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PERFORMANCE AND GENETIC NATURE OF ANTHER CULTURE RESPONSE TRAITS FOR SOME EGYPTIAN WHEAT CULTIVARS AND THEIR F1 CROSSES

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ABSTRACT

The objectives of this investigation were to study the performance and genetic nature of anther culture response traits of some Egyptian wheat (Triticum aestivum L.) cultivars and their F1 crosses. Eight out of the studied fifteen wheat genotypes showed anther culture response. Sahel 1 cultivar and the cross Sakha 8 X Giza 164 exhibited the highest callus induction and green plantlet regeneration from anther culture. Significant favorable heterosis estimates were shown by the two crosses Sids 1 X Giza 164 (460%) and Sakha 8 X Giza 164 (300%) for percentage of responding anthers and by the cross Sakha 8 X Giza 164 (483.5%) for number of regenerated plants. For both studied traits the best general combiners were Sakha 8 (female) and Giza 164 (male) and the best specific combining ability effect was shown by the crosses Sahel 1 X Gemmeiza 5 and Sakha 8 X Giza 164. Estimates of dominance (δ^2D) were appreciably larger than additive (δ^2A) genetic variance and the degree of dominance indicated the role of overdominance in the inheritance of both traits. The narrow-sense heritability estimate for percentage of responding anthers was 27.47%.

Key words: ***Bread wheat, Triticum aestivum, Anther culture, Inheritance, Androgenic traits, Regeneration, Responding anthers, Embryoids, Albino plantlets, Haploid plants.***

INTRODUCTION

The use of haploid plants, generated from anther culture technique could enhance the efficiency of crop breeding programs (Collins and Genovesi 1982). This is because desirable genetic recombinants resulting from microsporogenesis could be exploited by recovering haploid plants from anther culture. Promising recombinants, however, could be utilized as new varieties or homozygous breeding lines after chromosome doubling. Pure diploid lines of desirable recombinations have been developed via *in vitro* anther culture of F1 or F2 generations of crosses of wheat, rice and many other crops and have been utilized as new varieties in China and other countries (Hu 1996).

Successful wheat anther culture depends on callus initiation, and subsequent green plant regeneration. Marked role of genotype on wheat anther culture response has been reported by many investigators (Al-Naggar and Youssef 1990, Lu *et al* 1991, Cattaneo and Qiao 1991, Becraft and Taylor 1992, Ekiz and Konzak 1994a, Moieni *et al* 1997, Machii *et al* 1998 and Moussa *et al* 1999). They found that callus formation and plant regeneration from wheat anthers are genotype-dependent traits.

Previous studies (Agache *et al* 1989, Zhou and Konzak 1992 and Moieni *et al* 1997) found that most of the genetic variation for wheat anther culture response and plant regeneration was due to the additive gene action. However, Lazar *et al* (1984), Deaton *et al* (1987) and Ekiz and Konzak (1994b) indicated the role of dominance genetic variance in the inheritance of androgenic traits of wheat.

The objectives of this study were to test the performance and genetic nature of anther culture response traits of some Egyptian wheat cultivars and their F_1 crosses.

MATERIALS AND METHODS

Plants of 15 Egyptian wheat (*Triticum aestivum* L.) genotypes i.e. six cultivars, Sahel 1, Sids 1, Sakha 8, Gemmeiza 5, Giza 164 and Giza 168 and their nine F1 crosses (Sahel 1 x Gemmeiza 5, Sahel 1 x Giza 164, Sahel 1 x Giza 168, Sids 1 x Gemmeiza 5, Sids 1 x Giza 164, Sids 1 x Giza 168, Sakha 8 x Gemmeiza 5, Sakha 8 x Giza 164 and Sakha 8 x Giza 168) were used as the genetic material of the present study. Plants were grown under field conditions during the winter season 2001/2002 and properly watered and fertilized to maintain vigorous growth.

