J. Agric. Sci. Mansoura Univ., 31 (8): 5081 - 5091, 2006

ANTHER CULTURE AS A TECHNIQUE TO REGENERATE HOMOZYGOUS WHEAT *(Triticum aestivum L.)* GENOTYPES

Sabry, S.R.S.[**]; O.M. El-Shihy[*]; M.R.A. Nesiem[*] and D.S. Daghma[*]

[*] **Plant Physiology Section, Agricultural Botany Department, Faculty of Agriculture, Cairo Unvi., Giza.**

[**] **Wheat Research Department, Field Crops Research Institute, Agricultural Research Center (ARC), Giza.**

[***] **National Gene Bank and Genetic Resources (NGBGR), Ministry of Agriculture and Land Reclamation, Giza.**

ABSTRACT

Eleven wheat *(Triticum aestivum L.)* genotypes were screened for salinity tolerance. Seeds were germinated under six salinity levels (0 – 12000 ppm) with quarter strength of Hogland solution. Sea salt was used as a source for salinity. Three genotypes, i.e. Sids 1, Sakha 8 and line 25 were selected for performing crosses among the salinity tolerant (Sids 1 and Sakha 8) and the salinity sensitive Line 25. Three crosses were conducted between the selected genotypes and their F_1 plants were grown to obtain their anthers. Anther culture was used as a breeding technique to obtain pure lines in a short time. P-4S medium was used for callus induction. Great differences were observed in callus induction among the anthers of the three crosses. Embryogenic callus induction reached 10.1, 61.9 and 51.4 % for hybrid 1 (Sids 1 X Sakha 8), hybrid 2 (Sakha 8 X Line 25), and hybrid 3 (Sakha 8 X Line 25), respectively. Plant regeneration medium 190-2 was used. Frequency of regenerated plants reached 1.4, 8.5, and 9.0 % for crosses 1, 2, and 3, respectively. Root tips of regenerated plants were cytologically examined to determine their ploidy level. All the examined plants were haploid type. Haploid plants were treated by colchicine for chromosome doubling. The doubled haploid plants reached 22.3, 24.4, and 22.1 % for crosses 1, 2 and 3 respectively.

INTRODUCTION

Wheat is the most strategic crop in Egypt. It covers 3.064 million Feddan, with total production of 8.274 million Ton and average yield of 2.69 Ton/Feddan (Agricultural Statistics, 2006).

Haploid production is a method to obtain homozygous individuals in a single step (Inagaki,*et.al* 1998; Trop *et al.*, 2001; Kim *et al.*, 2003). Anther culture technique with an efficient plant regeneration system could be used in plant breeding programs. Chromosome doubling of haploid plants is potentially considered an efficient source for homozygous pure lines in wheat (Ball *et al.* 1992; Moieni *et.al* 1997; Tuvesson *et al.* 2000; Trop *et al.*, 2001; Konieczny[1], *et al.* 2003). The induction of androgenesis from wheat anther is strongly controlled by genetic make up and environmental factors as well as the interaction between them (Konieczny[1], *et al.* 2003).

In the gametophyte phase, microspore mother cells in anther locules undergo meiosis and that forms male gametophytes or pollen grains (Zheng, 2003). This process promoted by certain cues. Immature pollen grain (microspore) can be switched from gametophytic pathway to sporophytic pathway, leading to the formation of pseudoembryos commonly known as

embryoids. Embryoids germinate to produce either haploid or doubled haploid plants. This phenomenon is considered an excellent example of plant cell totipotency, and defined as androgenesis or microspore embryogenesis, these two terms are often used interchangeable (Zheng, 2003). Thus, the method is important for plant genetic improvement, genetic manipulation, and in many other areas of basic research related to plant development biology (He,and Ouvary, 1984; Kim, *et al.* 2003; Zheng, 2003). The attainment of homozygosity in one generation helps to reduce the numerous cycles of inbreeding necessary in conventional pure line breeding methods (Trop, *et al.*, 2001). Chromosome doubling of plants can now be effectively performed during the *in vitro* culture with colchicines or other chromosome doubling chemical agents (Inagaki, *et al.*, 1998; Trop,*et .al.* 2001; Kunz,*et.al* 2000).

