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GENETIC CONTROL OF EMBRYOID INDUCTION AND PLANT REGENERATION ABILITY IN EGYPTIAN WHEAT (*Triticum aestivum L.*) ANTHER CULTURE

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ABSTRACT

Low efficiencies of embryoid induction and green plant regeneration have limited the application of anther culture in breeding programs. However, this study was conducted to determine the inheritance of ability to embryoid induction and subsequently plant regeneration in Egyptian wheat anther culture. Eleven genotypes derived from a 3 x 2 line x tester mating system of five wheat cultivars adapted to Egyptian conditions were evaluated over two different media for their responding anthers, embryoid induction, green plant, albino plant and total plant regeneration ability.

Highly significant difference among genotypes and genotypes x medium interaction were observed for all studied traits. Most of genotypes gave a better response to anther culture on P2-induction medium than on MN6 induction medium. Giza 164 cultivar was, generally, the best one for responding anthers and regeneration ability, especially in the ratio of green plants. Also, it showed higher general combining ability effect for the ratio of anther response, embryoid induction and green plant regeneration. Similar modes of inheritance were observed for these traits, combining ability analysis demonstrated the predominance of dominance gene effects in the genetic control of studied traits. Therefore, most of crosses exhibited desirable significant heterosis for the ratio of anther response and regeneration ability, especially when the averages of the hybrids were compared to their mid-parents values.

In general, the present results confirm the fact of the dominating effects of genetic factors in the expression of anther response and plant regeneration ability. Thus the transfer of anther culture responsiveness to non-responsive breeding materials appear easier than testing numerous culture conditions when there is a possibility that each genotype might require its own specific medium.

INTRODUCTION

Doubled haploid plants have many uses in genetic studies and plant breeding. With recent progress in culture techniques, embryoid and/or calli induction from anthers and plant regeneration from the calli have been greatly improved by manipulation of growth conditions of the donor plants (Guzman and Zapata, 2000), the media composition (Navarro-Alvarez *et al.*, 1994; Ildiko and Bedo, 1997; Zheng and Konzak, 1999) the development stage of microspors and the culture conditions (Dhia and Liang, 1990). Although, the low yield of regenerated plants, which are mostly albinos have limited practical use of dihaploids in cereal crop improvement, new wheat varieties have been produced by the use of anther culture technique such as “Florin” in France (De Buyser *et al*., 1987); in China “Jinghua 1” (Hu, 1986) and “764” (Hu *et al.*, 1988); in Hungary “Gk Deibab” (Pauk *et al.*, 1995).

It was recognized from the beginning of *in vitro* tissue culture studies that responses are affected by the genotype of the plant. For wheat, marked effects of genotype on anther culture response have been observed (Abou-

Ghalia *et al.*, 1997 and Anapiiaev, 2000). It has also been demonstrated that overall wheat haploid plant production from anther culture is controlled by at least three different and independently inherited traits (Agache *et al.*, 1988): embryoid induction rate, embryoid regeneration ability and the ratio of green to albino plants (Henry and De Buyser 1985).

Furthermore, genotype and genotype x environmental conditions interaction effects have been reported (Lazar *et al.,* 1990; Abd El-Maksoud and Bedo, 1993; Dogramaci-Atuntpe *et al.,* 2001). However, studies of the inheritance of responsiveness to anther culture are difficult because of the large amount of uncontrollable and environmentally induced variations (Ockendon and Sutherland, 1987). For embryo formation and plant regeneration in wheat anther culture, it has been shown that nuclear genes are mainly involved, resulting in both additive and non-additive genetic variation, with the additive effects predominating (Deaton *et al*., 1987; Abd El-Maksoud, 1997).

The aim of this work is to provide information about the genetic control involved in androgenetic abilities on different media composition and their inheritance in order to predict the possibility of increasing the yield of haploid production via anther culture by genetic improvement in Egyptian wheat.

MATERIALS AND METHODS

Plant Materials:

In this investigation five wheat varieties adapted to the Egyptian conditions were used. These varieties were Giza 164 (1), Gemmieza 3 (2), Sakha 69 (3), Gemmieza 7 (4) and Sids 1 (5). During the growing season 1999/2000, the five varieties were sown and at the flowering time, the first two varieties were crossed as male parents (pollinators) to the other three varieties (female parents) in order to produce six top crosses. The crosses yielded 6 F_1 hybrids as following: Sakha 69/Giza 164 (3 x 1), Gemmieza 7/Giza 164 (4 x 1), Sids 1/Giza 164 (5 x 1), Sakha 69/Gemmieza 3 (3 x 2), Gemmieza7/Gemmieza 3 (4 x 2) and Sids 1/Gemmieza 3 (5 x 2). In addition, the five varieties were self pollinated to increase seeds from each one.