Primary tillers were collected when the top of the inflorescence was midway between the penultimate leaf and flag leaf. At this stage, microspores of anthers from the primary and secondary florets of the central spikelets are in mid- to late-uninucleate. Flag leaves were removed and the tillers were placed in flasks containing 40 ml of tap water. They were wrapped with aluminum foil to maintain high humidity and to exclude light, and stored for 4 days at 5°C. Before anther culture, sample anthers from the florets in the middle of the spike were stained with 4% acetocarmine and examined in light microscope to confirm the initial staging. Before the spikes were removed from the ensheathing leaves, tillers were surface sterilized with an aerosol 75 % (v/v) ethanol and then removed from leaves, sterilized in 10 % (v/v) commercial Clorox solution for 10 minutes and rinsed four times with sterile distilled water. Each spike was aseptically

excised from the sheath, and the poorly developed distal and basal spikelets were discarded. Anthers from the primary and secondary florets of the central spikelets were excised using fine tipped forceps. The anthers were cultured directly onto vessels containing induction medium. Three replications were used each containing 100 anthers. The culture vessels were sealed with Parafilm and incubated in a dark incubator at 28 ^{0}C (according to Marburger *et al* 1987).

Liquid potato-4 (P4) medium (Ouyang 1986) was used as a standard induction medium. The potato extract was freshly made using 100g peeled potato tubers boiled in 200 ml-distilled water. After continuous boiling for 10 minutes about 100-ml broth remained. The supernatant broth was poured off without disturbing the residue and used to prepare the induction medium. The standard induction medium contained 90 g/l sucrose, 1.5 mg/l 2,4-D and 0.5 mg/l Kinetin.

Embryoids developed from the anthers were transferred to MS regeneration medium for plant regeneration when they reached 1mm in diameter. The embryoids were transferred twice, the first after 30 days and the second after 40 days from anther plating. The standard regeneration medium contained MS salts, 30g/l sucrose, 0.5 mg/l Kinetin, 0.5 mg/l Naphthaleneacetic acid and solidified using 2.5g/l gelrite. Embryoids were cultured on the regeneration medium in a randomized complete block design with three replications in petri dishes, and kept at 26° C and 16-h photoperiod. Embryoids regenerating into green or albino plants were recorded 30 days later. For better root formation green plantlets were transferred into vials containing regeneration medium supplemented with 4g/l Charcoal. When several shoots and roots have been developed, plants were potted in small pots containing mixture of good soil and peatmoss and transferred to a greenhouse at 22 ^{0}C. To prevent plants from water stress, they were covered with plastic or glass caps for about one week.

The following data on anther culturability were recorded: (1) % of responding anthers, (2) number of pollen embryoids per 100 anthers, (3) % of regenerated green plantlets and (4) % of regenerated albino plantlets. Square-root transformation was used when data were in percentages and the normal analysis of variance of completely randomized design was done according to Gomez and Gomez (1984). Analysis of variance suggested by Comstock and Robinson (1952) was used to estimate the combining ability variances and effects and components of genetic variance for the percentage of responding anthers (%) and % of regenerated green plants characteristics. Narrow sense heritability, heterosis and degree of dominance were estimated too.

RESULTS AND DISCUSSION

Performance of anther culture traits

Eight wheat genotypes i.e. four parents (Sahel 1, Sakha 8, Gemmeiza 5 and Giza 164) and four crosses (Sahel 1 X Gemmeiza 5, Sahel 1 X Giza١٦٤, Sids 1 X Giza 164 and Sakha 8 X Giza 164) out of the 15 studied genotypes showed anther culture response (Table 1 and Figure 1). Among parents, Sahel 1 followed by Giza 164 and Sakha 8 exhibited the highest anther culture response i.e. 8.33, 5.0 and 5.0%, respectively. However, Sakha 8 X Giza 164 (Fig. 1), among F_1 crosses showed the highest percentage of responding anthers (20.00%) followed by Sids 1 X Giza 164 (14%).

The number of embryoids induced per 100 anthers was the highest in Sakha 8 X Giza 164 (25.00%), followed by Sids 1 X Giza 164 (15.67%) and Sahel 1 (13.33%). Moreover, for green plantlet regeneration, Sakha 8 X Giza 164 (Fig. 1) showed the highest percentage (11.67%) followed by Sahel 1 (10.33%). It should be noted that Sakha 8 X Giza 164 produced also the highest number of albino plants (7 plants from 300 anthers). Although, the cross Sids 1 X Giza 164 exhibited high percentage of responding anthers (14.00%) and of embryoids (15.67%), it showed low percentage of green plantlets regeneration (1.67%).