Many wheat genotypes show a total lack of embryo induction or high percentage of albinos among regenerated plants. The optimization of media and culture conditions for high androgenic response of such genotype is still a challenge (Heszky and Meseh 1976; Redha, *et al.* 1998; Kunz, 2000).

The goal of our research was to use anther culture for developing a simple and rapid technique for breeding wheat homozygous lines.

MATERIALS AND METHODS

Screening for salinity tolerance:

Eleven wheat (*Triticum aestivum L.*) genotypes, i.e. Giza 168, Sids 1, Yakora, Sakha 8, Sakha 61, Tosson, Giza 155, Giza 157, Gemmiza 5, Gemmiza 7 and Line 25 were germinated in petri dishes containing quarter strength of Hogland solution (Hogland and Arnon, 1950) under six salinity levels (0, 6000, 9000, 10000, 11000 and 12000 ppm). All genotypes seeds were provided by the Wheat Research Department, Field Crops Research Institute, Agriculture Research Center. Sea salt (Sigma #-S-9883) was used as a source of salt stress. Each treatment was represented by five petri dishes. Each dish contains one hundred seeds. The germination percentage was calculated after two weeks.

Donor plants and growth conditions:

Two genotypes i.e. Sakha 8 and Sids 1 were selected due to their efficient performance under salt stress (salinity tolerant). A third genotype (Line 25) was introduced due to its inefficient performance under salt stress (salinity sensitive). The three genotypes were sown as parents under open field conditions on 20th Nov., 2001. Three crosses were conducted among the selected genotypes, i.e. Sids 1 X Sakha 8, Sakha 8 X Line 25 and Sids 1 X Line 25. After six months, the seeds of wheat hybrids were harvested.

First experiment:

F_1 hybrid wheat seeds were sown under open field conditions on 25th Nov, 2002 to use their anthers in anther culture.

To identify the suitable spike age for anther culture, five spikes from each hybrid at booting stage were collected at -5.0, -3.0 and 0.0 cm as distance between the top of the spike (last floret) and flag leaf auricles. The proper spike age for each hybrid was cytologically determined according to

the method of (He and Ouyang, 1984). The suitable spike age produced the highest frequency of microspore cells in the mid-uninucleate. Three hundred anthers distributed in three replicates were tested for each hybrid.

To identify the duration of spikes cold treatment, ten spikes from each hybrid were collected at -5 cm of spike age. The basal parts of the spikes were immersed in distilled water. Then, they were covered with black plastic bag to keep humidity and incubated at 4° C for 5, 7, 14 and 20 days. After cold treatments, the spikes surface were sterilized by 70% ethanol for 2-3 minutes, washed for five times with sterilized water, followed by soaking in 0.1% mercuric chloride for 10 minutes and finally rinsed for five times by sterilized water.

Under laminar air flow cabinet, anthers were isolated from the lateral florets of each spikelet laying in the middle third of the spike, the anthers from the middle florets were discarded.

Isolated anthers were cultured on Petri dishes containing 15 ml of P4s medium (Ouyang *et al.* 1983) to induce embryogenic callus formation. Seven grams per liter agar was added as gelling agent and pH was adjusted to 5.8. Anthers were incubated in darkness at 28° C for two months. Each treatment was conducted in five replicates. Each replicate contained one hundred anthers. After two months, the number of anthers forming embryoids was counted.

Second experiment:

The obtained data from the applied treatments in the first experiment was exploited during the second experiment was explained.

Hybrid seeds were sown in open field under similar conditions of that in the first season. Spikes were collected at -5 cm in booting stage and incubated for seven days at 4° C as cold treatment. Anthers were isolated from spikes and cultured on P-4s medium for embryogenic callus induction as previously.

Approximately 1-2 mm in diameter embryogenic calli were cultured in glass jars (50 ml) containing 25 ml of 190-2 regeneration medium (Zhuhang and Jia, 1983). Seven grams per liter agar were added to regeneration medium and pH was adjusted to 5.8. Cultures were incubated for two months at 27° C and exposed to 12 hours photoperiod with a light intensity of 1500 lux using fluorescent tubes (white cool). After two months, the number of regenerated plantlets was scored.

Regenerated green plantlets were transferred to plastic pots filled with mixture peatmoos, vermiculite and clay (2:1:1, v/v/v). The plantlets were covered with transparent polyethylene bags for one week. The plantlets were grown in green house for another one week at 21 /16 ° C (day/night) and 14 h photoperiod.