In the growing season 2000/2001, the six F_1 hybrids and their five parental varieties were grown under field conditions. Thirty spikes from each entries were collected at the mid to late uninucleate microspore stages for anther culture procedure. The optimal microspore stage was determined based on the spike and anther morphology. Suitable tillers could be recognized by morphological traits, which are correlated to the stage of microspore development, when the upper part of the spike is half way up the flag leaf sheath. At this time, the microspores are approximately in the mid to late uninucleate stage of development, which is the preferred stage for anther inoculation. To verify the developmental stage of microspore, one or two anthers from each spike were squashed in a drop of acetocarmine on a glass slide for a microscopic test.

Anther culture procedure:

The collected spikes from each entry were removed from the flag leaf sheath and sterilized. Sterilization was carried out under sterile condition in

0.1% HgCl2 solution for 10 minutes and rinsed three times with sterile distilled water.

The induction media used in this study were Potato 2-medium (P2-medium) according to Ouyang (1986) and modified N6 medium (MN6-medium) recognized by Chu *et al.* (1990). The regeneration media were those recommended by Zhuang and Jia (1983), 190-2 medium for embryoids transferred from P2-medium and MN6 regeneration medium for the embryoids transferred from the MN6 induction media. Therefore, the first and second protocols symbolized by M1 and M2, respectively.

The experiment was designed as a randomized complete block with eleven genotypes, five replications and two media. In each replicate, the anthers of six spikes were distributed equally over four 10cm Petri dishes, each two with a different embryoid induction medium. Thus, each Petri dish containing about 80-110 anthers was considered as experimental unit. The dishes were sealed with parafilm and incubated in darkness at 28±2C^{o} for 42 days. After that period, the total number of anther responded and the total number of embryoids and/or calli were recorded. The embryoids were transferred to regeneration media (P2-medium was replaced by 190-2 regeneration medium, while MN6 induction medium was replaced by MN6 regeneration medium) for shoot and root development. The cultures were placed in controlled incubators with 16 hours white fluorescent illumination at 25±2C^{o} for four weeks, then the total number of green plants and total numbers of albino plants were scored. Meanwhile, the data were recorded on each replicate for the following traits: 1- Responding anthers, as ratio of the number of anthers which responded (producing at least one embryoid or callus) to the total number of anther cultured., 2- Embryoid induction, as ratio of the number of embryoids and/or calli originated from the responding anthers to the total number of anther responded (responding anthers usually produce more than one embryoid and/or callus)., 3- Green plants frequency, the number of green plants divided by the total number of embryoids transferred to regeneration medium., 4- Albino plants frequency, the number of albino plants divided by the total number of embryoids transferred to regeneration medium., 5- Total plants, total regenerated plants (green and albino) divided by the total number of embryoids transferred to regeneration medium.

Statistical analysis:

In order to normalize the distribution of the percentage data which fall between 0.00 to 1.00, the data were transformed by using the arcsine $x^{½}$ function prior to statistical analysis for all studied traits except for embryoid induction percentage, which generally exceeded one.

Analysis of variance was done for single and combined data over the two media according to Steel and Torrie (1980), and Kempthorne (1957) procedure was further followed to estimate combining ability and type of gene effects. To calculate additive genetic variance and dominance genetic variance, the coefficients of inbreeding for maternal and paternal varieties were considered equal to one.

The heterosis values were determined as a relative deviation of F_1 hybrids mean than their mid-parents and better parent.

The heritability estimates were determined from the combined data over two media according to the following equations:

$h^2_b = \sigma^2A + \sigma^2D/ \sigma^2A + \sigma^2D + \sigma^2Axm/M + \sigma^2Dxm/M + \sigma^2e$

$h^2_n = \sigma^2A/ \sigma^2A + \sigma^2D + \sigma^2Axm/M + \sigma^2Dxm/M + \sigma^2e$

where, σ^2e : is the error variance divided by the number of replication; and M is the number of media.

Dominance degree (D.d) was estimated as root squares of the ratio of dominance variance to additive variance $(\sigma^2D/ \sigma^2A)^{½}$.

RESULTS AND DISCUSSION

The results of the analysis of variance for all studied traits at two media and its combined analysis are presented in Table 1. Highly significant differences were existed among entries for all studied traits, indicating a large amount of variability in the response to anther culture and regeneration ability. Therefore, parents and crosses mean squares exhibited highly significant differences with respect to all studied traits at each media and their combined analysis. Parent Vs. crosses mean squares as an indication to average heterosis overall crosses were found to be highly significant for all studied traits except ratio of albino plants at each media and their combined.