Many wheat cultivars with good anther response traits have been identified in different laboratories around the world. However, the number of such cultivars among the large tested numbers of cultivated genotypes seemed to be very limited (Orlov *et al* 1993). This was confirmed by other workers who found significant genotypic variations for anther culture response, embryoid formation and green plantlets regeneration (Lu *et al* 1991, Cattaneo and Qiao 1991, Becraft and Taylor 1992, Ekiz and Konzak 1994a, Bruins and Snijders 1995, Moieni *et al* 1997, Machii *et al* 1998 and Moussa *et al* 1999). All of them assured the influence of genetic background on anther culture response, embryo induction frequency and regeneration ability.

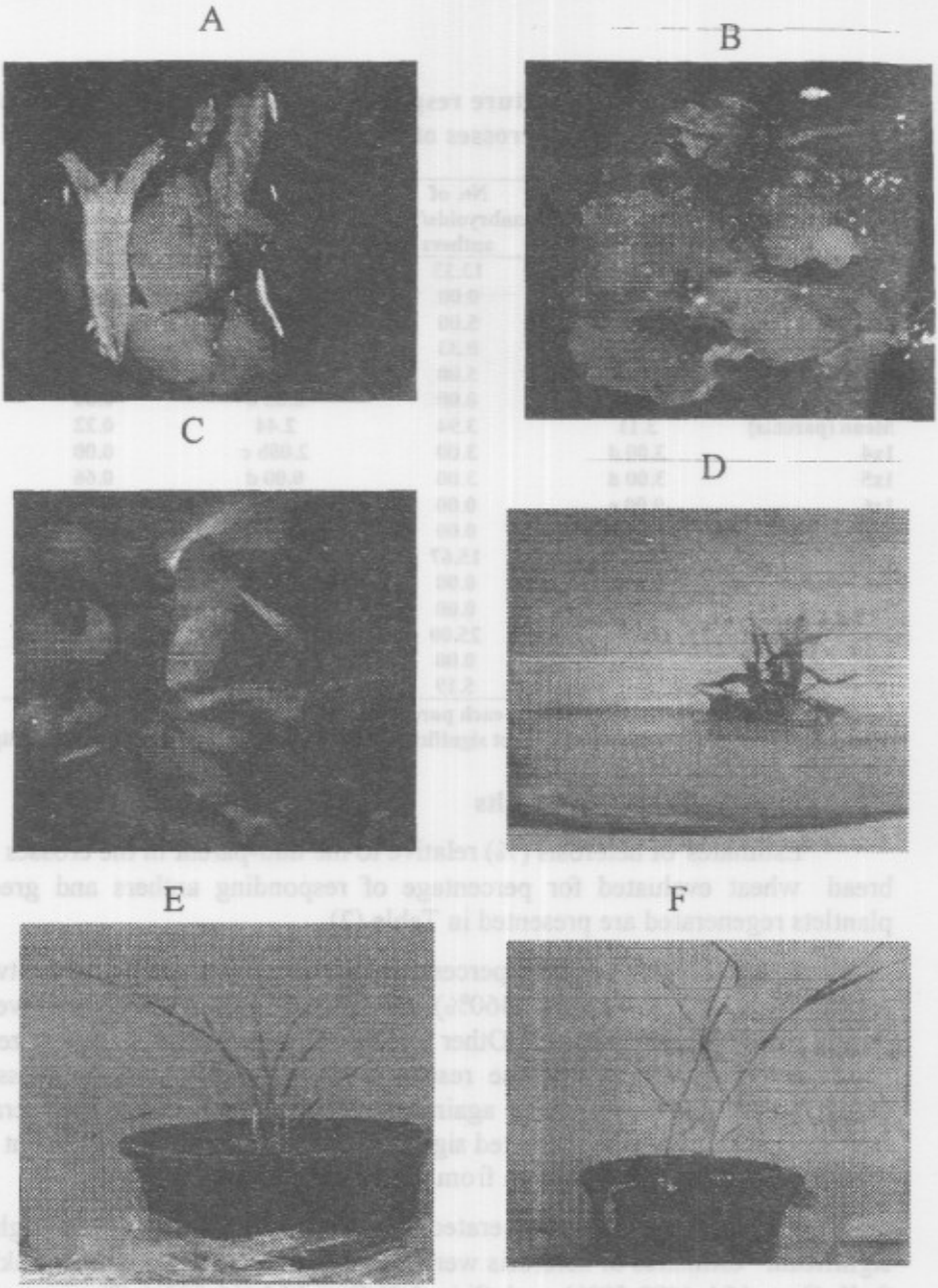

Fig. 1. Wheat anther culture and haploid production. Embryo-like structures (embryoids) formation 30 days from anther culture on Potato-4 medium supplemented with 1.5 mg /l 2, 4-D and 0.5 mg / l kinetin in A. Regenerable embryo-like structures showing green regions on MS medium supplemented with 0.5 mg / l kinetin and 0.5 mg / l NAA in B. Further development of green shoots in C. Regenerated plant at optimum size for transplanting into pots in D. Plants after transplanting from vessels to plastic pot in E. Further development of regenerated plants during adaptation in F.

Table 1. Means of anther culture response characteristics of six parents and their nine F_1 crosses of bread wheat.

Genotype	% of responding anthers	No. of embryoids/100 anthers	No. of green plantlets/100 anthers	No. of albino plantlets/100 anthers
Sahel 1 (1)	8.33 c	13.33	10.33 a	0.66
Sids 1 (2)	0.00 e	0.00	0.00 d	0.00
Sakha 8 (3)	5.00 c	5.00	1.00 bcd	0.33
Gemmeiza 5 (4)	0.33 e	0.33	0.33 cd	0.00
Giza 164 (5)	5.00 c	5.00	3.00 b	0.33
Giza 168 (6)	0.00 e	0.00	0.00 d	0.00
