انتاج نباتات بنصف العدد الكروموسومي من زراعة متوك تضريبات الجيل الثاني من الحنطة (*Triticum aestivume* L.)

سهيله عائد ابراهيم

الملخص

ان استخدام تقنية زراعة المتوك كاداة للانتاج السريع للخطوط النقية وراثيا في برامج التربية، يقلل الوقت المستغرق لتطوير الاصناف الجديدة. طبقت هذه التجربة بهدف دراسة: 1:اختبار استجابة متوك سبعة من تضريبات الجيل الثاني من الحنطة (*Triticum aestivum*) لانتاج نباتات تحوي نصف العدد الكروموسومي **Haploid plants**من خلال زراعتها على وسط غذائي صناعي .2:دراسة كفاءة عملية التضاعف الكروموسومي للنباتات الحاوية على نصف العدد الكروموسومي لتطوير نباتات حاوية على العدد الكروموسومي الكامل **Doubled haploid plants**. اختيرت سبعة من تضريبات الجيل الثاني **F2** من الحنطه. جمعت السنابل منها لغرض زراعة متوكها، وانتخبت السنابل التي كانت متوكها في مرحلة المايكروسبورات الاحادية النواة. زرعت متوك السنابل المنتخبة على وسط غذائي صناعي لغرض تحفيزها على تكوين الكالس. بعد بضعة اسابيع تم نقل انسجة الكالس المتكونة الى وسط غذائي اخر خاص بنشوء النباتات . نقلت النباتات الفتيه الحاوية على نصف العدد الكروموسومي الى التربة ثم اجريت لها المعاملة بالكولجسين لاحداث عملية تضاعف كروموسومي وتطويرنباتات حاوية على العدد الكروموسومي الكامل . أظهرت التراكيب الوراثيه اشكالا مختلفة من الاستجابة . لوحظ وجود اختلافات بين التراكيب الوراثية في عدد الكالس المتكون. كما اختلفت قابلية انسجة الكالس على تكوين النباتات باختلاف التركيب الوراثي . اظهرت اغلب التضريبات القدرة على تكوين نباتات خضراء بنصف العدد الكروموسومي، كما امكن اجراء عملية التضاعف الكروموسومي لها وتم انتاج نباتات متضاعفة لها عدد كروموسومي كامل. تسجل هذه النتائج اول نجاح لانتاج نباتات من متوك تضريبات الجيل الثاني في العراق.

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Some of the haploid green plants grew and developed well after transplantation to soil and the others died soon after transplantation to soil. Haploid green plants appeared healthy and produced many tillers with small spikes, which set no seeds.

Chromosome doubling effect is 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 killed some plants. Chromosome doubling efficiency was (4.35%). (4.35%) of colchicine treated plants set seed normally after self-pollination (doubled haploid plants). All the survival plants set seeds after self-pollination. Number of seed for doubled haploid plants ranged from (3 to 529) seed / plant. Colchicine delayed growth of survivors by at least one month. The doubled haploid plants represent genetically pure lines. The developed doubled lines will be used for breeding purposes.

This experiment represent the first successful in Iraq to develop doubled haploid plants from F2 plants.

The overall response of F2 bread wheat crosses observed in this study is encouraging, and the pollen-plants could be successfully induced from F2 plants. The results obtained for the response to anther culture are comparable to results obtained by other researchers. The genotypes used in this study could be considered as good responders, and the frequency of callus induction and overall haploid green plants production was sufficient to generate populations of haploid lines. Chromosome doubling can be used to develop doubled haploid plants.

In order to obtain the highest frequency of response, further studies may be needed.

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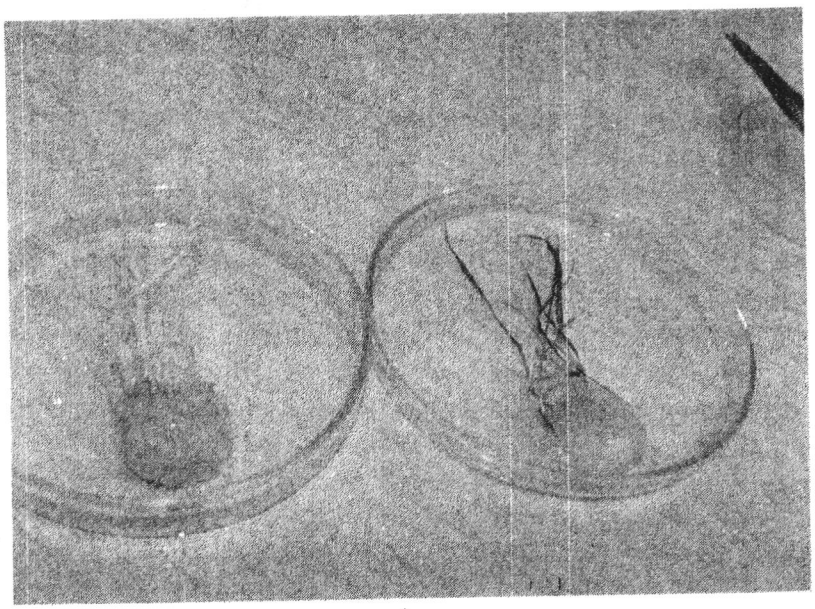

Fig.2: Green and albino plants regenerated from calli.

