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IN VITRO CULTURE OF IMMATURE EMBERYOS AND ANTHER CULTURES OF BREAD WHEAT

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ABSTRACT

This investigation was carried out at the Cell and Tissue Culture Lab. Agronomy Dept. Faculty of Agriculture , Al-Azhar University, during the period from 2005 to 2006 aiming to study the main factors affecting callus Induction and plant regeneration from cultured immature embryos of cultivars of wheat (*Triticum aestivum* L) namely Gemmeiza 7, Gemmeiza 9 , Gemmeiza 10, Giza 168 and Sakha 93 .Experiments were designed to identify cultivars of wheat and growth regulator most suitable for successful in vitro immature embryos and anther culture stages were also investigated.

Results indicated that there were significant effect for the cultivars , growth regulators and their interaction on callus induction from the immature embryos. The cultivar G168 recorded the highest significant effect for callus induction followed by Gemmeiza 10. The highest value of callus initiation recorded when 2.0 mg/L2,4-D+0.5 mg/L kin was added to the MS medium. Results indicated that there were significant effect for growth regulators and their interaction with genotypes on plant regeneration. Growth regulators, effected on the plant regeneration percent. The highest value of plant regeneration recorded when 1.0 mg/L kin +0.5 mg /L 2,4-D or 1.0 mg /L BAP +0.5 mg /L NAA were added to the MS medium. The interaction between cultivars and growth regulators was significant . The highest plant regeneration recorded for Gemmeiza 10 cultured on MS medium supplemented with 1.0 mg /L BAP +0.5 mg /L NAA. For anther studies the highest mean for plant regeneration (11.66%) was obtained from Sakha 93. G168 also yielded high (11.00%) mean number of plant regeneration .Albino plants were obtained from 3 of the 5 cultivars. The stage of development of the microspores at the time of culture was affected on plant regeneration percent . Uninucleate microspores stage was identified as producing the highest percentages of green plants(26.66%) compared with the tetrad(15.37%) stage. It is also indicated that the Sakha 93 cultivars showed the highest percentage of green plants from the nucleate stage (35.55%) follow by G 168 .

INTRODUCTION

Response of bread wheat (*Triticum aestivum* L.) to the *in vitro* culture has been shown to depend on medium , genotypes and explants tissue Calli obtained from immature embryo showed the best potential for shoot regeneration (Eapen and Rao 1982, Sears and Deckard 1982, Reddy and Reddy 1983, Heyser *et al.*, 1985, Lazar *et al.,* 1988, Galiba and Sutka 1989 and Sarker and Ashapurno 2002).) in a number of varieties of wheat. Plant regeneration from tissue cultures derived from immature embryos is predictable and stable when the appropriate genotype is used.

Anther culture**s** leading to callus production often generate genetic variation called gametoclonal variation, which describes phenotypically variant plants regenerated from gametophytic cells. Although the basis of gametoclonal and somaclonal variation is not understood, in vitro induced chromosomal aberrations may have contributed to such variation (Marburger and Jauhar 1989)..

Specialized plant tissue culture methods have enabled the production of completely homozygous breeding lines from gametic cells in a shortened time frame compared to conventional plant breeding . Several varieties developed via this method are grown on considerable acreage while others are being tested as candidates to replace varieties developed by conventional methods .In China, at least 20 cultivars of wheat have been produced using tissue culture techniques (Hu 1997). These cultivars, with superior agronomic traits (high yields and wide adaptation) are reported to be cultivated on more than 1 million hectares (Hu 1997). Lu *et al*., (2000) continue to use somatic tissue culture and anther culture to induce and stabilize variation to improve wheat breeding efficiency in China. Cell and tissue culture techniques such as anther culture have attracted considerable attention as supplementary tools to cereal crop improvement (Vasil and Vasil 1994). Anther culture involves the induction of embryoid formation from immature pollen and subsequent regeneration of embryoids into plantlets. Since Ouyang *et al.,* (1973) reported the in vitro regeneration of plants from pollen, the research on wheat anther culture has progressed rapidly. Anther culture -derived haploids have been used to produce homozygous lines, which accelerate breeding programs (Kasha *et al.,* 1990). The application of modern methods of direct gene transfer into crop plants also depends on the development of efficient systems for regeneration of full plants from cultured cells and tissues (Vasil and Vasil 1994). The development of efficient systems for regenerating haploid callus cultures may enable direct gene transfer into wheat. Although earlier workers obtained low frequencies (0.7%) of green plants (Ouyang *et al.,* 1973), the frequency of microspore embryogenesis in common wheat has been improved considerably (Chu *et al.,* 1990). Several factors are considered important for increasing the induction frequency of green plantlets from anther culture (Hu 1997), including the genotype of the donor plant and environmental conditions under which these plants are grown (Sibikeeva and Sibikeev 1996). However, not much is known about the anther- culture response (i.e., the ability to induce embryoids from microspores) of wheat. Studies on wheat reported the production of some albino plants (Zhu *et al.,* 1979). For anther culture of wheat to be useful, a large number of anther culture -derived regenerates must be obtained. The objective of this study was to investigate the effect of the genotype of the donor plant, growth regulators, on callus initiation and plant regeneration from the immature embryos and anther stages on anther culture .

