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ESTIMATING HETEROSIS AND COMBINING ABILITY FOR *in vivo* AND *in vitro* TRAITS USING DIALLEL CROSS IN MAIZE (*Zea mays L.*)

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ABSTRACT

Combining ability and heterosis studies were performed for in vivo and in vitro traits in a diallel cross involving five maize inbred lines. Mean squares of genotypes were found to be significant for all in vivo and in vitro studied traits except callus formation percentage. Mean squares of parents were found to be significant for all in vivo and in vitro studied traits except grain yield per plant, callus fresh weight II and callus formation percentage. Mean squares of the resultant ten hybrids combination were found to be significant for all in vivo and in vitro studied traits except callus formation percentage. Mean square estimates of parent vs. crosses were found to be highly significant for all studied traits except callus growth rate III. Both general (GCA) and specific combining ability (SCA) variances were found to be highly significant for all in vivo and in vitro studied traits except callus formation percentage and callus growth rate II. The GCA/SCA ratios were found to be less than unity for all traits except callus growth rate III. The inbred line ***L173*** *was considered to be good general combiner for all studied traits (plant height, ear height, ear length, ear diameter, number of rows per ear, grain yield per plant, callus fresh weight I, callus fresh weight II, callus fresh weight III, callus formation percentage, callus growth rate I, callus growth rate II and callus growth rate III). The correlation coefficient was positive and highly significant between in vitro and in vivo characters except callus growth rate III. Information generated from this study can be useful for selecting parents and hybrids to maximize the grain yield and its components in maize.*

Keywords: Maize (*Zea mays L.*), Diallel cross, Heterosis, Combining ability, *in vivo* and *in vitro* traits.

INTRODUCTION

Maize (*Zea mays L.*) is one of the most important cereal crops and widely cultivated in world today. It is a good source for chemical industry, animal feed, and human food. With the expanding population and limitation of land, the great demand for maize both quality and quantity requires more rapid genetic improvement of maize. As it is known, that the first step in hybrid maize development programs, is to identify new lines that when crossed with other parents, produce hybrids with superior performance (Abdel-Hady *et al.* 2004). . In Egypt, maize is the second cereal crops after wheat. The production and consumption is decreasing due to population increase, limited land, biotic and abiotic stresses.

Over years, classical breeding methods have been used as a tool to overcome these constraints. For some traits which improvement through classical breeding holds little promise, biotechnology methods now provide viable alternatives in several crops, including maize (Frame *et al*., 2002). In cereal such as wheat, barley and maize, embryos have been the favorite explants for *in vitro* culture (Armstrong and Green, 1985, Green and Phillips, 1975, Ray and Ghosh, 1990). In order to improve productivity, one of the most important steps in a breeding program is the choice of suitable parents.

Diallel crosses have been widely used in genetic research to investigate the inheritance of important traits among a set of genotypes. These were devised, specifically, to investigate the combining ability of the parental lines for the purpose of identification the superior parents for use in corn hybrid breeding programs. Analysis of diallel data is usually conducted according to the methods of Griffing (1956) which partition the total variation of diallel data into GCA of the parents and SCA of the crosses (Yan and Hunt, 2002).

Biotechnology offer several valuable techniques such as cell, anther and tissue culture which develop the breeding methods to improve the genetic characters including drought tolerance in the economical crops. Tissue culture generates a wide range of genetic variation in plant species, which can be incorporated in plant breeding programs. By *in vitro* selection, mutants with useful agronomic traits, i.e., salt or drought tolerance or disease resistance can be isolated in a short duration. However, the successful use of somaclonal variation is very much dependent on its genetic stability in the subsequent generations (Mercado *et al*., 2000, Jain, 2001, El-Aref, 2002). To achieve remarkable gains in the biotechnology of maize using embryo culture, combining abilities for *in vitro* and *in vivo* traits are necessary.

The objectives of the present study were to (i) estimate GCA and SCA and heterosis effect for some *in vivo* and *in vitro* traits, (ii) examine the relationship between *in vivo* and *in vitro* studied traits and (iii) identify the best inbred lines which can be used in corn breeding programs.