The crosses tested were able to produce green plantlets except the cross (Hamrah x IPA99). Differences in green plants induction frequency existed among genotypes (Table .1). On the average (IPA 99 x Salli), (IPA99x Mexipak) and (Mexipak x Salli) had the most green plantlets rate (11.1%, 10.6%, 9.1%) respectively, followed by (IPA99 x Adnania) and (Mexipak x Adnania) with green plantlets rate (8.8%, 8%,) respectively. The cross (IPA99 xAbu-graib) responded poorly, it generated fewer green plants than did the other crosses (1.5%). 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 (6, 7). Green plants regeneration is not correlated with callus formation frequency, since, only two crosses (IPA 99 x Salli) and (IPA99x Mexipak) with high calli induction rate (31.8% and 16.3%) showed high green plants regeneration rate (11.1% and 10.6%) respectively. This confirms previous reports by (3, 12, 18) 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.

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 (Table 1). Effect of genotype on albino plants production is clearly recognizable. More albino yielded from (IPA 99 x Salli) (IPA99x Mexipak) . Very few albino plants (0.1%, 0.2%, 0.4%) were produced from (IPA 99 x Abu-graib), (Mexipak x Salli) and (IPA99 x Adnania). While no albino plants were developed from the cross (Mexipak x Adnania) . These results were in agreement with other reports of (5,6).

The formation of albino plantlets is both genetically and environmentally controlled (14,16).

induction rate (0.6%). The other crosses were found to be different from each other in their response.

Table.1: Response of F2 wheat crosses to anther culture.

Mexipak x Adnania	4.7	8.0	8.0	0.0
Mexipak x Salli	6.5	9.3	9.1	0.2
IPA99 xAbu-graib	0.6	1.6	1.5	0.1
IPA99 x Salli	31.8	13.3	11.1	2.2
IPA99 x Mexipak	16.3	12.0	10.6	1.4
IPA99 x Adnania	4.0	9.2	8.8	0.4
Hamrah x IPA99	4.0	0.0	0.0	0.0

Plantlets formation was observed approximately 10 days after the transfer of calli to the shoot regeneration medium. The ability of callus to produce plantlets varied with genotypes. All crosses tested demonstrated the capability for producing plantlets except the cross (Hamrah x IPA99). Plantlets regeneration did not depend on callus size. This process was of the same frequency for both, big and small calluses. Liang et al. (13) reported similar observation. Anthers within the same cross did not respond similarly. Some anthers dedifferentiated and redifferentiated, others showed dedifferentiation only, and still others did not respond at all. Liang et al. (13) also found anthers of some genotypes responded *in vitro* better than others. Two kinds of plants have been developed (Fig.2), green plants and albino plants (white, chlorophyll defective plants). There were no cases of regeneration of green plants and albino plants from the same callus. All pollen plants recovered in this study were developed indirectly through callus tissue, no direct formation was appeared. Developing green plants are shown in (Fig.1).

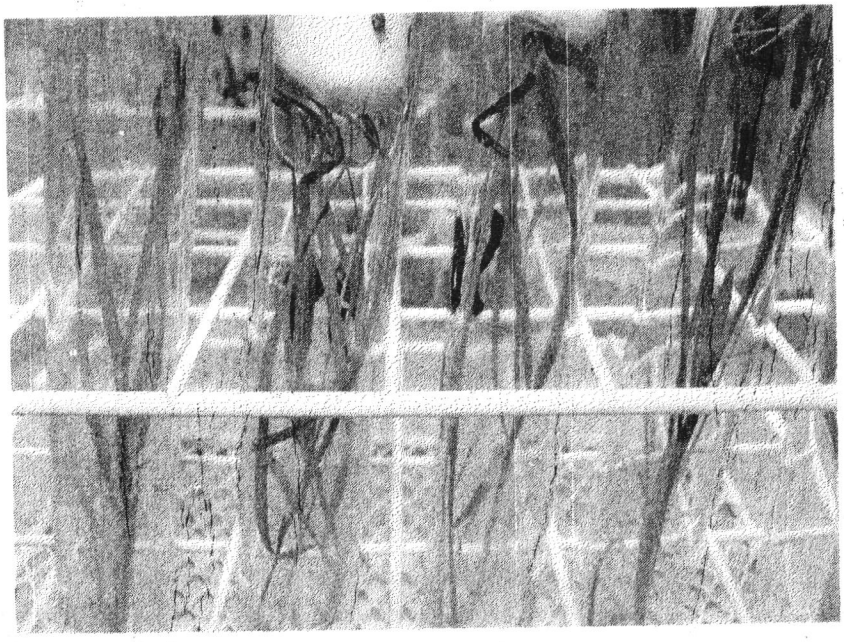

Fig.1: Developing haploid (anther – derived) plantlets.