To determine ploidy level, root tips of two weeks old plantlets were collected and soaked in ice-cold water for 24 h (Ram, 2002). Then, root tips were transferred into Carnoy solution ethanol : glacial acetic acid (3:1, v/v) for fixation 24 h. The root tips were preserved in aceto-carmine for one week. Root tips were heated for 30 seconds and squashed in acetic acid 45%.

Chromosome number was counted by using light microscope to identify the ploidy levels of green plants.

Haploid plants (five weeks old) were removed from pots and roots were washed with tap water and soaked in colchicine solution (500 mg/l. colchicine and 20 ml/l. dimethylsulphoxide) for five hours at room temperature in darkness (Saber, 1991). Plants were removed out from colchicine solution and carefully rinsed with tap water for two hours. Plants were again transplanted in pots

After three weeks, root tips from colchicine treated plants were collected for cytological test to identify the ploidy level of the obtained plants as previously mentioned.

Statistical analysis:

In both experiments, the obtained data were analyzed using two factor completely randomized design according to MSTAT-C (1990) computer program.

RESULTS AND DISCUSSION

Screening for salinity tolerance:

Data in table 1 showed that seed germination percentage in all tested genotypes significantly decreased with increasing salinity level. Sids 1 and Sakha 8 cultivars showed significant higher seed germination percentage over the other tested genotypes under all salinity levels. The germination percentage for Sakha 8 and Sids 1 decreased from 86.1 and 86 % at the control level to 25 % at 12000 ppm. Both cultivars were significantly the highest cultivars in the germination percentage under 12000 ppm salinity level. Line 25 was introduced to the crosses program as recommended by the Wheat Research Department, Agriculture research Center for its higher yield capacity, in spite of its low germination percentage under 12000 ppm salinity levels (6 %).

Table (1): **Seed germination percentage of eleven bread wheat genotypes under different sea salt levels.**

Salinity level (ppm) / Genotype	0.	6000	9000	10000	11000	12000	Mean (a)
Giza 168	87.0	60.2	٣٦,٣	٢٠.2	٢٥.0	١٣,1	٣٩.5
Sids 1	86.0	٦٠,٣	٥٠,٨	٤٠,٩	٣٣.0	٢٥.0	٤٩,٥
Yacora	95.0	٥٠.0	٣١.0	٣١,1	٢٠.0	١١,1	٤٠,٥
Sakha 8	٨٦,١	٦٦.0	٦١.0	٥٣.0	٤١,1	٢٥.0	٥٥,4
Line 25	٩٣,٤	٤٥,١	٢٩.0	٢٣.0	١٠.0	٦.0	٣٤,5
Sakha 61	٨٨.5	٤٠.0	٢٦.0	٢٦,1	٢٢,١	١٦,1	٣٦,5
Gemmieza 7	٨٨,5	٥٣.0	٣٨.0	١٨.0	١٣.0	١٠.0	٣٦,8
Toson	٩١.5	٤٦.0	٤٠.0	٣٨.0	٣٠.0	٢٣.0	٤٤,8
Giza 155	٨٨,5	٦٥.0	٣٨.0	٣٣,١	٢٨.0	٢٠.0	٤٥,4
Giza 157	٨٥.0	٦١.0	٣٧.0	٣٣.0	٢٨.0	٢٠.0	٤٤,0
Gemmieza 5	٧٠.0	٢٥.0	٢٠.0	١٥.0	١٠.0	١٠.0	٢٥.0
Mean (b)	87.1	52.1	37.5	30.1	23.2	16.2	

L. S. D. $_{0.05}$ a = 0.6 b = 0.6 a X b = 1.6

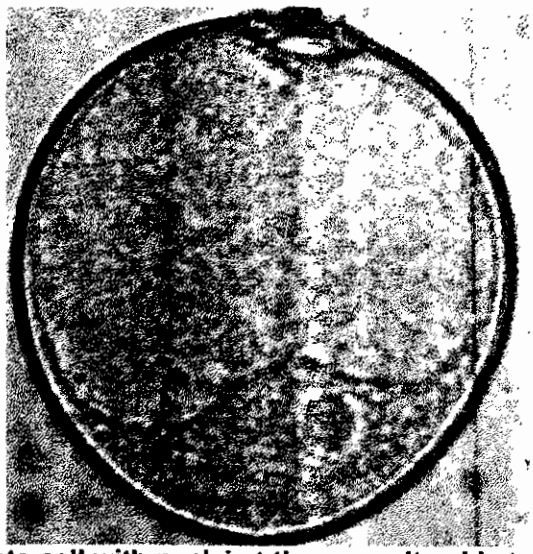
Suitable spike age for anther culture:

Consequently, according to He and Ouyang (1984), it was proposed that mid-uninucleate is the proper stage for microspore cells to induce embryogenic callus. Data in table 2 revealed that spikes at -5 cm stage in the three tested hybrids possessed higher frequency of microspore cells in the mid-uninucleate stage as compared to the other two older spikes ages, i.e. -3 and 0 cm. The mean percentage of anthers/spike produced microspore cells in the mid-uninucleate stage reached 89.9, 67 and 11 for spike ages -5, -3 and 0 cm, respectively.

Table (2): The effect of spike age on the percentage of the three wheat ybrids anthers possess microspores in mid-uninucleate stage.

Spike age (cm) / Genotype	- 5	-3	0	Mean a
Hybrid 1	86.3	57.3	10.6	**51.4**
Hybrid 2	94.3	73.6	12.0	**60.0**
Hybrid 3	89.0	70.0	10.3	**56.4**
Mean b	**89.9**	**67.0**	**11.0**	

L. S. D. $_{0.05}$ a = 2.78 b = 2.78 a X b = 4.83



Mid-uninucleate cell with nuclei at the opposite side to the cell pore, which consider the proper stage for induction of embryogenic callus by anther culture.

Cold treatments of spikes:

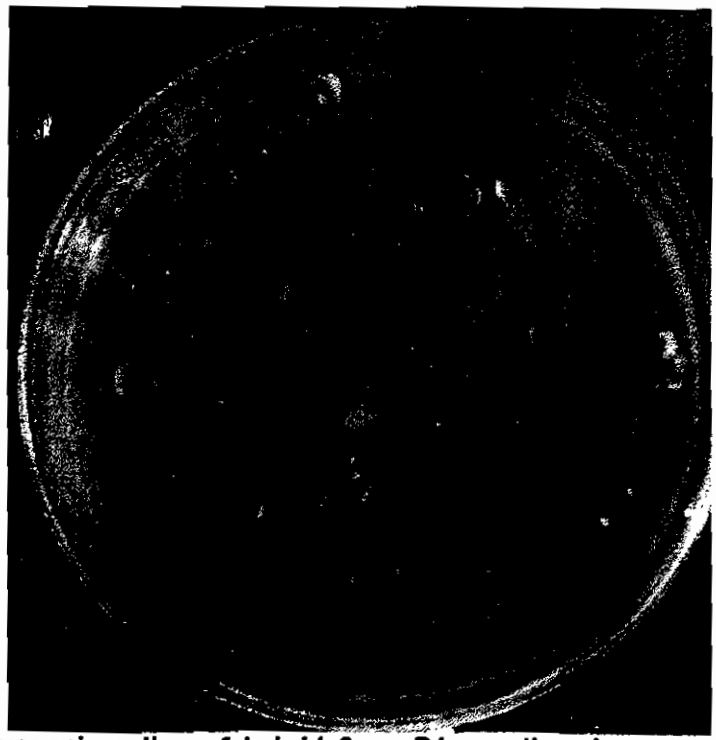
Table 3 showed that cold incubation of spikes for 7 or 14 days induced significant higher number of embryos compared with the other incubation periods (5 and 20 days).This result was in agreement with those obtained from Datta and wenzel(1987), Henry and Buyser (1990) and Chu and Hill (1996). Cold treatment was used to starve the microspore cells inside their

anthers to switch on their normal pathway (pollen grain formation) to the embryos formation pathway (Cistue, *et al.*, 1999 and Konicny, *et.al.* 2003). On the other hand, starving microspores for short time, i.e. 5 days could not be enough to change the normal pathway of microspore cells to form embryos. Meanwhile, starving microspores for long period, i.e. 20 days might kill them in the spikes. Therefore, it seams that under our experiment conditions as well as the tested hybrids the incubation of spikes for 7 or 14 days was highly recommended.

