EFFECTOF GENOTYPE ON THE RESPONSE OF WHEAT (*Triticum aestivume* L.) F1 CROSSES TO ANTHER CULTURE *IN VITRO* IN THE MIDDLE OF IRAQ

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ABSTRACT

The present research was conducted to evaluate the effect of genotype on the response of seven wheat F1 crosses to anther culture and their ability to initiate callus and haploid green plants (plants with half number of chromosomes) *in vitro* and the efficiency of chromosome doubling to produce the doubled haploid plants under the conditions predominant in the middle region of Iraq. Anthers at uninucleate stage were excised and plated in the callus induction medium. Few weeks later, developed calli were transferred to plant regeneration medium. Regenerated haploid plants were transferred to the soil in a growth chamber. Chromosome doubling was applied and doubled haploid plants were produced. The genotype clearly affected callus induction and green plantlets frequency. The overall response of F1 crosses grown under environmental conditions predominant in the middle part of Iraq is encouraging, and the pollen-plants could be successfully induced.

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INTRODUCTION:

Anther culture is a technique in which haploid plants are obtained from pollen grains, by placing anthers on a suitable, synthetic culture medium. The technique was discovered by Guha and Maheshwari (9). It is one of the various tissue culture techniques or methods used. The use of anther culture method in breeding program shortens the time needed for cultivar development through rapid production of pure lines. The haploid plants technique is a potential tool for a range of breeding and biotechnological applications in many of the major small grain cereals such as wheat, barley and rice. In wheat, the first anther culture derived haploid plant was obtained in the early 1970's and the use of anther culture has since become successfully established in many wheat breeding programs, and doubled haploid cultivars have been released (21). The successful of anther culture is depends on various factors: species, genotype of donor plants, age of donor plants, physiological status of the donor plants, growing conditions of donor plants, culture medium, culture conditions, and other factors. The genetic variation and the environmental influence on the success rate in anther culture response is a major limitation for wide application of this technology to crop improvement. Large variation among wheat genotypes for anther culture response has been demonstrated (1) and genotype x environment interaction has been reported (17 and 16). Temperature is the most effective environmental factor for anther culture response in bread wheat. Generally, donor plants grown at higher temperature showed a reduced in vitro response. Growing the donor plants at low temperature has been reported to substantially improve plants (embryoids) production rate (22), and the frequency of green plants production can be influenced by the donor plant environment (1). Higher temperatures (20-22^{o} C) showed a reduced anther culture response versus lower temperature (14^{o} C) as reported by (7). Donor plants of 50 field-grown F1 barley crosses produced (10%) embryoids, but only (0.4%) green plants regenerated (24). Therefore,

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growth chamber and green-house grown plants are used for anther culture (24, 11, 15).

In order to use the anther culture technique for breeding purposes, the production of donor plants in the growth chambers is too expensive. Field–grown plants might be an alternative. Although environmental conditions in Iraq is ideal for wheat growth, however, the sharp rise in spring temperature during spike collection period and the temperature variation in the field which could not be controlled may reduce the possibility of using field- grown plants for anther culture. Therefore, this experiment was conducted to evaluate the effect of genotype on the response of seven wheat (*Triticum aestivum* L.*)* F1 crosses to anther culture and their ability to initiate callus and haploid green plants *in vitro* and the efficiency of chromosome doubling to produce the doubled haploid plants under the conditions predominant in the middle region of Iraq.

MATERIALS AND METHODS

Genotypes considered in this study included seven F1 Crosses (Adnania x Abu-graib), (Mexipak x Sali), (IPA99 x Abu-graib), (IPA99 x Sali), (Mexipak x Adnania), (IPA99 x Adnania), (Adnania x Sali).

Spikes were collected at the uninucleate stage. The developmental stage of anthers was assessed by microscopic examination by dissecting one anther and squashing it in a drop of acetocarmine stain on a glass slide. Spikes having anthers with pollen at the uninucleate stage were chosen as explants. Spikes were sterilized according to the usual sterilization method and then rinsed with distilled water. Anthers were excised aseptically in the laminar air flow-cabinet using sterilized forceps and cultured in the callus induction medium (Table 1) according to (2) . Developing calli were collected and transferred to differentiation medium for plant regeneration and maintained in a 16/8 hrs. photoperiod in growth room. All these operations were carried out in a sterile air flow chamber. The developing plantlets are haploids with half number of chromosomes. The developing haploid plantlets were ultimately transferred to soil. A colchicine treatment was applied to double the chromosome number of the haploid plants according to (3). The colchicine treated plants were replanted in the soil until maturity. The total number of callus and haploid plants was recorded.

Table 1: Induction medium.