MATERIALS AND METHODS

Plant materials

Five maize inbred lines (four Egyptian and one Indian origins) were used in the present investigation namely i.e., L71, L85, L101, L120 and L173 obtained from Agriculture Research Center (ARC), Giza, Egypt. The name, origin and pedigree are presented in (Table 1). 5 x 5 half diallel (without reciprocal) mating system by hand emasculation and pollination was followed to obtain ten hybrids.

Table (1): Name, origin and pedigree of the five maize inbred lines used in the present investigation.

No.	Name	Origin	Pedigree
1	L 71	Egypt	R9-27 syn. Laposta × 303 × (G2-216× M0 2 RF)
2	L 85	Egypt	R9-42 sanjuan × 307 × (s.c 14)
3	L 101	Egypt	R9-58 (syn. Laposta × 307) × (s.c.14)
4	L 120	Egypt	L-57 B locally developed
5	L 173	India	C. M. 400 imported

Field experiments

A half diallel set of crosses involving five maize inbred lines was carried out in 2007 growing season. The parents and ten F_1 hybrids were evaluated under field conditions at the Agricultural Research Station, Genetic Engineering and Biotechnology Research Institute (GEBRI), Sadat City, Minufiya University, Egypt during the maize growing season 2008. The experiment was arranged in a randomized complete block design (RCBD), with three replicates. The experimental plot consisted of a single row, 3.5 m long and 30 cm width. Plants were over planted and thinned at one plants per hill after about 30 days from planting. The plants were spaced at 20 cm within row. Ordinary cultural practices for maize production under sand soil were applied. At maturity, ten guarded plants were selected at random from each plot for subsequent characters as follows: plant height, ear high, ear length, ear diameter, number of rows per ear, number of kernels per row, 1000-kernel weight and grain yield per plant.

Tissue culture analysis

Surface sterilization

Callus culture for five maize inbred lines and their F_1 hybrids were induced from immature embryos in late milk to dough stage 14 days after anthesis as the following procedure. Kernels were rinsed in 0.1% Mercuric Chloride (MC) for 5 min., then kernels wished one time by sterile distilled water, the kernels were sterilized in 50% Clorox (Sodium hypochlorite) for 20 min. and washed by sterile distilled water for three times (Zhao *et al*., 2008). The embryos were extracted by cutting the top of kernels with a sharp blade and placed on solid agar medium attached with scutellum of the embryo for 15 days to induced callus. Five embryos were separately cultured in each jar. Each genotype was cultured in eight jars. Stock culture maintained by subculture to fresh medium every 15 days for three times. Harvested ears can be stored for up to 3 days at 4ºC before dissection although the embryos are best isolated immediately.

Media preparation and callus induction

The basal medium of Murashige and Skoog (1962) supplemented with 1 mg/L 2, 4-dichlorophenoxy acetic acid (2.4-D), 0.5 mg/L thiamin-HCL, 150 mg/L asparagines, 30 g/L sucrose and 6 g/L agar. The pH was adjusted to 5.8 by using 0.1N KOH or 0.1N HCL and autoclaved for 20 min. at 1.5 psi, 121ºC. The embryos were evaluated for callus formations after 15 days of initial culturing and then subcultured onto fresh media. Furthered, subculturing of calli occurred every 15 days interval for three times. All subcultured materials were grown in a growth chamber with light intensity of 1500 Lux from white fluorescent lights with photoperiod of 16/8 h at 25ºC. Data were recorded for the following callus characteristics according to (Hunt, 1978): Callus formation percentage was record as: $\frac{\text{Number of callused embry}}{\text{Total number of embryo}}$. Callus fresh weight (CFW) (mg) was recorded three times. First callus fresh weight (CFW I) was recorded after 15 days from embryo culturing on (MS) media. Second callus fresh weight (CFW II) was recorded after 15 days from the first subculture. Third callus fresh weight (CFW III) was recorded after 15 days from second subculture. Callus Growth Rate (CGR) was estimated as:

CGR I= $\frac{w2-w1}{T2-T1}$, CGR II= $\frac{w3-w2}{T3-T2}$ and CGR III= $\frac{w4-w3}{T4-T3}$

Where W1, W2, W3 and W4 were immature embryo, CFW1, CFW2 and CFW3, respectively. While , T1, T2, T3 and T4 were 0, 15, 30 and 45 days, respectively.