Mean (parents)	3.11	3.94	2.44	0.22
1x4	3.00 d	3.00	2.00b c	0.00
1x5	3.00 d	3.00	0.00 d	0.66
1x6	0.00 e	0.00	0.00 d	0.00
2x4	0.00 e	0.00	0.00 d	0.00
2x5	14.00 b	15.67	1.67 bc	0.33
2x6	0.00 e	0.00	0.00 d	0.00
3x4	0.00 e	0.00	0.00 d	0.00
3x5	20.00 a	25.00	11.67 a	2.33
3x6	0.00 e	0.00	0.00 d	0.00
Mean (crosses)	4.33	5.19	1.70	0.37

Number of anthers cultured was 300 for each parent or cross
Means followed by the same letter are not significantly different according to Duncan's multiple range test.

Heterosis in anther culture traits

Estimates of heterosis (%) relative to the mid-parent in the crosses of bread wheat evaluated for percentage of responding anthers and green plantlets regenerated are presented in Table (2).

Heterosis estimates of percentage of responding anthers of the two crosses Sids 1 X Giza 164 (460%) and Sakha 8 X Giza 164 (300%) were positive and highly significant. Other crosses showed either negative or zero heterosis (non-favorable). These results confirm the fact that some crosses had hybrid vigor performance against their parents as revealed by Becraft and Taylor (1992) who detected significant heterosis only from one out of three crosses for callus initiation from anther culture.

For number of regenerated green plantlets positive and highly significant estimates of heterosis were also shown by the two crosses Sakha 8 X Giza 164 (483.50%) and Sids 1 X Giza 164 (11.33%). These results indicated that these two crosses had useful hybrid vigor in both studied androgenic traits and transgressive segregants for high percentages of anther culture response and green plant regeneration could be selected from their segregating generations.

Table 2. Estimates of heterosis (%) relative to the mid-parent in nine crosses of bread wheat for % of responding anthers and % of regenerated green plantlets.