Media, entries x media, parents x media and crosses x media mean squares exhibited highly significant difference for all studied traits. This might indicate that the behavior of these genotypes would differ from medium to another with respect to their responding to anther culture and regeneration ability. In addition, the interactions of parent Vs. crosses x media were significant for all studied traits except ratio of green plants, indicated that the test of potential parents for the expression of heterosis would be necessarily conducting over different environmental conditions. While, in the case of the ratio of green plants, the average heterosis overall crosses was stable on the different studied media.

Further partitioning of crosses mean squares i.e. line x tester analysis indicated that the difference due to both females and males were highly significant for all studied traits except due to males for ratio of embryoid induction at first medium (P2-medium) and combined as well as the ratio of albino plants at two media and combined. This reveals that greater diversity existed among male and female parents, especially in their ability to anther culture and green plants regeneration. At the same time, female x male interactions were significant for all studied traits except ratio of embryoid induction in the two media as well as the combined data, indicating that female parents differed in their order of performance in crosses with each of the male parents. Furthermore, highly significant mean square interactions of female x media, male x media and female x male x media were detected for all attributes except female x male x media and male x media for the ratio of embryoid induction and albino plants, respectively. These interactions with media might be caused mainly through different ranking of genotypes (females, males and top-crosses) from medium to another.

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Mean performance of the five parents and their 6 F_1 hybrids for all studied traits were determined over each media and from their combined data and the obtained results are shown in Table 2. Significant differences were observed among genotype means for most of studied traits. In addition, the means showed that most of studied entries gave a better response on P2-induction medium, which replaced by 190-2 regeneration medium (M1) than on MN6 induction and regeneration media (M2). Of the five parental lines, the greatest mean frequencies of responding anthers, embryoid induction, green plants and total regenerated plants were observed in the variety Giza 164 (1) at each media as well as the combined data, and the mean values were 6.44, 3.64, 26.22 and 44.94 from the combined data, respectively, indicating that this variety was the best one for responding anthers and regeneration ability. This supports previous report that Giza 164 highly responded to anther culture (Abd El-Maksoud, 1997 and Abou-Ghalia *et al.*, 1997). However, the inferior variety for anther culture on the two media was Sids 1 (5), which exhibited the lowest mean values for frequencies of anther responded, embryoid induction and green plants, with means of 0.73, 1.47 and 8.75 from the combined data, respectively. Regarding albino plant frequency, the best one for this phenomenon was Gemmieza 3 (2), which showed the lowest frequency of albino plants (7.01 from combined data), but it has also the lowest values of total regenerated plants. Also, the results showed that, the greatest overall values for responding anthers, green plants and total regenerated plants frequencies were recorded in the combination between Sids 1 x Giza 164 (5 x 1) with the means of 10.35, 26.15 and 45.25 from the combined data, respectively. Although, the cross Gemmieza 7 x Giza 164 (3 x 1) was the best for embryoid induction frequency, it ranked as the second one for responding anthers, green plants and total regenerated plants frequencies. This makes it possible to use varieties with a low frequency of haploid plant induction of microspore origin for anther culture purpose through crossing it with a high responded varieties, for instance by crossing Sids 1 (low response) with Giza 164 (high response).

In general, the overall means of crosses exceeded their mid-parents for all studied traits. Furthermore, some crosses, such as Sids 1 x Giza 164 (5 x 1) and Sids 1 x Gemmeiza 3 (5 x 2) showed superiority over their better parents with respect to frequency of anther responded and plant regeneration. This fact could be confirmed by the estimated amount of heterosis over mid-parents and better parent from the data at two media in addition to the combined data, which presented in the Tables 3 and 4, respectively.

The obtained results showed that most of studied crosses exhibited different heterotic values with different media, this may be due to the difference in the performance of the genotypes with different media. Therefore, it could be more precise to concentrate on the results derived from the combined data. All studied hybrids showed significant positive heterosis relative to their mid-parents over two media for the frequency of anther responded and embryoid induction, with heterotic values ranged from 11.31% (4 x 1) to 235.29% (5 x 2) and from 13.39% (4 x 1) to 75.19% (5 x 2), respectively.

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However, the crosses Sakha 69/Giza 164 (3 x 1) and Gemmieza 7/Giza 164 (4 x 1) exhibited desirable negative heterotic values relative to mid-parents for frequency of albino plants, while the hybrids remain showing undesirable positive values.

