.S.D. 0.05	6.26	3.02
L.S.D. 0.01	8.57	4.13

effects for grain yield. Similar results were obtained by Haggag and El-Hennawy<sup>[9]</sup>, El-Shouny *et al*<sup>[5]</sup> and Abdel-Hady *et al*<sup>[11]</sup> who suggested that breeding and selection could result in the use of callus growth rate as genetic marker.

**General and specific combining ability:** General and specific combining ability shown in (Table 3). Results indicated that there were highly significant differences due to general combining ability (GCA) and specific combining ability (SCA) for callus growth rate and also for grain yield. To determine the genetic effects of greatest importance, GCA/SCA ratio was computed. High ratios of GCA/SCA mean squares for callus growth rate were detected, indicating that the magnitude of GCA (48-913) variance was more than that due to SCA (32-456) variance. For grain yield, however, the GCA/SCA ratio of less than unity was observed, revealing that the largest part of the total genetic variability associated with grain yield was due to non additive gene action.

Therefore, it could be concluded that the inheritance of callus growth was mainly controlled additive genetic effects of genes and these results can be exploited to improve plant materials for tissue culture research. Similar results also reported by Keyes *et al*<sup>[12]</sup> for callus growth in tobacco, Haggag and El-Hennawy *et al*<sup>[9,10]</sup> for callus growth of wheat and maize respectively.

**Effect of general combining on parents:** Evaluation of general combining ability was made from the values of GCA (Table 4). The highest positive GCA value was observed for inbred wheat line MSWD-2 followed by MSWD-9, therefore, it can be used as promising progenitor for high callus growth and grain yield.

On the contrary, Benysweif-2 and NRCWD-16 proved to be poor general combiner for callus growth *in vitro* and for grain yield *in vivo* as shown from their highly significant negative GCA value. The ranking

**Table 2:** Heterosis in callus growth and grain yield as percentage of mid-parent ( $\overline{MP}$ ) and better parent ( $\overline{BP}$ ) in four-parents diallel.

Crosses	Callus growth rate Heterosis over %		Grain yield/plant Heterosis over %	
	$\overline{MP}$	$\overline{BP}$	$\overline{MP}$	$\overline{BP}$
Benysweif-2 XMSWD-2	49.91**	12.37**	42.47**	6.39**
Benysweif-2 XMSWD-9	47.52**	11.18**	46.43**	16.30**
Benysweif-2 XNRCWD-16	40.63**	7.87**	47.06**	26.05**
MSWD-2 XMSWD-9	103.17**	101.55**	109.24**	92.35**
MSWD-2 XNRCWD-16	82.61**	76.67**	82.65**	54.41**
MSWD-9 XNRCWD-16	73.64**	69.30**	62.46**	48.14**
Average	66.25	46.49	65.05	40.61
L.S.D. 0.05	5.71	6.24	5.83	6.18
L.S.D. 0.01	9.13	8.53	9.22	8.41

**Table 3:** Mean squares for general (GCA) and specific (SCA) combining ability.

Source	d.f.	Callus growth rate	Grain yield/plant
GCA	3	48.913**	18.994**
SCA	6	32.456**	21.171**
Error	18	1.749	1.030
GCA/SCA ratio		1.51	0.90

\*\* Significant at 0.01 level of probability.

**Table 4:** Estimates of general combining ability (GCA) effects of parents.

Parents	<i>In vitro</i> callus growth rate	<i>In vivo</i> Grain yield per plant (gm)
Benysweif-2 (P <sub>1</sub> )	-7.428**	-2.589**
MSWD-2 (P <sub>2</sub> )	5.093**	1.409**
MSWD-9 (P <sub>3</sub> )	3.415**	0.823**
NRCWD-16 (P <sub>4</sub> )	-1.080**	0.357**
SE (gi)	0.745	0.085
SE (gi-gi)	1.216	0.138

**Table 5:** Estimates of specific combining ability (SCA) effects on callus growth rate and grain yield/plant of crosses.

Crosses	<i>In vitro</i> callus growth rate	<i>In vivo</i> grain yield/plant (gm)
Benysweif-2 XMSWD-2	13.895	2.670
Benysweif-2 XMSWD-9	14.829	2.576
Benysweif-2 XNRCWD-16	11.780	3.063
MSWD-2 XMSWD-9	19.402	3.581
MSWD-2 XNRCWD-16	11.780	2.827
MSWD-9 XNRCWD-16	12.001	2.690
S.E. (Sij)	1.333	0.151
S.E. (Sij-sik)	2.720	0.309

parents according to the effects of GCA almost agree with the ranking of parent according to their mean values. This shows a possibility of predicting the combining ability of parents based on their mean values.