MATERIALS AND METHODS

The present investigation was carred out at the Cell and Tissue Culture lab., Agronomy Dept. Faculty of Agriculture, Al-Azhar Univ. during the period from 2005-2006.; Five cultivars (Gemmeiza 7, Gemmeiza 9 , Gemmeiza 10, Giza 168 and Sakha 93) of wheat (*Triticum aestivum* L.) were used as anther donors. Each experiment was set up with these five genotypes,

Callus induction and plant regeneration from the immature embryos:

Plants grown in the field were used for immature embryos . To initiate callus in the five wheat genotypes, the immature embryos excised from spike after 12 days old pollination and surface sterilized by treatment with 70% ethanol for 5 min, and then with 20% Clorox (sodium hypochlorite 5.25%) for 20 min and finally washed in sterilized water. Immature embryos were then sown , in jars on Murashige and Skoog**,**s**(**MS**)** medium , 0.8% agar .Jars were kept in continuous dark at 25 °C for three weeks before transfer on a 16-h photoperiod growth chamber.

Anther culture

Immature spikes were collected when the awns first emerged from the flag leaf, placed in a beaker containing enough distilled water to cover the stem at or just below the node prior to the spike, covered with plastic wrap, labeled, and refrigerated at 4°C for 7 days. After cold treatment, spikes were sterilized with 70% ethyl alcohol for 1 min, followed by 1% sodium hypochlorite and 0.1% Tween 20 for 8 min, then rinsed with sterile double-distilled water four to five times.

Each spike was staged by squashing its centermost anther in 1% acetocarmine. Anthers containing microspores at tetrad or the mid-uninucleate stage were cultured. Fifteen anthers were cultured in jars containing 20 ml of one of the induction media which contend MS medium supplemented with different concentration of growth regulators (Table 1). Cultures were sealed with aluminum foil and kept in an incubator at 28°C in the dark. Culture were observed weekly for callus and/or embryoid development. Cultures were automatically timed fluorescent lights with a 16-h photoperiod. After plantlet regeneration, the cultures were transferred to jars containing 25 ml of modified MS medium without growth regulators and 30 g/L sucrose) (Murashige and Skoog, 1962**)** and kept in the same incubation room.

Data obtained were analyzed according to Snedecor and Cochran (1981) and the treatments were compared by the least significant difference test (L.S.D) at 5% level.

RESULTS AND DISCUSSION

A-Immature embryos experiment

1- Callus induction

Effect of MS medium supplemented with different auxin and cytokinins on callus induction from the immature embryos of wheat genotypes are presented in Table (1). Results indicated that there were significant effect for the genotypes and growth regulators on callus induction.. The genotype G168 recorded the highest significant effect for callus induction (Fig.1) followed by Gemmeiza 10 (Fig. 2).While the lowest callus obtained from the genotype Gemmeiza 9. The present results are mainly due to the differences in the genetical structure of the genotypes under study.

Table (1): Effect of MS medium supplemented with different auxin and cytokinins on callus induction from five immature embryos of wheat genotypes in all jar.

Growth regulators	Gemmeiza 7	Gemmeiza 9	Gemmeiza 10	Giza 168	Sakha 93	Mean
2.0 mg/L 2,4-D	3.000	2.333	3.666	4.666	3.666	3.428
2.0 mg /L NAA	3.666	3.000	4.000	4.333	4.333	3.857
2.0 mg/L2,4-D+0.5 mg/L kin.	4.000	3.333	4.666	5.000	3.666	4.142
2.0 mg /L NAA+0.5 /L BAP	3.333	3.000	4.000	4.000	3.666	3.571
2.0 mg /L IAA	2.666	2.666	3.000	3.333	3.000	2.928
Mean	3.333	2.866	3.866	4.266	3.666	3.585