Statistical analysis

Better-parent heterosis (BPH) for each trait of individual cross was expressed as the percentage increase of F_1 performance above the better-parent (BP) performance. Heterosis over the better-parent % was estimated as follows:

$$\text{BPH \%} = \frac{F1-BP}{BP} \times 100$$

Where: F_1 = mean value of the first generation and BP = mean value of the better-parent.

General (GCA) and specific combining ability (SCA) analysis were computed according to Griffing (1956) designated as Method 2, Model 1.

Correlation studies

To study possibility of predicting heterotic effects of F_1 hybrids, simple phenotypic correlation coefficients were calculated for all pairs of F_1 characters studied *in vivo* and *in vitro* experiments. Correlation was estimate according to pearson's formulae:

$$r = \frac{n(\sum xy) - (\sum x)(\sum y)}{\sqrt{[n\sum x^2 - (\sum x)^2][n\sum y^2 - (\sum y)^2]}}$$

RESULTS AND DISCUSSION

The genotypes mean performance for all *in vivo* and *in vitro* studied traits are presented in (Table 2). The mean squares analysis of variance for *in vivo* and *in vitro* traits of the diallel cross are presented in Table 3.

Mean squares of genotypes were found to be significant for all *in vivo* and *in vitro* studied traits except for callus formation percentage. Mean squares of parents were found to be significant for all *in vivo* and *in vitro* studied traits except grain yield per plant, callus fresh weight II and callus formation percentage. Mean squares of the resultant ten hybrids combination were found to be significant for all *in vivo* and *in vitro* studied traits except callus formation percentage.

Therefore, no genotypic differences among genotypes, parents and their F_1 were detected for callus formation percentage. There is no need to proceed the second step analysis (combining ability analysis) for this trait.

Mean square estimates of parent vs. crosses, as an indication to average heterosis overall crosses, were found to be highly significant for all *in vivo* and *in vitro* studied traits except in callus growth rate III.

I. Heterosis

Useful heterosis, expressed as the percentage deviations of F_1 mean performance from better–parents for all *in vivo* and *in vitro* studied traits are presented in Table 4. These effects were observed in all studied traits but the degree of heterosis showed variation from trait to trait. High positive values of heterosis would be of interest in most traits under investigation except plant height and ear height the negative values would be useful from the corn breeder's point of view.

For plant height and ear height, no hybrids showed significant negative desirable heterosis. In respect of ear length, all hybrids showed significantly positive desirable heterosis which ranged from 16.31% to 53.99% for the two hybrids L85 × L120 and L85 × L101, respectively. Regarding ear diameter, all hybrids showed significantly positive desirable heterosis which ranged from 12.16% to 32.98%. For number of rows per ear, all hybrids showed significantly desirable heterosis which ranged from 3.23% to 28.86% for the hybrids L85 × L120 and L71 × L120, respectively. As for number of kernels per row, all hybrids showed significantly positive desirable heterosis which ranged from 43.91% to 144.16% for the hybrids L85 × L120 and L71 × L101, respectively. Concerning 1000-kernel weight, all hybrids showed significantly positive desirable heterosis which ranged from 23.67% to 66.40% for the hybrids L101 × L120 and L85 × L101, respectively. Regarding to grain yield per plant, all hybrids showed significantly positive desirable heterosis which ranged from 92.12% to 207.56% for the hybrids L71 × L101 and L85 × L101, respectively. Similar results were obtained by Mahmoud *et al*. (1990), El-Shamarka (1995), Li and Lu (1997), Roy *et al*. (1998), El-Hosary *et al.* (1999), Khalil (1999), Yasein (1999), El-Sheikh and Ahmed (2000), El-Bagoury *et al*. (2004), Amer and Mosa (2004), Tollenaar *et al*. (2004), Alam *et al.* (2008), Sharief *et al.* (2009), Assuncao *et al*. (2010) and Iqbal *et al*. (2010).