Components	Quantity
KNO3	13.9 mM
CaCl2.2H2O	0.6
MgSO4.7H2O	0.4
KH2PO4	1.5
(NH4)2SO4	1.8
KI	0.0024
ZnSO4.7H2O	0.017
MnSO4.4H2O	0.033
H3BO3	0.08
NaMoO4.2H2O	0.00006
CuSO4.5H2O	0.00005
CoCl2.6H2O	0.00005
FeSO4.7H2O	0.1
Na2EDTA	0.1
Thiamine HCL(B1)	0.0074
Nicotinic acid	0.004
Pyridoxine (B6)	0.0024
Glycine	0.013
Biotine	0.001
Panthine	0.0028
Ascobic acid	0.0028
L-glutamine	6.8
Myo-inositol	1.7
2,4 D	0.0023
Kintine	0.0023
Maltose	0.25 M

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Table 2: Plantlets growth medium.

Components	Quantity
KNO3	1000
Ca(NO3)2.4H2O	500
MgSO4.7H2O	71.5
KH2PO4	300
NH4NO3	1000
KI	0.75
ZnSO4.7H2O	2.7
MnSO4.H2O	4.9
H3BO3	1.6
NaMoO4.2H2O	0.2
CuSO4.5H2O	0.076
CoCl2.6H2O	0.05
FeSO4.7H2O	0.1
Na2EDTA	0.1
Thiamine HCL(B1)	1
Nicotinic acid	5
Pyridoxine (B6)	5
Glycine	2
Myo-inositol	100
IAA	1
Sucrose	20 gm
Agar	7 gm
PH	5.9

RESULTS AND DISCUSSION

The anther culture responses of wheat F1 crosses are presented in (Fig.1). Three response traits, the callus induction frequency, plant regeneration capacity and frequency of green plantlets regeneration together determine the overall *in vitro* androgenic response. It can be observed from (Fig.1), that the tested genotypes varied in their response to anther culture revealing the presence of genetic differences in the genotypes tested. Both callus induction and plant regeneration frequency varied among genotypes tested. Time required for callus induction was genotype independent. Pollen derived calli are distinguished by compactness, dense consistence, and white color.

Differences in callus induction frequency existed among genotypes (Fig.1). Callus number ranged from (14 to 207). Of the seven genotypes tested in this experiment, crosses (IPA99 x Sali), (Mexipak x Sali), and (Mexipak x Adnania) had better androgenic ability than the other crosses, they are very responsive, with maximum number of callus induction rate (207,186, 137) respectively. The cross (IPA99 x Abu-graib) had poor androgenic ability, it had (14) callus only. The other crosses were found to be different from each other in their response, with callus induction number equal to (100, 78, 65) for the crosses (Adnania x Abu-graib), (Abu-graib x Sali) and (IPA99 x Adnania) respectively.

Ability of callus to produce plantlets varied with genotypes. All crosses tested demonstrated the capability for producing plantlets. Plantlets formation was observed after the transfer of calli to the shoot regeneration medium. Callus size did not affect plantlets regeneration. This process was of the same frequency for both, big and small calluses. Liang et al., (1982)(19) reported similar observation. Anthers within the same cross did not respond similarly. Some anthers dedifferentiated and redifferentiated, others showed dedifferentiation only, and still others

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did not respond at all. Pauk et al., (1995)(20) also found plants of some genotypes responded *in vitro* better than others.

Two kinds of plants have been developed, green plants and albino plants (white, chlorophyll defective plants). In some cases, green plants and albino plants regenerated from the same callus. All plants were developed indirectly through callus tissue, no direct formation was appeared. Developing green plants are shown in (Fig. 2).