Regarding green and total regenerated plants frequency, 5 out of six crosses exhibited positive heterosis relative to mid-parents. These values were highly significant and ranged from 10.65% (4 x 2) to 49.56% (5 x 1) for green plant frequency, but it was insignificant in two crosses (3 x1) and (4 x 2) for total plants frequency. The highest heterotic effect for total plant regeneration was observed in the cross Sids 1/Gemmieza 3 (5 x 2) with value 45.70% followed by the cross Sids 1/Giza 164 (5 x 1) with value of 31.47%. On the other hand, significant positive values of heterosis relative to better parent, which would be of interest were found in the following crosses, Sids 1/Giza 164 (5 x 1), Sakha 69/Gemmieza 3 (3 x 2), Gemmieza 7/Gemmieza 3 (4 x 2) and Sids 1/Gemmieza 3 (5 x 2) for frequency of anther responded, with heterotic values ranged from 60.57% to 148.16%. While, the crosses which involved Gemmieza 3 (2) as one of their parents exhibited highly significant positive heterosis for the frequency of embryoid induction. Concerning plant regeneration ability, the cross (5 x 2) showed highly significant positive heterosis relative to better parent for green and total plant regeneration, but it also showed undesirable positive heterotic effect for the frequency of albino plants. The positive heterosis relative to better parents indicates that the dominant genes controlling these characters are more frequent in these crosses. These results were in agreement with the results obtained by Lazar *et al.* 1984; Tuvesson *et al.* 1989; and Abd El-Maksoud, 1997. Thus, these results confirmed that heterosis for frequency of anther responded (while is only present when the anther donor plants are heterozygous) is mainly influenced by the anther wall genotype, but not by the genotype of the pollen. This suggests that the frequency of anther responded as well as embryoid induction in wheat may be controlled by genes in the diploid anther wall tissue, as recognized in petunia (Raquin, 1982).

Estimates of general combining ability effects of the five parents for all studied traits from the combined analysis are given in Table 5. Positive or negative estimates would indicate that a given inbred is much better or much poorer than the average of the group involved with it in line x tester crossing. Among the female parental lines, Sids 1 (5) appeared to be the best combiner for the frequency of anther responded and regeneration ability, but it was the inferior for albino plant regeneration, which showed undesirable highly significant positive GCA effect value. Although, Sakha 69 appeared to be the best combiner among the female parents for embryoid induction, it ranked as the second one for plant regeneration ability. On the other hand, the best combiner over all studied varieties was Giza 164 (1), which exhibited highly significant positive values for the frequency of anther responded, green plants and total plants in addition to desirable insignificant value for the frequency of albino plant. Therefore, it could be mentioned that the best combiner for regeneration ability among this group is Giza 164.Thus, the best general combiner varieties posses favorable genes for improving hybrids and

could be utilized in a traditional breeding program for improving the ability to anther culture response as well as plant regeneration.

Table 5: General combining ability effects for each female and male parents for all studied traits from the combined data over both two media

	Anther responded	Embryoid ind.	Green plants	Albino plants	Total Plants
Females					
3	-0.49	0.138	1.183*	-0.480	1.002
4	-1.35**	-0.187	-2.840**	-1.539*	-5.195**
5	1.84**	0.049	1.657*	2.019**	4.194**
S.E (gi)	0.206	0.077	0.367	0.424	0.650
Male					
1	1.57**	0.071	4.566**	0.083	5.500**
2	-1.57**	-0.071	-4.566**	-0.083	-5.500**
S.E (gj)	0.163	0.094	0.300	0.346	0.531

***,** denote significant at 0.05 and 0.01 levels of probability, respectively.**
Note: The percentages data were transformed by arcsin $x^{½}$ prior to statistical analysis for all traits except embryoid induction percentage.

Specific combining ability effects of the top-crosses for the studied traits from the combined analysis are presented in Table 6. The highest desirable positive SCA effects were detected in the crosses, Sids 1/Giza 164 (5 x 1) and Gemmieza 7/Gemmieza 3 (4 x 2) with respect to the frequency of anther responded, green plants and total plants. Also, these crosses formed the best combination for decreases the frequency of albino plants, which exhibited the highest desirable negative SCA effect values. Although, the crosses Sakha 69/Giza 164 (3 x 1) and Sids 1/Gemmieza 3 (5 x 2) were the best combinations for the frequency of embryoid induction (number of embryoid per anther responded), they were the inferior for anther response and regeneration ability. From this results, it is worth-noting that parents possessing good GCA effect may not exhibit a good SCA effect, but the best SCA effect was obtained from crosses between parents with good x good GCA or poor x good GCA effect. This suggests the varieties with low response to anther culture process, could be used for anther culture purpose by crossing it with high responded varieties.