Crosses	% of responding anthers	% of regenerated green plantlets
Sahel 1 X Gemmeiza 5	-30.71	-62.50
Sahel 1 X Giza 164	-55.00	-100.00
Sahel 1 X Giza 168	-100.00	-100.00
Sids 1 X Gemmeiza 5	-100.00	-100.00
Sids 1 X Giza 164	460.00**	11.33**
Sids 1 X Giza 168	00.00	00.00
Sakha 8 X Gemmeiza 5	-100.00	-100.00
Sakha 8 X Giza 164	300.00**	483.50**
Sakha 8 X Giza 168	-100.00	-100.00

Inheritance of anther culture traits

The analysis of variance (not presented) showed the existence of significant genetic variability among wheat parents and crosses for the two traits. These results confirm the fact that the genotype is a major determinant of embryoid production and plant regeneration as mentioned by previous findings of Abdel-Maksoud and Bedo (1993) and Oılov *et al* (1993).

Mean squares due to specific combining ability (SCA) were highly significant for anther culture response trait. While mean squares due to general combining ability (GCA) were highly significant for only % of responding anthers in males. These results suggest that both additive and dominance variances control the inheritance of % of responding anthers.

Other studies (Agache *et al* 1989 and Zhou and Konzak 1992), indicated that most of the genetic variation for wheat anther culture response was due to general combining ability. Moieni *et al* (1997) also found that GCA variance was significant for all androgenic traits, except for albino plant regeneration, however, specific combining ability was not significant. On the other hand, significant SCA variances were also observed for wheat anther culture response in other investigations (Lazar *et al* 1984, Deaton *et al* 1987, and Ekiz and Konzak 1994b). The inconsistency between different results might be attributed to the difference in genetic background of the wheat genotypes used in different studies.

GCA effects for anther culture traits

Estimates of GCA effects for percentage of responding anthers and percentage of regenerated green plantlets are presented in Table (4). The results indicated that the female Sakha 8 and the male Giza 164 had positive and significant GCA effects (favorable) for percentage of responding anthers and percentage of regenerated green plantlets, hence, they are good combiners for these two traits

Table 4. Estimates of general combining ability effects from Comstock and Robinson (1952) design II analysis of six wheat parents and their nine F1 crosses for % of responding anthers and % of regenerated green plantlets.

Parents	% of responding anthers	% of regenerated green plantlets
	Females	
Sahel 1	-0.28*	-0.20
Sids 1	0.01	-0.23
Sakha 8	0.27**	0.42**
	Males	
Gemmeiza 5	-0.67**	-0.20
Giza 164	1.67**	0.67**
Giza 168	-1.00**	-0.47*
S.E.gi	0.14	0.14
S.E. (gi-gj)	0.20	0.19

*** and ** indicate significance at 5 and 1% probability levels, respectively.**

SCA effects for anther culture traits

Estimates of specific combining ability (SCA) effects for % of responding anthers and % of regenerated green plantlets are presented in Table (5). The results showed that only the three crosses Sahel 1 X Gemmeiza 5, Sids 1 X Giza 164 and Sakha 8 X Giza 164 had the positive and significant SCA effects (favorable) for % of responding anthers. However, for % of regenerated green plantlets the two crosses Sahel 1 X Gemmeiza 5 and Sakha 8 X Giza 164 only had positive and significant SCA effects.

Table 5. Estimates of specific combining ability (SCA) effects from Comstock and Robinson (1952) design II analysis of six wheat parents and their F_1 crosses for % of responding anthers and % of regenerated green plantlets.

Crosses	% of responding anthers	% regenerated green plantlets
Sahel 1 X Gemmeiza 5	0.97**	0.74**
Sahel 1 X Giza 164	-1.24**	-0.94**
Sahel 1 X Giza 168	0.27	0.20
Sids 1 X Gemmeiza 5	-0.36	-0.05
Sids 1 X Giza 164	0.36**	-0.18
Sids 1 X Giza 168	0.00	0.23
Sakha 8 X Gemmeiza 5	-0.60*	-0.70**
Sakha 8 X Giza 164	0.86**	1.12**
Sakha 8 X Giza 168	-0.26	-0.42
S.E. (Sij)	0.25	0.24
S.E. (Sij-Sik)	0.34	0.32
S.E. (Sij-Skl)	0.35	0.34

Type of gene action and heritability:

Estimates of dominance genetic variance (δ^2D) were appreciably larger than additive genetic variance (δ^2A) for both traits: number of responding anthers and number of regenerated green plantlets (Table 6). The degree of dominance "a" indicated the role of overdominance in the inheritance of both studied traits with the difference that it was overdominance for the higher parent (1.57) concerning % of responding anthers and for the lower parent (-6.36) concerning % of regenerated plants. The results obtained by Zhou and Konzak (1992) and Ekiz and Konzak (1994b) from F_1's suggested that additive genetic effects predominated for embryoid induction and plant regeneration, in addition epistasis and dominance were also observed in some crosses. Our results agreed with the findings of Lazar *et al* (1984), Deaton *et al* (1987) and Ekiz and Konzak (1994 b) who reported that dominance gene actions were predominant.