L S D at 5% for Genotypes (G) 0.450
L S D at 5% for growth regulators (R) 0.450
L S D at 5% for (G) x (R) N.S

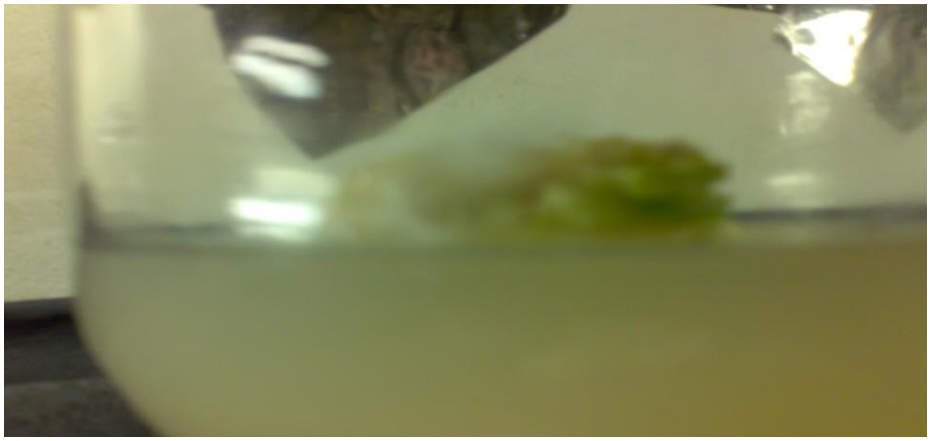

Fig.(1) .Callus induction from the immature embryos of Giza 168 with starting of plant regeneration on medium containing 1.0 mg /l kin. 0.5 mg /l 2,4-D.

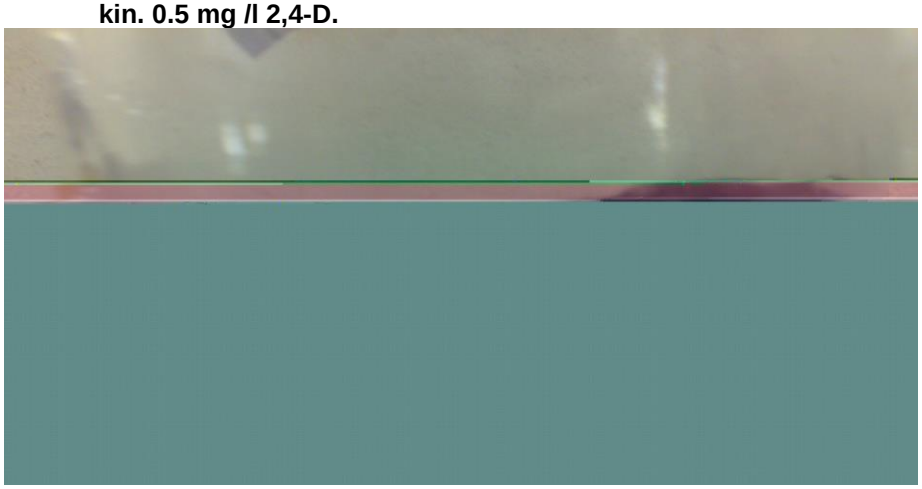

Fig.(2): Callus induction from the immature emberyos of Gemmeiza 10 on medium containing 2 mg /l 2,4-D+ 0.5 mg /l kin. Intiated

Growth regulator concentrations in culture medium are critical for the control of growth and morphogenesis. Generally, high concentration of auxins and low cytokinins in the medium promote abundant cell proliferation with the formation of callus

Growth regulators also, effected on the callus initiation .The highest value of callus initiation recorded when 2.0 mg/L2,4-D+0.5 mg/L kin was added to the MS medium. While the lowest callus induction obtained when 2.0 mg /L IAA was added to the MS medium.These results in agreement with those reported by Sarker and Ashapurno (2002).

2- Plant regeneration

Effect of MS medium supplemented with different auxin and cytokinins on plant regeneration from the immature embryos of wheat genotypes are presented in Table (2) and fig. (1 and 2).

To obtain rapid regeneration and study combined effect of auxin and cytokinins on morphogenetic response, the calli were excised and cultured on MS medium . Cytokinins used were BAP and Kin. In order to study the combined effect of cytokinins and auxins on regeneration , different concentrations of BAP and Kin were used in combination with 2,4-D and NAA.