High positive values of heterosis would be of interest in most *in vitro* characters under investigation. For callus fresh weight I, nine crosses showed significantly desirable heterosis which ranged from 16.47% to 46.75% for the hybrids L71 × L120 and L101 × L73, respectively.

Concerning callus fresh weight II, eight crosses showed significantly desirable heterosis which ranged from 27.78% to 52.985% for the hybrids L71 × L85 and L101 × L73, respectively. With regard to callus fresh weight III, the hybrid L101 × L120 showed significant desirable heterosis (34.60%) followed by L101 × L73 (38.65%). In respect of callus growth rate I, all hybrids showed significant desirable heterosis which ranged from 11.86% to 46.88% for the hybrids L71 × L173 and L101 × L73, respectively. Regarding, callus growth rate II, all hybrids showed significant desirable heterosis which ranged from 9.96% to 57.31% for the hybrids L85 × L173 and L101 × L73, respectively. Concerning callus growth rate III, two hybrid L85 × L173 and L101 × L120 showed significant positive desirable heterosis. Similar results were obtained by Green *et al.* (1974), Tuberosa and Landi (1991), Invantsov *et al*. (1983), Haggag and El-Hennawy (1992), Willadino *et al*. (1996), 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 as a genetic marker in maize.

II- Combining ability

Both general (GCA) and specific (SCA) combining ability variances were found to be highly significant for all *in vivo* and *in vitro* studied traits except callus formation percentage and callus growth rate II. The GCA/SCA ratios for all *in vivo* and *in vitro* studied traits were less than unity, indicated that non-additive gene action had a greater importance in the inheritance for all *in vivo* and *in vitro* traits studied. For callus growth rate III, the GCA/SCA was found more than unity, indicating that additive gene action had a greater importance in inheritance of this trait. For *in vivo* traits similar results were obtained by Mahmoud *et al*. (1990), El-Shamarka (1995), Li and Lu (1997), Roy *et al*. (1998), El-Hosary *et al.* (1999), Khalil (1999), Yasein (1999), El-Sheikh and Ahmed (2000), El-Bagoury *et al*. (2004), Amer and Mosa (2004), Tollenaar *et al*. (2004), Alam *et al.* (2008), Sharief *et al.* (2009), Kanagarasu *et al.* (2010) and Kumar and Bharathi (2010). For *in vitro* traits similar results were also reported by Haggag and El-Hennaway (1992), Barakat and Shehab El-Din (1993) and El-Shouny *et al.* (1999)

II. a. *General combining ability (GCA)*

Estimates of the GCA effects (gi) for the parental inbred in each trait are presented in Table 5. High positive GCA effects would be of interest in most traits under investigation except for plant height and ear height as the negative values would be useful from the corn breeder's point of view.

Concerning plant height and ear height one inbred line L173 showed a significant negative GCA effect. Significant positive GCA effects were found for all other studied traits. Based on GCA estimates, it could be concluded that the best combiners for ear length, two inbred lines L85 and L120, for ear diameter, two inbred lines L71 and L173, for number of rows per ear, 1000-kernel weight, and grain yield per plant, two inbred lines L85 and L173 . As for number of kernels per row, one inbred line L120 showed a significant positive GCA effects. Generally, the parental maize inbred lines L85 and L173 were considered to be good general combiner for most *in vivo* studied characters for improving maize breeding programs. The results are in accordance with Mahmoud *et al*. (1990), El-Shamarka (1995), Li and Lu (1997), Roy *et al*. (1998), El-Hosary *et al.* (1999), Khalil (1999), Yasein (1999), El-Sheikh and Ahmed (2000), El-Bagoury *et al*. (2004), Amer and Mosa (2004), Tollenaar *et al* (2004), Alam *et al.* (2008), Sharief *et al.* (2009), Kanagarasu *et al* (2010), and Kumar and Bharathi (2010).

