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EARLY PREDICTION FOR HETEROSIS AND COMBINING ABILITY IN WHEAT (TRITICUM AESTIVUM L.)USING TISSUE CULTURE TECHNIQUES

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ABSTRACT

This investigation studied the ability of tissue culture technique as early testing for heterosis and combining ability in wheat. Genetic determination of callus growth *in vitro* of four genotypes of wheat were in a diallel crosses. 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 the Parents P_2, P_3 and p_4 and crosses ($p_2 \times p_3$), ($p_2 \times p_4$) and ($p_3 \times p_4$) showed the best results of the studied characters. Tissue culture technique could be 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: Wheat, tissue culture, heterosis, general combining ability, specific combining ability.

INTRODUCTION

Tissue culture techniques provide a new and available approach in cereal crops improvement and for genetic studies as they save effort and time to the breeder as well as providing material that can never be obtained through the conventional breeding methods. Plant cell and tissue culture techniques are offering very good tools to the breeder which enables genetic improvement of several crops with less effort and in shorter time compared to the conventional breeding methods *(Haggag, 1983 and Martin, 2009).*

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.

Heterosis in tissue cultures of various plant species, was reported by *Izhar and Power (1977), Haggag and El-Hennawy (1991) and Abdel-Hafez and Hamad (2000).*

Genotypic and phenotypic correlation indicated the possibility of early prediction of heterosis and combing ability for yield and some agronomic traits of maize by measuring callus growth in tissue (*Haggag and El-Hennawy, 1991 and Abdel-Hady et al 2004*).

Heterosis refers to the phenomenon that progeny of diverse varieties of a species or crosses between species exhibit greater biomass, speed of development, and fertility than both parents (*Brichler et al. (2010*)

The main objective of this study is to evaluate the ability of tissue culture technique to predict heterosis in F_1 hybrids for the choice of parents at early stages in the program to save both money and effort.

MATERIALS AND METHODS

Four wheat (*Triticum aestivum L.*) genotypes namely Gamiza-9(local variety) and the introduced lines MSWB-19, MSWB-20, MSWB-21 were used in this study. These wheat genotypes were crossed in a diallel combination. These six F_1 hybrids and their parents were grown in a randomized complete blocks design with three replications at the Experimental Station of the National Research Centre at Nobaria in the two seasons (2008-2009) and (2009-2010). 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 of the parents and F_1 were induced from immature embryos (13-16 days after anthesis) as indicated by *Haggag (1983)*. The caryopses were removed from the spike; surface-sterilized for 5min. in 5% Clorox (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 (1962) inorganic components and B5-vitamins *(Gamborg et al., 1968)* 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^{o}C for 15 min. and incubated at 22^{o}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., (1983).*

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 analyzed according to *Griffing (1956)*, 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.,* (1970).

RESULTS AND DISCUSSION

Analysis of variance showed that highly significant differences among entries for callus growth rate and also for grain yield per plant. Table (1) show that the parents ranged from 132.23 gm. for Gamiza-9 (P_1) to 190.92 gm. for MSWB-21 (P_4), with an average of 168.65 gm. The most performing hybrid was MSWB-20 X MSWB-21 (390.20 gm and 39.60 gm.) revealed a highly significant response to callus growth rate and grain yield, respectively than the other hybrids under investigation, while the hybrid (Gamiza-9 X MSWB-19) 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.

Table 1: Mean performance for the four parents and their F_1 hybrids for *in vitro* callus growth rate and *in vivo* grain yield/plant (gm.)

Parents and crosses		*In vitro* callus growth rate	*vivo* grain yield/plant (gm)
Gamiza - 9	P_1	132.23	11.21
MSWB-19	P_2	169.81	14.10
MSWB-20	P_3	181.62	17.61
MSWB-21	P_4	190.92	19.83
Mean		168.65	15.69
Gamiza - 9 XMSWB-19		199.16	14.42
Gamiza - 9 XMSWB-20		270.35	19.10
Gamiza - 9 XMSWB-21		291.51	23.31
MSWB-19	XMSWB-20	354.43	28.78
MSWB-19	XMSWB-21	373.61	34.27
MSWB-20	XMSWB-21	390.20	39.60
Mean		313.21	26.58
L.S.D. 0.05		6.31	3.02
L.S.D. 0.01		8.59	4.14

Many investigators reported also desirable heterosis for callus growth rate and grain yield per plant, in different crops (*Austin et al., 1985, Haggag and El-Hennawy 1991, Haggag and El-Hennawy 1992, Abdel-Hady et al., 2004, Abdel Hady 2006 and Vivek et al 2009).*

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 31.88% and 13.90% to 109.48% and 111.54% with an average 83.81% and 65.26% for callus growth rate and grain yield/plant, respectively. The progeny of MSWB-20 X MSWB-21 gave the highest mid-parent heterosis 109.48% for callus growth rate and 111.54% for grain yield/plant.