Table 6: Specific combining ability effects for each cross from the data combined over the two media for all studied traits

	Anther responded	Embryoid ind.	Green plants	Albino plants	Total Plants
3 x 1	-0.483	0.138	0.018	-0.144	0.081
4 x 1	-1.234**	-0.012	-2.436**	0.860	-2.108*
5 x 1	1.717**	-0.126	2.418**	-0.716	2.026*
3 x 2	0.483	-0.138	-0.018	0.144	-0.081
4 x 2	1.234**	0.012	2.436**	-0.860	2.108*
5 x 2	-1.717**	0.126	-2.418**	0.716	-2.026*
S.E (sij)	0.292	0.133	0.519	0.560	0.919

***,** denote significant at 0.05 and 0.01 levels of probability, respectively.**
Note: The percentages data were transformed by arcsin $x^{½}$ prior to statistical analysis for all traits except embryoid induction percentage.

The additive (σ^2A), dominance (σ^2D), additive x media (σ^2Axm) and dominance x media (σ^2Dxm) variances in addition to heritability in broad (h^2_b) and narrow (h^2_n) sense as well as dominance degree ratio (D.d) were estimated from the combined analysis for all studied traits and the obtained results are shown in Table 7. The results revealed that the magnitude of both additive and non-additive (including dominance) genetic variances were positive for all studied traits, indicating to the contribution of both components in the inheritance of these traits. However, the magnitude values of dominance genetic variance were larger than the corresponding values of additive genetic variance for responding anthers, embryoid induction, green plants and albino plants, suggests the predominance of dominance gene effects in the inheritance of these traits. While, additive genetic variance was larger than those of dominance genetic variance for total plant regeneration and this indicated the predominance of additive gene action in the genetic control of this trait. This fact could be emphasized by dominance degree ratio, which were more than one for all studied traits except total plant regeneration with ratio less than one, revealing the importance of over dominance in the expression of these traits. While, in the case of total plant regeneration, partial dominance is important and the additive gene action plays a major role in the inheritance of this trait. These results may explain the absence of heterosis relative to better parent in most of crosses in the case of the frequency of total regenerated plants, and the presence of heterosis over better parent in most of crosses in other traits. These findings are in agreement with the results previously reported for *in vitro* androgenesis of triticale (Charmet and Bernard, 1984), rice (Quimio and Zapata, 1990, Shigeru *et al.*, 1998) and wheat (Lazar *et al.*, 1984; Deaton *et al*., 1987; Zhou and Konzak, 1992).

Furthermore, the results also showed that the variances due to additive x media interactions were negative for regeneration ability (green, albino and total plants), while the dominance x media interactions were positive for these traits. The magnitudes of variance of dominance x media interactions were positive and larger than those of additive x media interaction for the frequency of anther responded. These findings indicate that the additive effects are more stable over different media (environmental conditions) than dominance effects with respect these traits.

High heritability value in broad sense with low ones in narrow sense were detected for the frequency of anther responded and green plants due to the major role of dominance gene effects in the inheritance of these traits. Broad sense heritability value was moderate and close to narrow sense heritability for the frequency of total plant regenerated, exploring the major role of additive gene effects in addition to ecological factors in the expression of this trait. However, in the case of frequency of embryoid induction and albino plants, both broad and narrow sense heritabilities were low and the values of heritability in broad sense were higher than those of narrow sense heritability. This suggests that these traits are highly influenced by ecological factors (media composition and culture conditions) and emphasize the major role of dominance gene effects in their expression.

Table 7: Genetic parameters for all studied traits from the data combined over two media

	Anther responded	Embryoid ind.	Green plants	Albino plants	Total plants
σ^2A	2.22	0.01	21.8	1.82	47.0
σ^2D	9.32	0.05	23.3	2.19	16.3
σ^2A x m	0.08	0.08	-0.40	-11.06	-9.26
σ^2D x m	7.25	0.03	12.73	86.8	120.5
h^2_b	0.75	0.46	0.87	0.08	0.51
h^2_n	0.14	0.08	0.42	0.04	0.38
D.d	2.05	2.24	1.04	1.09	0.59

From the results of this investigation in addition to the previously reported results dealing with the effect of culture conditions, it could be concluded that there are two principal ways of improving anther culture response, namely the adjustment of culture conditions and the genetic improvement of anther donor plants. Because of the dominating effects of genetic factors, which were demonstrated above, the latter way is (at the present time) more useful for practical application. The transfer of anther culture responsiveness to non-responsive breeding material appears to be easier than testing numerous culture conditions when there is a possibility that each genotype might require its own specific medium.