In respect of *in vitro* traits, significant positive GCA effects were found for all studied traits. Based on GCA estimates, it could be concluded that the best combiners for callus fresh weight 1, two inbred lines L85 and L101, for callus fresh weight 2, three inbred lines L101, L120 and L85, for callus fresh weight III, only inbred line L85, for callus growth rate I, two inbred lines L85 and L101, for

callus growth rate II, two inbred lines L101 and L120 and callus growth rate III, two inbred lines L85 and L173. Generally, the parental maize inbred line L85 was consider to be good general combiner for most *in vitro* studied characters for improving maize breeding programs. The results are in accordance for *in vitro* traits in maize with Haggag and El-Hennawy (1992), El-Shouny *et al*. (1999), Milad *et al.* (2001), Abdel-Hady (2006). and Abdel-Hady *et al.* (2004).

II. b. *Specific combining ability (SCA)*

Specific combining ability effect (si) for F_1's new genetic combination in each trait is presented in Table 6. For plant height and ear height one hybrid showed significant negative SCA effect L71× L85 and L85× L101, respectively. Regarding to ear length, ear diameter and number of kernels per row all hybrids showed significantly positive SCA effect. As for number of rows per ear, five hybrids expressed significantly positive SCA effect. Concerning 1000-kernel weight, nine hybrids showed significantly positive SCA effect.

For callus fresh weight I, callus fresh weight II, callus fresh weight III, callus growth rate I, all hybrids showed significantly positive SCA effect. Regarding to callus formation percentage, callus growth rate II, nine hybrids expressed significantly positive SCA effect. Concerning callus growth rate III, two hybrids L101 × L120 and L101 × L173 showed significantly positive SCA effect.

In general, the eight crosses L71 × L85, L71 × L101, L71 × L120, L85 × L101, L85 × L120, L85 × L73, L101 × L120 and L120 × L173 could be considered as the most superior crosses in their SCA effects for all *in vivo* and *in vitro* traits studied, indicating that these genetic materials could be useful in maize breeding programs. This finding was also found by Haggag and El-Hannawy (1992), El- Shouny *et al*. (1999), Abdel-Hafez and Hamad (2000), Milad *et al*. (2001), Abdel-Hady (2006) and Abdel-Hady *et al.* (2004). Moreover, this would allow the definition of early screening methods based on *in vitro* tests, which could aid in speeding up selection work for the production of large numbers of inbred lines and hybrids.

III. Correlation between *in vitro* and *in vivo* characters.

Estimate of phenotypic correlations between *in vitro* and *in vivo* characters are presented in Table 7. The obtained data reveal that, phenotypic correlation was positive and highly significant between *in vitro* and *in vivo* characters, except callus growth rate III with all agronomic characters. These indicate that the tissue culture technique might be valuable for predicting the combining ability. Our results are agreement with those obtained by Haggag and El-Hannawy (1991), Haggag and El-Hannawy (1992), Shouny *et al* (1999) and Abdel-Hady (2006) and Abdel-Hady *et al.* (2004) who found significant positive

phenotypic correlation between callus growth and yield of grain in wheat and maize, respectively.

In conclusion, this study indicated that the *in vitro* traits are very effective for prediction of heterosis. Results recorded in this study may be contributed to the development of an effective method to select components for heterosis and combining ability of quantitative traits in maize breeding program.