The hybrid (MSWB-20 X MSWB-21) followed by (MSWB-19 X MSWB-21) scored the highest heterosis over all crosses relative to better parent values 104.38%, 95.69% (callus growth rate), 99.70% and 72.82% (grain yield/plant) , respectively (Table 2). These results indicated that heterosis at cellular level may be relative to heterotic effects for grain yield. Similar results were obtained by *Haggag and El-Hennawy (1991), El-Shouny et al., (1999) and Abdel-Hady et al (2004)* who suggested that breeding and selection could result in the use of callus growth rate as genetic marker.

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

Crosses	Callus growth rate Heterosis over %		Grain yield/plant Heterosis over %	
	$\overline{MP}$	$\overline{BP}$	$\overline{MP}$	$\overline{BP}$
Gamiza - 9 XMSWB-19	31.88	17.28	13.90	2.27
Gamiza - 9 XMSWB-20	72.27	48.85	32.55	8.46
Gamiza - 9 XMSWB-21	80.41	52.69	50.19	17.55
MSWB-19 XMSWB-20	101.70	95.15	81.46	63.43
MSWB-19 XMSWB-21	107.14	95.69	101.94	72.82
MSWB-20 XMSWB-21	109.48	104.38	111.54	99.70
Average	83.81	69.01	65.26	44.04
L.S.D. 0.05	5.63	6.19	5.92	6.35
L.S.D. 0.01	9.16	8.56	9.51	8.42

General and specific combining ability.

General and specific combining ability are 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 the 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 (49.913) variance was more than that due to SCA (33.435) variance. For grain yield, however, the GCA/SCA ratio of less than 0.89 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 were reported by *Keyes et al., (1981)* for callus growth in tobacco, *Haggag and El-Hennawy (1991),* *(1992), Abdel Hady (2006), El Sayed et al (2007) and Musila et al (2010)*, for callus growth of wheat, maize and barley , respectively.

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

Source	d.f.	Callus growth rate	Grain yield/plant
GCA	3	49.913**	19.651**
SCA	6	33.435**	22.132**
Error	18	1.752	1.041
GCA/SCA ratio		1.49	0.89

** Significant at 0.01 level of probability.

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 MSWB-21 (P_4)followed by MSWB-20 (P_3); Therefore, it can be used as promising progenitor for high callus growth and grain yield.

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

Parents	*In vitro* callus growth rate	*In vivo* Grain yield per plant (gm)
Gamiza - 9 (P_1)	-7.428	-2.591
MSWB-19 (P_2)	-1.192	0.367
MSWB-20 (P_3)	3.419	0.833
MSWB-21 (P_4)	5.101	1.419
SE (gi)	0.755	0.089
SE (gi-gi)	1.226	0.141

On the contrary, Gamiza-9 and MSWB-19 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 parents according to the effects of GCA almost aggress 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 MSWB-20 X MSWB-21 scored the highest positive SCA effect over all crosses under study. It is worthy to note that the highest specific combination MSWB-20 XMSWB-21 involved two good combiner parents and was also the best hybrid for callus growth *in vitro* and grain yield *in vivo*. These results indicated that specific intra-allelic gene interactions for these crosses that promoted *in vitro* callus growth were related to gene combinations that promoted grain yield. Similar results were obtained by *Abdel Hady (2006) and Martin et al (2009)* in wheat.

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

Crosses	*In vitro* callus growth rate	*In vivo* grain yield/plant (gm)
Gamiza - 9 XMSWB-19	11.511	2.461
Gamiza - 9 XMSWB-20	11.621	2.531
Gamiza – 9 XMSWB-21	12.031	2.642
MSWB-19 XMSWB-20	13.212	2.872
MSWB-19 XMSWB-21	14.830	3.011
MSWB-20 XMSWB-21	19.401	3.152
S.E. (Sij)	1.340	0.152
S.E. (Sij-sik)	2.721	0.308

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 were obtained by *Haggag and El-Hennawy (1991), (1992), Abdel-Hafez and Hamed (2000), Abdel-Hady et al (2004)* and *Musila et al (2010)*; they revealed that tissue culture technique could be a useful tool for early screening for combining ability analysis in the plant breeding programs.

REFERENCES

Abdel Hady, M.S. (2006). Hetrosis and combining ability effects for callus growth of wheat *(Triticum durum, Desf.) in vitro*. Journal of Applied Sciences Research, 2 (6) : 360-362.

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.

Abdel-Hafez, A.G. and Th. Hamad, 2000. Heterosis in anther culture of bread wheat: performance under sodium chloride stress. Third International Crop. Sci, Aug. pp. 1-7, Congress, Hamburg.