Results indicated that there were significant effect for growth regulators and their interaction on plant regeneration. Growth regulators, effected on the plant regeneration .Highest value of plant regeneration recorded when 1.0 mg/L kin +0.5 mg /L 2,4-D or 1.0 mg /L BAP +0.5 mg /L NAA were added to the MS medium.

The interaction between genotypes and growth regulators was significant . The highest plant regeneration recorded for Gemmeiza 10 cultured on MS medium supplemented with 1.0 mg /L BAP +0.5 mg /L NAA.

These results are supported by those of Springer Wien (1982), Varshney *et al* ., (1996), they worked on immature emberyos of wheat (*Triticum aestivum* and *Triticum durum* L.) and Mohammad, *et al*., *(2003) .* In contrast Chen, *et al*., (1992) observed plant regeneration in hybrids between *Triticum aestivum* and *Agropyron cristatum* on hormone free MS medium.

Table (2): Effect of different auxin and cytokinins on regeneration potential of wheat callus tissue initiated from five immature embryos in all jar used .

Growth regulators	Gemmeiza 7	Gemmeiza 9	Gemmeiza 10	Giza 168	Sakha 93	Mean
0.5 BAP+0.1 NAA	1.666	1.000	2.666	2.000	2.000	1.866
1.0 BAP+0.5 NAA	2.333	2.000	3.333	2.000	2.666	2.466
2.0 BAP+1.0 NAA	2.000	1.666	2.666	1.333	1.666	1.866
0.5Kin.+0.1 2,4-D	1.666	2.000	1.666	2.333	2.333	2.000
1.0 Kin.+0.5 2,4-D	2.666	3.000	3.000	2.000	2.333	2.600
2.0 Kin.+1.0 2,4-D	2.000	2.333	2.333	1.000	1.666	1.866
Mean	2.055	1.997	2.606	1.770	2.111	

L S D at 5% for Genotypes(G) N.S
L S D at 5% for growth regulators (R) 0.526
L S D at 5% for(G) x (R) 1.176

A-Anther culture experiment

Anther culture response and callus production of the total of 1500 anthers cultured from 5 genotypes, 1072 responded (71.46 %) (Table 3) and fingers (3 and 4). Microspores that responded to culture developed either into embryoids or formed calli, although in some cases both embryoids and calli were derived from a single anther. Many of the anthers produced only a single callus, while others produced several calli. Callusing of the anther filament was common..

Plant Regeneration five to six weeks after culture initiation, calli and embryoids were transferred to differentiation medium containing MS medium supplemented with 1 mg /l kin.+0.1mg /l 2-4,D for shoot and root initiation fig(4). Cultures were then kept in the dark at 26°C until differentiation took place, usually within 9–12 days. While some plantlets started developing chlorophyll uring differentiation, others turned green only calli showed an abundance of rooted plantlets , which were separated and subcultured. However, some plantlets never developed chlorophyll and remained albino .

Table (3): Androgenesis of wheat varieties in isolated microspore culture

Genotypes	No. of anthers cultured	Callus initiation	Callus %	No. of plantlets	Albino	Green plants
Gemmeiza 7	300	218	72.66	35	3	25
Gemmeiza 9	300	213	71.00	28	-	23
Gemmeiza10	300	183	61.00	24	5	18
Giza 168	300	225	75.00	40	2	33
Sakha 93	300	233	77.66	43	-	35
Mean	300	214.4	71.46	34	2	26.8

Table (4): Effect of anther stages on plant regeneration capacity of the genotypes tested.

Genotypes	Tetrad stage			Uninucleate stage		
	No. of anthers cultured	Green plants obtained	Green plants %	No. of anthers cultured	Green plants obtained	Green plants %
Gemmeiza 7	45	6	13.00	45	10	22.22
Gemmeiza 9	45	7	15.00	45	11	24.44
Gemmeiza 10	45	5	11.11	45	10	22.22
Giza 168	45	8	17.77	45	13	28.88
Sakha 93	45	9	20.00	45	16	35.55
Mean	45	7	15.37	45	12	26.66

For no. of 144 plants (9.61%) regenerated from 1500 **culture**d anthers, 134 were green and 10 were albino (0.66%) (Table 3). The highest mean for plant regeneration (11.66%) was obtained from Sakha 93 (Fig. 3), while Gemmeiza 10 gave the lowest mean (6.00%).G168 also yielded the highest(11.00%) mean number of plant regeneration .Albino plants were obtained from 3 of the 5 genotypes. For root induction, shoots of 3 - 5 cm in length were excised and cultured on half strength of MS. In this medium more

than 85% of the shoots of all varieties produced roots without the addition of any hormonal supplements.The shoots that failed to grow roots were transferred to the rooting medium i.e. half strength of MS with 0.2 mg/l IBA successfully produced roots.