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تقدير قوة الهجين والقدرة على الائتلاف لبعض الصفات الحقلية والمعملية باستخدام الهجن التبادلية فى محصول الذرة الشامية

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أجرى هذا البحث بمزرعة معهد الهندسة الوراثية والتكنولوجيا الحيوية - جامعة المنوفية- مدينة السادات- مصر خلال موسمي ٢٠٠٧، ٢٠٠٨ وقد استخدم لتنفيذ هذا البحث خمسة سلالات من الذرة الشامية أربعه منها مصرية هي L 71، L 85، L 101، L 120 وسلالة هندية L 173. أجرى التهجين التبادلي بينهما (ماعدا العكسى) فى الموسم ٢٠٠٧ ولقد تم تقييم الأباء والهجن فى موسم ٢٠٠٨ وتم تحليل البيانات باستخدام طريقة جرفنج للأباء والهجن (١٩٥٦) الطريقة الثانية الموديل الأول ولقد اجرى هذا البحث بهدف:

(١) تقدير القدرة العامة والخاصة على الائتلاف وقوة الهجين لبعض الصفات الحقلية والمعملية.

(٢) دراسة العلاقة بين الصفات الحقلية والمعملية تحت الدراسة.

(٣) تحديد أفضل السلالات والتي يمكن استخدامها في برنامج تربية الذرة الشامية.

وكانت الصفات تحت الدراسة هى (طول النبات، ارتفاع الكوز، طول الكوز، قطر الكوز، عدد الصفوف بالكوز،عدد الحبوب بالصف، وزن الالف حبه، محصول النبات الفردى،الوزن الطازج للكالس فى ثلاث فترات I ، II ،III،النسبة المئوية لتكوين الكالس، معدل نمو الكالس I ، معدل نمو الكالسII ومعدل نمو الكالسIII) وفيما يلى ملخص لأهم النتائج المتحصل عليها:

١- كانت قيم التباين الراجعة الى التراكيب الوراثية عالية المعنوية لجميع الصفات الحقلية والمعملية تحت الدراسة ما عدا صفة نسبة تكوين الكالس .

٢- كانت قيم التباين الراجعة الى الاباء عالية المعنوية لجميع الصفات تحت الدراسة ما عدا صفة محصول النبات الفردي و الوزن الطازج للكالسII و نسبة تكوين الكالس

٣- كانت قيم التباين الراجعة الى الهجن عالية المعنوية لجميع الصفات تحت الدراسة ما عدا صفة نسبة تكوين الكالس.

٤- كانت قيم التباين الراجعة الى قوة الهجين عالية المعنوية لجميع الصفات تحت الدراسة ما عدا صفة معدل نمو الكالس III.

٥- كانت قيم التباين الوراثى الراجع لكل من القدرة العامة والخاصة على الائتلاف عالي المعنوية لجميع الصفات تحت الدراسة ماعدا صفة نسبة تكوين الكالس و معدل نمو الكالس II .

٦- اظهرت النسبة بين تباينى القدرة العامة والقدرة الخاصة على الائتلاف تأثير اكبر للقدرة الخاصة على الائتلاف لجميع الصفات تحت الدراسة ماعدا صفة معدل نمو الكالس III.

٧- كانت السلالتين L85 ، L173 افضل الاباء لبعض الصفات الحقلية و المعملية مثل طول النبات ، ارتفاع الكوز ، طول الكوز ، قطر الكوز، عدد الصفوف بالكوز، محصول النبات الفردى وكذلك بعض الصفات المعملية مثل الوزن الطازج للكالس I، الوزن الطازج للكالس II، الوزن الطازج للكالس٣ونسبة تكوين الكالس ، معدل نمو الكالس I، معدل نموالكالس II و معدل نمو الكالس III .

٨- أظهرت الدراسة وجود ارتباط موجب عالي المعنوية بين الصفات الحقلية والمعملية تحت الدراسة ما عدا صفة معدل نمو الكالس III مع جميع الصفات الحقلية .

٩- توضح هذه الدراسة أهمية استخدام الصفات المعملية فى التنبؤ بقوة الهجين لإنتاج هجن او إنتاج سلالات ذرة جديدة فى برامج التربية لصفة المحصول ومكوناته وتوفير للوقت والنفقات لاختيار الآباء الداخلة فى التهجين.