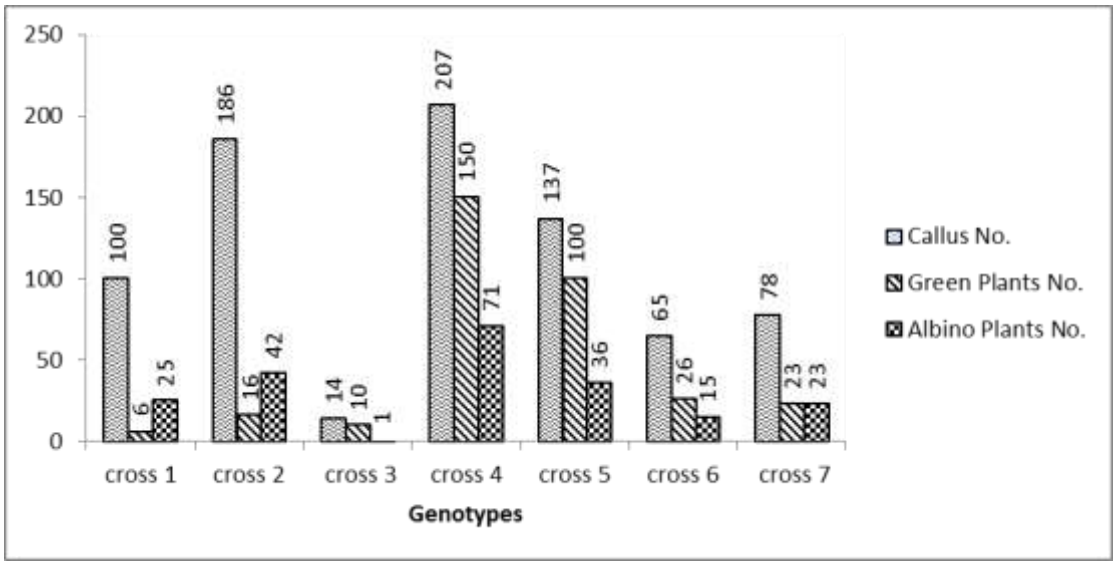

Fig.1: Response of F1 wheat crosses to anther culture.

Adnania x Abu-graib	Cross 1
Mexipak x Sali	Cross 2
IPA99 x Abu-graib	Cross 3
IPA99 x Sali	Cross 4
Mexipak x Adnania	Cross 5
IPA99 x Adnania	Cross 6
Adnania x Sali	Cross 7

Fig.2: Developing green plantlet.

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All crosses tested were able to produce green plantlets. Differences in green plants induction frequency existed among genotypes (Fig .1). On the average the crosses (IPA99 x Sali) and (Mexipak x Adnania) produced the most green plantlets (150,100) respectively, followed by the crosses (IPA99 x Adnania), (Abu-graib x Sali), (Mexipak x Sali) and (IPA99 x Abu-graib) which produced (26, 23, 16, 10) plants respectively, while cross (Adnania x Abu-graib) produced the lowest number of plants. These results indicate that frequency of anthers capable of producing green plantlets was very dependent on genotype. A similar observation in wheat was reported by (12). Green plants regeneration was not correlated with callus formation frequency, since, only two crosses (IPA99 x Sali) and (Mexipak x Adnania), with high calli formation (207, 137) showed high green plants regeneration (150, 100) respectively. This confirms previous reports by (19 and 4) that haploid production from anther culture seems to be controlled by at least three different independently inherited traits: callus (embryoid) induction, plant regeneration, and frequency of green plant regeneration. Also, (5) stated that plant regeneration from callus cultures was found to be highly heritable. Genetic control of plant regeneration has been observed in several plant species and exploited in some cases to improve plant materials for use in tissue culture research (7, 21).

Exploitation of haploid and doubled haploid (DH) as a powerful breeding tool requires the availability of reliable tissue culture protocols that can overcome several methodology problems, such as low frequencies of embryo induction, albinism, plant regeneration, plant survival and the genotype-dependent response, in order to improve the regeneration efficiency in a wider range of genotypes (8). There is no single standard condition or protocol for inducing pollen-derived plant formation (8).

The highest response for anther culture was obtained from genotypes combining high rate of callus induction and good regeneration capacity, while the low response resulted from poor callus frequency and\ or low regeneration rate.

Frequency of albino plantlets production was found to be fairly low in all genotypes (Fig. 1). Effect of genotype on albino plants production is clearly recognizable. On the average the crosses (IPA99 x Sali) and (Mexipak x Sali) produced the most albino plants (71,42), only one albino plant was produced from cross (IPA99 x Abu-graib), while (36, 25, 23, 15) albino plants were developed from the crosses (Mexipak x Adnania), (Adnania x Abu-graib), (Abu-graib x Sali) and (IPA99 x Adnania) respectively. These results were in agreement with other reports (12,13). The formation of albino plantlets is both genetically and environmentally controlled (23), growing the donor plants at low temperature (18/12^{o} C) has been reported to produce low albino plants frequency in comparison with high temperature (23/17^{o} C) (16) . Some of the haploid green plants grew and developed well after transplantation to soil and the others died soon after transplantation to soil. Chromosome doubling effect was measured indirectly by the fertility and seed set of the colchicine treated plants. Colchicine can cause high mortality due to the toxic effect of the agent. Mechanical damage during handling and colchicine treatment may kill some plants.

Chromosome doubling efficiency was (35%). (35%) of colchicine treated plants set seed normally after self-pollination (doubled haploid plants). Most of the survival plants set seeds after self-pollination. Number of seed for doubled haploid plants ranged from (1 to 1038) seed / plant. Colchicine delayed growth of survivors by at least one month. The doubled haploid plants represent genetically pure lines. The developed doubled haploid lines will be used for breeding purposes.

The response obtained in this study is better than that observed by (12, 13) with growth chamber and green-house grown plants. The overall response of F1 crosses grown under environmental conditions predominant in the middle region of Iraq is encouraging, and the pollen-plants could be

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successfully induced. Colchicine was efficiently induced chromosome doubling, and many doubled haploid plants were produced.

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