Table 6. Estimates of additive (δ^2A) and dominance genetic variances (δ^2D), heritability in the narrow sense (h^2_{ns}) and degree of dominance "a" for % of responding anthers and % of regenerated green plantlets of bread wheat.

Genetic components	% of responding anthers	% of regenerated green plantlets
(δ^2A)	0.38	-0.02
(δ^2D)	0.94	0.81
"a"	1.57	-6.36
h^2 ns	27.47	0.00

Narrow-sense heritability estimate in the present study was 27.47% for the percentage of responding anthers. While for percentage of regenerated green plantlets the h^2_{ns} estimate was zero, since the additive ($\delta^2 A$) variance showed a negative estimate (-0.02) and therefore was considered zero. These results indicated that the percentage of responding anthers trait is governed by both dominance and additive genetic variance and is strongly heritable.

Several investigators confirmed the fact that the percentage of anther culture response and other androgenetic traits are highly heritable and rapid gain from selection should be possible (Lazar *et al* 1984 and Moieni *et al.* 1997).

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أداء وطبيعة توارث صفات الاستجابة لزراعة المتوك لبعض أصناف القمح المصرية وهجنها

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تم إختبار صفتى الاستجابة لزراعة المتوك وإستيلاد النباتات الخضراء معمليا فى بعض أصناف القمح المصرية (ستة) وهى سلحل ١ وسدس ١ وسخا ٨ وجميزه ٥ وجيزه ١٦٤ وجيزه ١٦٨ وهجنها (تسعة) من حيث الآداء وطبيعة التوارث. استجابت ثمانية تراكيب وراثية لزراعة المتوك من الخمس عشر تركيب وراثي تحت الدراسة. أظهر الصنف سلحل١ والهجين سخا ٨ × جيزة ١٦٤ أعلى استجابة لزراعة المتوك وأعلى قدرة على إستيلاد النباتات الخضراء. كانت أفضل التقديرات لقوة الهجين (الموجبة وعالية المعنوية) قد ظهرت فى الهجينين سدس ١ × جيزة ١٦٤ (٤٦٠%) وسخا ٨ × جيزة ١٦٤ قوة هجين (٣٠٠%) بالنسبة لصفة الاستجابة لزراعة المتوك والهجين سخا ٨ × جيزة ١٦٤ (٤٨٣,٥%) لصفة إستيلاد النباتات الخضراء. كانت متوسطات المربعات الراجعة للقدرة الخاصة على التآلف عالية المعنوية لكل من صفتى استجابة المتوك والنسبة المئوية لاستيلاد النباتات الخضراء بينما كانت متوسطات المربعات الراجعة للقدرة العامة على التآلف عالية المعنوية فقط فيما يتعلق بالنسبة المئوية لإستجابة المتوك فى الآباء الذكور. كانت أفضل الآباء فى القدرة العامة على التآلف هى سخا ٨ (كأم) وجيزة ١٦٤ (كأب) وأفضل الهجن فى القدرة الخاصة على التآلف هى سلحل ١ × جميزة ٥ وسخا ٨ × جيزة ١٦٤ وذلك للصفتين المدروستين. وكانت تقديرات التباين الوراثى الراجعة للسيادة أكبر من تلك الراجعة لفعل الجين المضيف (حيث كانت السيادة من نوع السيادة الفائقة) فى كل من صفتى الاستجابة لزراعة المتوك واستيلاد النباتات الخضراء. وقد بلغت كفاءة التوريث فى المعنى الخاص ٢٧,٤٧ % بالنسبة لصفة الاستجابة لزراعة المتوك.

المجلة المصرية لتربية النبات ٨: ٢٨٧-٢٩٨ (٢٠٠٤)