The results are in agreement with those of Hossain (1993). Eapen and Rao (1982) reported that plantlets rooted when they were transferred to MS supplemented with 1.0 mg/l NAA. On the other hand, Springer Wien (1982) eported that plantlets rooted when they were cultured on half strength of MS supplemented with 1.0 mg/l NAA .The genotype of the donor plant is known to affect anther culture response in wheat (Moieni and Sarrafi, 1995)..

Fig. (3): Callus iduction(in right) and plant regereration(in left) from anthers cultured for sakha 93 variety.

Fig. (4): Callus iduction(in right) and green plants with rooting(in left) for the variety Giza 168 emerging from cultured anthers at uninucleate stages.

Effect of anther stages

Plant regeneration in cultures from the different stages in the wheat genotypes are presented in Table (4).The ideal microspore development stage is the first important step of androgenesis induction in microspore culture. The collected donor tillers consisted of microspore with tetrad or uninucleate stages in middle part of spikes .The stage of development of the microspores at the time of culture was affected on plant regeneration percent. Uninucleate microspores stage was identified as producing the highest percentages of green plants (26.66%) compared with the tetrad(15.37%) stage.

It is also indicated that the genotype Sakha 93 showed the highest percentage of green plants from the nucleate stage (35.55%) follow by G 168(Fig. 4) .These results agreement with those reported by Martha *et al*., (1991) and Haggag and El-Hennawy (1996) and Hassanein (1998).

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زراعة الأجنة غير الناضجة والمتوك لقمح الخبز في أنابيب

إبراهيم الوصيف البيلي

قسم المحاصيل بكلية الزراعة -جامعة الأزهر- القاهرة

أجرى هذا البحث بمعمل مزارع الأنسجة والخلايا بقسم المحاصيل بكلية الزراعة جامعة الأزهر بالقاهرة خلال الفترة من ٢٠٠٥-٢٠٠٦ بهدف دراسة العوامل الرئيسية المؤثرة علي إنتاج كالس وإمكانية توجيهه لتكوين نباتات جديدة من القمح باستخدام الأجنة غير الناضجة وزراعات المتوك وذلك لخمسة تراكيب وراثية من قمح الخبز وهي :جميزة ٧، جميزة٩، جميزة ١٠، جيزة ١٦٨ وسخا ٩٣

وقد أوضحت النتائج ما يلي :

١- وجود فروق معنوية بين التراكيب الوراثية وكذلك بين منظمات النمو المستخدمه في إنتاج كالس من الاجنة غير الناضجة وقد سجل التركيب الوراثى جيزه ١٦٨ أعلى القيم في إنتاج الكالس يليه التركيب الوراثي جميزه ١٠وكان تأثير منظمات النمو معنوى على إنتاج الكالس و قد سجلت المعاملة ٢ ملجم/لتر ٤,٢- د + ٥ ., ملجم/ لتر كينتين أعلى قيم لإنتاج الكالس.

٢- وجود فروق معنوية بين منظمات النمو المستخدمه في اعادة تجديد نباتات من الأجنة غير الناضجة و كذلك التفاعل ما بين التراكيب الوراثية و منظمات النمو وقد سجلت المعاملة ١ ملجم /لتر كينتين + ٥ ., ملجم ٤,٢- د أو١ ملجم /لتر BAP+ ٥ ., ملجم NAA أعلي معدل لإنتاج النباتات٠ وأعطي التركيب الو راثي جميزة ١٠ أعلي معدل لتجديد النباتات علي البيئة المحتوية علي ١ ملجم /لتر BAP+ ٥ ., ملجم NAA ٠

٣-أظهرت نتائج زراعة المتوك أن أعلي معدل لإعادة تجديد النباتات من المتوك سجل للتركيب الوراثي سخا ٩٣ (٦٦, ١١ %) كذلك سجل التركيب الوراثي جيزة١٦٨ قيما مرتفعة بالمقارنة بالأصناف الأخرى (١١%) ٠

٤-كان لعمر زراعة المتوك غير الناضجة لتأثير ا علي إعادة تجديد نباتات من المتوك حيث أعطت مرحلة Uninucleate stages أعلي قيم لتجديد النباتات إذا قورنت بمرحله Tetrad stages .وتفوق الصنف سخا ٩٣ عن باقي الأصناف في نسبة تجديد النباتات من المتوك المنزرعة.