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التحكم الوراثى فى القدرة على إنتاج أشباه الأجنة وانبثاق النباتات من زراعة المتوك للأقماح المصرية

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نظراً للكفاءة المنخفضة لعديد من التراكيب الوراثية فى معدل إنتاج أشباه الأجنة وما يتبعها من إنتاج نباتات خضراء يحد من إستخدام زراعة المتوك فى برامج التربية. فقد أجريت هذه الدراسة بهدف تحديد السلوك الوراثى للقدرة على الإستجابة لزراعة المتوك من خلال إنتاج أشباه الأجنة وما يتبعها من إنتاج النباتات فى الأقماح المصرية. ومن أجل هذا الغرض فقد تم أستخدام أحد عشر تركيباً وراثياً شملت خمسة آباء من أصناف القمح المصرية بالإضافة الى ستة هجن ناتجة من نظام التزاوج 3 × 2(سلالة X كشاف).

وقيمت هذه التراكيب الوراثية على بيئتين مغذيتين مختلفتين من حيث القابلية لزراعة المتوك والقدرة على إنتاج أشباه الأجنة وقدرتها على التكشف إلى نباتات ولذلك كانت الصفات المدروسة تشمل: معدل إستجابة المتوك لإنتاج أشباه أجنة، معدل أشباه الأجنة لكل متك، معدل النباتات الخضراء لأشباه الأجنة المنزرعة، معدل النباتات الألبينو لأشباه الأجنة المنزرعة ومعدل النباتات الكلية لأشباه الأجنة المنزرعة.

ويمكن إيجاز أهم النتائج المتحصل عليها فيما يلى:

أظهرت إختبارات المعنوية أن هناك إختلاف عالى المعنوية بين التراكيب الوراثية وأيضا تداخل التراكيب الوراثية مع البيئات وذلك فى كل الصفات المدروسة.

كانت معظم التراكيب الوراثية أكثر إستجابة لزراعة المتوك على البيئة المغذية P2 منها على البيئة المغذية MN6 .

أظهر الصنف جيزة 164 أنه أكثر الأصناف المستخدمة قدرة على إنتاج أشباه أجنة وقدرتها على التكشف الى نباتات كاملة وكان معظمها نباتات خضراء. بالإضافة الى ذلك أظهر هذا الصنف أنه الأكثر قيمة للقدرة العامة على التآلف لهذه الصفات.

ومن تحليل القدرة العامة على التآلف تبين أن هذه الصفات يتحكم فيها نظام وراثى متماثل يسود به تأثير الفعل الجينى السيادى ولذلك كانت تقديرات معامل السيادة تزيد عن الواحد الصحيح فى كل الصفات فيما عدا صفة معدل النباتات الكلية الناتجة التى كان كل من الفعل الجينى المضيف والسيادى له دور فعال فى إظهارها مع زيادة الفعل المضيف.

أظهرت الهجن قوة هجين مرغوبة فى كل الصفات خاصة عندما قورن متوسط الهجن بمتوسط آبائها.

ويمكن أن نستخلص من ذلك أن هذه النتائج تؤكد الدور الفعال الذى تلعبه العوامل الوراثية فى تعبير هذه الصفات ولذلك يمكن تحسين القدرة على الإستجابة لزراعة المتوك وإنتاج أشباه أجنة وقدرتها على التكشف الى نباتات خضراء عن طريق نقلها من الأصناف الأعلى إستجابة الى الأصناف ضعيفة الإستجابة، وهذا يكون أسهل بكثير من معالجة البيئة المغذية وظروف الزراعة داخل المعمل خاصة وأنه ثبت من الدراسات السابقة أن كل تركيب وراثى قد يحتاج الى ظروف محددة تختلف عن الآخر لكى يمكنه إنتاج نباتات أحادية.

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Table 1: Analysis of variance for all studied traits at single medium and their combined data over two media
Note: The percentages data were transformed by arcsin $x^{1/2}$ prior to statistical analysis for all traits except embryoid induction percentage.