In cereals, callus induction and plant regeneration abilities have been observed to be governed by genetic and environmental characteristic of different genotypes (4). Large variation among wheat genotypes for anther culture response has been demonstrated (1) and genotype (x) environment interactions have been reported (12,8).

Breeder may use any filial (generation) for screening and selection desired genotypes. No information are available about response of F2 wheat crosses to anther culture in Iraq. Therefore, this experiment was conducted to. 1: examine the response of anthers of seven F2 crosses of wheat (*Triticum aestivum* L.), to produce plants with half number of chromosomes (haploid plants) via anther culture *in vitro*. 2: to examine the efficiency of chromosome doubling to produce plants with full number of chromosomes (doubled haploid plants).

MATERIALS AND METHODS

Genotypes considered in this study included seven F2 Crosses (IPA99 xAbu-graib), (IPA99 x Salli), (IPA99 x Adnania), (IPA99 x Mexipak), (Mexipak x Salli), (Mexipak x Adnania), (Hamrah x IPA99).

Spikes were collected for anther culture. The developmental stage of anthers was assessed by microscopic examination by dissecting one anther from a spikelet midway along the spike 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 surface sterilized with sodium hypochlorite and then rinsed with sterile distilled water for three times. Anthers were excised aseptically inside the laminar air flow-cabinet using forceps and cultured in the callus induction medium containing macro, micro elements, vitamins and other components. Few weeks later, developing calli were collected and transferred to differentiation medium for plant regeneration and maintained at a 16/8 hr photoperiod in growth room. All these operations were carried out in a sterile air flow chamber. The developing plantlets are haploid with half number of chromosomes. The developed haploid plantlets were ultimately transferred to soil. A colchicine treatment was applied to double the chromosome number of the haploid plants according to (2). The colchicines treated plants were replanted in the soil until maturity. The total number of calli and plantlets expressed per 100 anthers cultured.

RESULTS AND DISCUSSION

The response of F2 crosses to anther culture is presented in Table.1. Three response traits, the callus induction frequency, plantlet regeneration capacity and frequency of green plantlets regeneration together determine the overall *in vitro* androgenic response. It can be observed from Table.1, that the tested genotypes varied in their response to anther culture. Both callus induction and plant regeneration frequency varied among genotypes tested. In general, time required for callus induction was genotype independent. Pollen derived calli are distinguished by compactness, dense consistence, and white colour.

Differences in callus induction frequency existed among genotypes Table(1). Callus induction rate ranged from (0.6% to 31.8%). Of the seven genotypes tested in this experiment the crosses (IPA99 x Salli) and (IPA99x Mexipak) had better androgenic ability than other crosses, they were very responsive, with maximum callus induction rate (31.8% and 16.3%) respectively. The cross (IPA99 x Abu-graib) showed poor androgenic ability, it had the lower callus

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PRODUCTION PLANTS WITH HALF NUMBER OF CHROMOSOMES THROUGH ANTHER CULTURE OF F2 WHEAT (*Triticum aestivume*_L.) CROSSES *IN VITRO*

S. A. Ibraheem

ABSTRACT

Utilization of anther culture method as a tool for a rapid production of pure lines in breeding program, usually shortens the time needed for cultivar development. The objectives of the present research were : 1: to examine the response of anthers of seven F2 crosses of wheat (*Triticum aestivum* L.), to produce plants with half number of chromosomes (haploid plants) via anther culture *in vitro*. 2: to examine the efficiency of chromosome doubling to produce plants with full number of chromosomes (doubled haploid plants). Spikes with anthers at uninucleate stage were selected, anthers from these spikes were excised and plated in the induction medium. Few weeks later, calli were transferred to regeneration medium. Regenerated plantlets were transferred to soil in a growth chamber. Colchicine treatment was applied to double the chromosome number of the haploid plants.

The tested genotypes showed different responses to anther culture. Differences in callus induction frequency existed among genotypes. Ability of callus to produce green plantlets varied in relation to different genotypes. Most crosses tested demonstrated the ability to produce green plantlets. Colchicine was efficiently induced chromosome doubling. This experiment represent the first successful in Iraq to develop doubled haploid plants from F2 crosses.

INTRODUCTION

***In vitro* haploid plants induction 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.**

Haploid plants are sporophytes containing the gametic chromosome number (9). The value of haploid plants production for breeders is its potential to produce homozygous progenies from heterozygous parents in a single generation. This property can increase the efficiency of production of new varieties in two ways. Firstly, particularly in self – pollinating species, it can reduce the time required from making the original crosses to the testing of homozygous recombinant progenies derived from them as potential varieties. Secondly, the testing of completely homozygous rather than heterozygous and consequently heterogeneous families can increase selection efficiency by facilitating the easier identification of superior genotypes within a field nursery (17).

Anther culture is a technique for the production of haploid plants in cultivated crops. 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 (15).

General Company for Horticulture and Forestry –Baghdad, Iraq.