Table (3): Number of embryogenic callus produced from anthers of the three hybrids in response to cold pretreatment durations.

Cold pretreatment durations (day) / Genotype	5	7	14	20	Mean a
Hybrid 1	14.00	36.60	34.80	0.01	21.35
Hybrid 2	20.40	59.80	62.20	8.00	37.60
Hybrid 3	17.00	52.20	51.80	0.60	30.40
Mean b	17.13	49.53	49.60	2.87	

L. S. D. $_{0.05}$ a = 1.698b = 1.959 a X b = 3.39411



Embryogenic callus of hybrid 2 on P4s medium in response to anther culture

Second Experiment:

In the second experiment, anthers from the three wheat hybrids differed in their response to callus induction medium. The number of embryos induced from the embryogenic callus showed small differences among the three hybrids. The percentage of embryogenic callus induction reached 10.1, 61.9 and 51.4 for the three hybrids 1, 2, and 3, respectively. The number of embryos reached 8784, 10764, and 10147 for the three hybrids 1, 2, and 3 respectively. These results could be ascribed to the genotype effect and / or the culture medium composition on both embryogenic callus and number of embryos. These results are in agreement with those obtained from Ouyang *et al.*, (1983) and Weiguo *et al* .(2002).

Table (4): Number of embryogenic callus and derived embryos induced from anthers of the three hybrids cultured for two months on P4s medium.

	No. of cultured anthers	No. of embryogenic callus	Embryogenic callus %	No. of embryos
Hybrid 1	8000	807	10.1	8784
Hybrid 2	8000	4957	61.9	10764
Hybrid 3	8000	4113	51.4	10147

The results in table (5) showed that number of total regenerated plants (green and albino plants) reached 29.2, 13.3 and 16.0 % for hybrids 1, 2, and 3, respectively. However, the percentage of green regenerated plants did not take the same trend. It reached 1.4, 8.5 and 9.0 % for the three hybrids 1, 2, and 3 respectively. These results might reveal that, the parent genotypes in each hybrid could affect the response of each hybrid. Similar results were obtained by Ouyang *et a.*, (1983), and Weiguo (2002). In this regard, genotype variation and differential responses of genotypes to changes in growth and culture conditions make it difficult to devise growth and culture procedures suitable for all genotypes. The dominant and additive gene effects could provide opportunity to improve androgenic response through cross breeding and recurrent selection.

Table (5): Number and percentage of green and albino plants derived from embryos of three wheat hybrids cultured for two months on 190-2 medium.

	No. of embryos	No. of regenerated green plants	Regenerated green plants %	Regenerated albino plants	No. of regenerated albino plants %	Total regenerated plants	Total regenerated plants %
Hybrid 1	8784	121	1.4	2437	27.8	2558	29.2
Hybrid 2	10764	921	8.5	503	4.7	1424	13.3
Hybrid 3	10147	915	9.0	709	7.0	1624	16.0

Data in terms of embryogenic callus, number of embryos, number of regenerated green plants, number of regenerated albino plants and number of total regenerated plants showed that, hybrid one was the lowest in response to embryogenic callus and green plants while gave the highest percentage of total regenerated plants with highest percentage in albino plants. These results revealed that, the parent involved in the hybrids might have genotype had negative performance under the described technique of anther culture. Meanwhile, the parent genotypes involved in uniform of both hybrids 2 and 3 showed high performance in response to anther culture.

The Cytological examination of root tips from all regenerated plants revealed that all tested plants possessed 21 chromosomes in their somatic cells (haploid plants) as shown in fig. 3. Several research groups i.e. Wang *et al.* (1973), Craig (1974), Schaeffer *et al* (1979), Henry and De Buyser (1990) found that both haploid and with very low frequency of spontaneous double haploid plants were produced from anther culture.

Somatic cell of root tip of wheat green haploid plant with 21 chromosomes (1n = 3x = 21).

Colchicine treatment:

Table 6 showed that colchicine treated plants produced double haploid plants after soaking of roots in colchicine for 5 hours. The percentage of double haploid plants was almost identical for the three hybrids. The results in the present study could imply that, the genotype effect on the response to colchicine treatment is very low. These results were in agreement with those obtained by Saber (1991).