Austin, S.; M.A. Beer; M.K. Elenfeldt, P.J. Kazmierczak, and J. P. Helgeson, 1985. Intraspecific fusion in *Solanum tuberosum.* Thear. Appl. Genet 71, 172-175.

Bhaskaran, S.; R.H. Smith, and K. Schertz, 1983. Sodium chloride tolerant callus of *Sorghum bicolar* (L.). Z. Pflanzenphysiol. 112, 459.

El Sayed, A.A., G.A. morshed, A.M Hassanein and H.A. Ashmawy (2007). Combining ability in the F_1 and F_2 generations of certain hul-less barley crosses. Egypt, J.Plant Breed. 11(1):271-279.

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.

Gamborg, O.L.; K. Miller, and K. Ojima, 1968. Nutrient requirements of suspension cultures of soybean root cells. Expt. Cell. Res. 50: 151-158.

Griffing, B., 1956. Concept of general and specific combining ability in relation to diallel crossing systems. Aust. J. Biol. Sci., 9: 463-493.

Haggag, M.E.A., 1983. Application of cell culture technique in crop improvement. EMCIP. Pub. No. 72: 62-70.

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.

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.

Izhar, S. and J.B. Power, 1977. Genetical studies with Petunia leaf protoplasts. 1-Genetic variation to specific growth hormones and possible genetic control on stages of protoplast development in culture. Plant Sci. Letters, 8: 375-383.

Brichler J. A., Hong Yao, S., Chudalayandfi, D. Vaiman and R. A. Veitia (2010). Heterosis. The plant cell 9:1102-1108

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.

Martin A, Fabrizius, Ropert H, Busch Khalil Khan and Linda Huckle (2009) Genetic diversity and heterosis of spring wheat crosses. Crop sci. 12, 1108-1112.

Murashige, T. and F. Skoog, 1962. A revised medium for crops growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473-497.

Musila R. N., A. O. Diallo, Dan Makumbi and Kiarie (2010). Combining ability of early-maturing quality protein maize inbread lines adapted to Eastern Africa. Field Crops Research,119, 231-237.

Vivek B.S., Crossa J and Alvardo J (2009) Heterosis and combining ability among (IMMYT'S) mid-altitude early to intermediate maturing maize *(Zea mays L.)* populations. Maydica 54 : 97-107.

Wynne, J.C.; D.A. Emery and P.W. Rice, 1970. Combining ability estimates in archis hypogeal. II-Field performance of F_1 hybrids. Crop Sci., 10: 713-715.

التنبؤ المبكر لقوة الهجين والقدرة علي التآلف في القمح باستخدام تقنية زراعة الأنسجة

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أجري هذا البحث بهدف إمكانية إستخدام تقنية مزارع الأنسجة في التنبؤ بقوة الهجين والقدرة علي التآلف في مراحل مبكرة من النمو وذلك في ستة هجن من القمح السداسي الناتجة من التهجين بين ثلاثة سلالات قمح مستوردة وصنف محلي (جميزة 9) وكانت أهم النتائج المتحصل عليها:-

1- أظهرت لكل الهجن قوة هجين عالية موجبة ومعنوية بالنسبة لصفة نمو الكالس وأيضاً لصفة نمو المحصول في الحقل.
2- التباين الوراثي لكل من القدرة العامة والخاصة علي التآلف معنوياً لصفتي نمو الكالس والمحصول.
3- كان تباين القدرة العامة علي التآلف أعلي من تباين القدرة الخاصة علي التآلف لصفة نمو الكالس مما يدل علي أن الجزء الأكبر من الإختلافات الوراثية المرتبطة بهذه الصفة راجع الي فعل الجينات من النوع المضيف.
4- أظهرت السلالة الأبوية MSWB21 يليها MSWB20 قدرة عالية علي التآلف العام بالنسبة لصفة نمو الكالس وأيضاً لصفة نمو المحصول بينما أظهر الصنف جميزة 9 يليه السلالة MSWB19 قيمة منخفضة من ناحية القدرة العامة علي التآلف لهاتين الصفتين.
5- أظهر الهجين (MSWB-20XMSWB-21) تأثير عالي المعنوية للقدرة الخاصة علي التآلف بينما سجل الهجين (جميزة 9 - × MSWB-19) أقل قيمة للقدرة الخاصة علي التآلف لصفتي نمو الكالس والمحصول في الحقل.
6- يتضح مما سبق أن الإرتباط بين صفة نمو الكالس في المعمل والمحصول في الحقل موجبة ومعنوية مما يؤكد أهمية إستخدام تقنية زراعة الأنسجة في التنبؤ بقوة الهجين والقدرة علي التآلف لمحصول الحبوب لإختيار أفضل الأباء وكذلك الهجين في مرحلة مبكرة من برنامج التربية مما يساعد المربي علي توفير الوقت والمال والجهد اللازم.