**Effect of specific combining on callus growth and grain yield:** Specific combining ability (SCA) effects calculated for each hybrid are presented in (Table 5). The cross combination MSWD-2 XMSWD-9 scored the

highest positive SCA effect over all crosses under study. It is worthy to note that the highest specific combination MSWD-2 XMSWD-9 involved two good combiner parents and was also the best hybrid for callus growth *in vitro* and grain yield *in vivo*. These results indicate that specific intra-allelic gene interactions for these crosses that promoted *in vitro* callus growth were related to gene combinations that promoted grain yield.

Genotypic and phenotypic correlation were positive (0.961 and 0.890) and highly significant between callus growth and grain yield/plant respectively. Genotypic correlation coefficient (G.C.V.) was higher than corresponding phenotypic correlation coefficient (P.C.V.). However, both G.C.V. and P.C.V. were the same direction. This indicated that the strong genetic association between the traits. Moreover, this would also allow the definition of early screening methods based on *in vitro* tests. Similar results obtained by Haggag and El-Hennawy *et al*<sup>[9,10]</sup>, Abdel-Hafez and Hamed<sup>[2]</sup> and Abdel-Hady *et al*<sup>[11]</sup>. They revealed that tissue culture technique is a useful tool for early screening for combining ability analysis in the plant breeding programs.

## REFERENCES

1. Abdel-Hady, M.S., A.A. Abdel-Sattar and I.M. Mahmoud, 2004. Prediction of heterosis and combining ability in maize using tissue cultures. Bull. NRC. Egypt, Vol. 29, No. 1, pp: 109-119.
2. Abdel-Hafez, A.G. and Th. Hamad, 2000. Heterosis in another culture of bread wheat: performance under sodium chloride stress. Third International Crop. Sci, Aug. pp. 1-7, Congress, Hamburg.
3. Austin, S., M.A. Beer, M.K. Elenfeldt, P.J. Kazmierczak and J.P. Helgeson, 1985. Intraspecific fusion in *Solanum tuberosum*. Theor. Appl. Genet 71, 172-175.

4. Bhaskaran, S., R.H. Smith, and K. Schertz, 1983. Sodium chloride tolerant callus of *Sorghum bicolor* (L.). Z. Pflanzenphysiol. 112, 459.
5. El-Shouny, K.A., A.A. Mohamed, S.M. Abdel-Rahman, 1999. Prediction of heterosis and combining ability in maize through tissue culture techniques. Annals of Agric. Sci. Cairo, 44(2): 537-548.
6. Gamborg, O.L., K. Miller, and K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean root cells. Expt. Cell. Res. 50: 151-158.
7. Griffing, B., 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Austral. J. Biol. Sci., 9: 463-493.
8. Haggag, M.E.A., 1983. Application of cell culture technique in crop improvement. EMCIP. Pub. No. 72: 62-70.
9. Haggag, M.E. and M.A. El-Hennawy, 1991. Heterosis and combining ability effects in callus growth of wheat (*Triticum aestivum* L.) in vitro. Al-Azhar J. Agric. Res., 13: 33-45.
10. Haggag, M.E. and M.A. El-Hennawy, 1992. Early testing for heterosis and combining ability in maize using tissue culture techniques. Annals Agric. Sci., Ain Shams Univ., Cairo, 37(1): 77-83.
11. Izhar, S. and J.B. Power, 1977. Genetical studies with *Petunia* leaf protoplasts. I-Genetic variation to specific growth hormones and possible genetic control on stages of protoplast development in culture. Plant Sci. Letters, 8: 375-383.
12. Keyes, G.J., W.R. Deston, G.B. Collins and P.D. Legg, 1981. Hybrid vigor in callus tissue cultures and seedlings of *Nicotiana tabacum* L. J. Heredity, 72: 172-174.
13. Murashing, T. and F. Skoog, 1962. A revised medium for crops growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.
14. Wynne, J.C., D.A. Emery and P.W. Rice, 1970. Combining ability estimates in archis hypogeal. II-Field performance of F<sub>1</sub> hybrids. Crop Sci., 10: 713-715.