S.O.V	d.f		Anther responded			Embryoid induction			Green plants			Albino plants			Total plants		
	S.	C.	M1	M2	Comb	M1	M2	Comb	M1	M2	Comb	M1	M2	Comb	M1	M2	Comb.
Media(m)	-	1	-	-	127.79**	-	-	8.99**	-	-	351.49**	-	-	7.55*	-	-	596.57**
R./m.	4	8	0.57	0.29	0.44	0.06	0.01	0.04	4.01*	0.66	2.34	4.04	3.66*	3.85*	2.92	3.39	3.16
Entries(E)	10	10	47.73**	47.35**	79.35**	5.19**	1.45**	5.36**	273.2**	143.8**	391.7**	78.32**	78.7**	97.22**	543.16**	349.17**	756.85**
Parents(P)	4	4	56.50**	8.93**	54.49**	5.10**	2.04**	6.57**	326.9**	182.1**	481.2**	98.96**	124.2**	203.20**	707.01**	457.15**	1089.7**
P.VS.C	1	1	182.39**	58.57**	223.83**	27.6**	3.38**	25.2**	142.7**	118.6**	260.7**	3.97	4.79	0.02	260.35**	78.14**	311.88**
Crosses(C)	5	5	13.78**	75.85**	70.35**	0.78**	0.59**	0.43**	256.2**	118.2**	346.1**	76.68**	56.97**	31.88**	468.63**	316.99**	579.57**
Females(F)	2	2	19.98**	45.40**	54.73**	1.45**	0.76**	0.56**	98.1**	50.1**	122.1**	56.26**	46.33**	66.77**	420.37**	232.47**	455.82**
Males (M)	1	1	17.88**	168.79**	148.27**	0.14	1.32**	0.30	813.5**	461.9**	1250.7**	0.15	1.68	0.41	1153.9**	690.72**	1815.11**
F x M	2	2	5.53*	59.83**	47.02**	0.42	0.05	0.35	135.7**	14.59**	117.8**	135.4**	95.25**	12.72**	174.25**	214.64**	85.55**
E. x m.	-	10	-	-	15.73**	-	-	1.28**	-	-	25.30**	-	-	59.74**	-	-	135.47**
P x m	-	4	-	-	10.95**	-	-	0.57**	-	-	27.63**	-	-	19.97**	-	-	74.47**
P.VS.Cxm.	-	1	-	-	17.12**	-	-	5.83**	-	-	0.55	-	-	8.74*	-	-	26.61*
C x m.	-	5	-	-	19.28**	-	-	0.94**	-	-	28.39**	-	-	101.77**	-	-	206.05**
F x m.	-	2	-	-	10.65**	-	-	1.65**	-	-	26.13**	-	-	35.81**	-	-	197.02**
M x m.	-	1	-	-	38.40**	-	-	1.16**	-	-	24.70**	-	-	1.42	-	-	29.55**
Fx M x m.	-	2	-	-	18.35**	-	-	0.12	-	-	32.49**	-	-	217.90**	-	-	303.34**
Error	40	80	0.73	0.12	0.42	0.14	0.04	0.09	1.53	1.17	1.35	2.34	1.25	1.80	5.21	3.24	4.23

***,** denote significant at 0.05 and 0.01 levels of probability, respectively.**

Table 2: The mean performances of all entries at each medium and their combined data for all studied traits

Entries.	Anther responded			Embryoid ind.			Green plants			Albino plants			Total plants		
	M1	M2	Comb.	M1	M2	Comb.	M1	M2	Comb.	M1	M2	Comb	M1	M2	Comb.
1	8.86	4.03	6.44	4.01	3.26	3.64	29.23	23.21	26.22	14.36	16.26	15.31	47.5	42.4	44.94
2	2.18	0.86	1.52	2.46	2.34	2.40	10.95	9.67	10.31	8.17	5.86	7.01	19.4	15.7	17.53
3	2.35	1.58	1.97	1.96	2.42	2.19	18.43	10.49	14.46	19.77	18.67	19.22	40.3	29.1	34.68
4	0.93	1.27	1.10	2.05	1.82	1.94	12.71	12.01	12.36	17.1	14.94	16.02	30.9	29.1	30.02
5	0.66	0.79	0.73	1.31	1.62	1.47	9.33	8.17	8.75	12.36	16.50	14.43	22.1	26.0	24.04
3 x 1	7.94	3.68	5.81	3.95	3.32	3.63	27.40	19.15	23.28	18.91	8.75	13.83	51.7	28.6	40.21
4 x 1	5.06	3.35	4.20	3.28	3.04	3.16	17.22	16.38	16.80	13.07	14.48	13.78	31.5	32.2	31.82
5 x 1	9.28	11.42	10.35	3.92	2.64	3.28	29.09	23.21	26.15	12.48	19.03	15.76	44.6	46.1	45.35
3 x 2	5.99	1.27	3.63	3.63	2.8	3.22	16.33	11.89	14.11	12.83	15.07	13.95	30.3	27.8	29.05
4 x 2	5.16	1.89	3.53	3.52	2.56	3.04	14.48	10.60	12.54	11.30	12.48	11.89	26.5	23.6	25.04
5 x 2	6.49	1.05	3.77	4.40	2.38	3.39	11.66	12.71	12.18	20.75	13.30	17.02	33.8	26.8	30.29
LSD 0.05	1.09	0.43	0.81	0.47	0.26	0.37	1.58	1.38	1.44	1.95	1.43	1.66	2.92	2.30	2.55
0.01	1.46	0.58	1.06	0.63	0.35	0.49	2.11	1.84	1.89	2.61	1.91	2.19	3.90	3.07	3.35

Note: The percentages data were transformed by arcsin $x^{½}$ prior to statistical analysis for all traits except embryoid induction percentage.

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Table 3: Estimates of heterosis relative to mid-parents (M.P) for all studied traits at each medium and their combined data

	Anther responded			Embryoid ind.			Green plants			Albino plants			Total plants		
	M1	M2	Comb.	M1	M2	Comb.	M1	M2	Comb.	M1	M2	Comb	M1	M2	Comb.
3 x 1	41.53**	31.57**	38.24**	31.97**	16.90**	24.63**	14.99**	13.67**	14.44**	10.78*	-49.91**	-19.90**	17.89**	-19.75**	1.00
4 x 1	3.22	26.26**	11.31*	8.12	19.69**	13.39**	-17.88**	-6.97*	-12.90**	-16.92**	-7.17	-12.07**	-19.69**	-10.06**	-15.07**
5 x 1	94.92**	373.86**	188.68**	47.37**	8.20	28.63**	50.88**	47.94**	49.56**	-6.57	16.20**	5.97*	28.28**	34.68**	31.47**
3 x 2	165.22**	-6.32	108.85**	64.25**	17.65**	40.09**	11.16*	17.96**	13.93**	-8.13	22.94**	6.39*	1.44	24.35**	11.24**
4 x 2	233.03**	76.64**	169.16**	55.75**	23.08**	39.77**	22.40**	-2.18	10.65**	-10.50	19.96**	3.26	5.25	5.37	5.35
5 x 2	357.04**	28.54	235.29**	132.80**	20.20**	75.19**	14.97*	42.49**	27.85**	102.2**	18.96**	58.81**	63.07**	28.40**	45.70**
LSD 0.05	0.95	0.38	0.70	0.41	0.23	0.32	1.37	1.19	1.25	1.69	1.24	1.44	2.53	1.99	2.21
0.01	1.27	0.50	0.92	0.55	0.30	0.42	1.83	1.60	1.64	2.26	1.66	1.89	3.38	2.66	2.90

***,** denote significant at 0.05 and 0.01 levels of probability, respectively.**
Note: The percentages data were transformed by arcsin $x^{1/2}$ prior to statistical analysis for all traits except embryoid induction percentage.

Table 4: Estimates of heterosis relative to better parent (B.P) for all studied traits at each medium and their combined data

	Anther responded			Embryoid ind.			Green plants			Albino plants			Total plants		
	M1	M2	Comb.	M1	M2	Comb.	M1	M2	Comb.	M1	M2	Comb	M1	M2	Comb.
3 x 1	-10.36	-8.59	-9.81**	-1.60	1.84	-0.06	-6.25*	-17.48**	-11.22**	-4.35	-53.13**	-28.04**	8.97**	-32.36**	-10.53**
4 x 1	-42.91**	-16.97**	-34.80**	-18.3**	-6.75	-13.12**	-41.09**	-29.42**	-35.92**	-23.58**	-10.93*	-14.01**	-33.67**	-24.20**	-29.19**
5 x 1	4.72	183.37**	60.57**	-2.24	-19.02**	-9.77**	-0.48	0.01	-0.26	-13.08	15.36**	2.89	-5.99	8.59**	0.90
3 x 2	155.06**	-19.37	84.94**	47.56**	15.70**	33.96**	-11.39*	13.35*	-2.39	-35.08**	-19.27**	-27.40**	-24.85**	-4.34	-16.25**
4 x 2	136.79**	48.82**	131.97**	43.09**	9.40	26.67**	13.93*	-11.71*	1.47	-33.89**	-16.49**	-25.78**	-14.35**	-18.96**	-16.60**
5 x 2	197.71**	22.56	148.16**	78.86**	1.71	41.25**	6.47	31.44**	18.12**	67.86**	-19.39**	17.97**	53.18**	2.91	26.01**
LSD 0.05	1.09	0.43	0.81	0.47	0.26	0.37	1.58	1.38	1.44	1.95	1.43	1.66	2.92	2.30	2.55
0.01	1.46	0.58	1.06	0.63	0.35	0.49	2.11	1.84	1.89	2.61	1.91	1.89	3.90	3.07	3.35

***,** denote significant at 0.05 and 0.01 levels of probability, respectively.**
Note: The percentages data were transformed by arcsin $x^{1/2}$ prior to statistical analysis for all traits except embryoid induction percentage.

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