Kasem *et. al.,* (1998) reported that the different genotypes varied widely in response to colchicine-treatment and that the genetic differences affect the sensitivity of those plants to colchicine treatment.

Table (6): Number of double haploid plants obtained from haploid plants of three-wheat hybrids treated with colchicine for 5 hours.

	No. of haploid green plants	No. of double haploids	Percent of Double Haploids (%)
Hybrid 1	121	27	22.3
Hybrid 2	921	225	24.4
Hybrid 3	915	203	22.1

In summary, double haploid production is providing plant breeders with a good technique which offers the advantages of saving time and producing high homozygozity simultaneously and both highly needed by plant breeders.

REFERENCES:

Agricultural Statistics (2006).Sector of Economic Affairs.Ministry of Agriculture and Land Reclamation ,EGYPT

Ball S. T., H. Zhou and C. F. Konzak, (1992). Sucrose Concentration and Its Relationship to Anther Culture in Wheat. Crop Science 32: 149-154.

Chu C. C. and. Hill R.D., (1996). An Improvement Anther Culture Method for Obtaining Higher Frequency of Pollen Embryoids in 175-181 *Triticum aestivum L.* Plant Science, 55. 175 - 81.

Cistue, L., A. Ramos, and A. M. Castillo, (1999). Influence of anther pretreatment and culture medium composition on the production of barley doubled haploids from model and low responding cultivars. Plant Cell Tiss. Org. Cult. 55: 159-166.

Craig L. (1974). Haploid plant (n=21) from in vitro anther culture of wheat *Triticum aestivum* L. Can J Genet Cytol, 16:697-700.

Datta S. K. and G. Wenzel, (1987). Isolated Microspore derived plant Formation via Emberyogenesis in *Triticum aestivum L.* PLANT SCI., 48: 49-56.

He D. G. and J. W. Ouyang (1984). Callus and plantlet formation from cultured wheat anther s at different developmental stages. Plant Science Lett, 33: 71-79.

Henry, Y. and J. De. Buyser, (1990). Wheat Anther Culture: Agronomic Performance of Doubled Haploid Lines and Released of a New Variety "Florin". In: Biotechnology in Agriculture and Forestry. vol. 13. Bajaj, Y. P. S. (Ef.) Springer-Verlag, Berlin, pp. 285-352.

Heszky L. and J.Mesch, (1976). Anther culture investigations in cereal gene bank collection. Z. Planzenzüchtg. 77: 187-197.

Hoagland, D. R. and D. I.Arnon, (1950). The Water Culture Method of Growing Plants Without Soil. University of California, Berkeley College of Agriculture, Cricular No. 347.

Inagaki M. N., G. Varughese, S. Rajaram, M. Van Ginkel and A.Mujeeb-Kazi, (1998). Comparison of Bread Wheat Lines selected by Doubled Haploid, Single-Seed Descent and Pedigree Selection Methods. Theoretical and Applied Genetic 97: 550-556.

Kasem Z. A., Hassan Z. A., Ali M. M., and Mohamed S.A.A. (1998). Diplodization of anther culture-derived haploid plants in some Egyptian wheat (*Triticum aestivum* L) cultivars. Proceding of the 26th annual meeting of genetics Alex. 29-30 Sept.

Kim k., P. S. Baenziger, J. J. Rybczynski, and K.Arumuganathan, (2003). Characterization of Ploidy Levels of Wheat Microspore-Derived Plants Using Laser Flow Cytometry. In vetro Cell. Dev. Biol.-plant 39:663-668, November – December 2003.

Konieczny R., A. Z.Czaplicki2, H. Golczyk & L. Przywara, (2003). Two Pathways of Plant Regeneration in Wheat Anther Culture. Plant Cell, Tissue and Organ Culture . 73: 177-187.

Kunz C., Islam S. M. S., Berberat J., Peter S. 0., Buter B., Stamp P., and Schmid J. E., (2000) Assessment and Improvement of Wheat Microspore derived Embryo Induction and Regeneration. Plant Physiol.:. Vol. 156. pp. 190-196.

Moieni A., K. Lokos-Toth, and A. Sarrafi , (1997). Evidence for Genetic Control and Media Effect on Haploid Regeneration in The Anther Culture of Hexaploid Wheat (*Triticum aestivum L.*). Plant Breeding 116, 502-504.

MSTAT-C (1990) computer program.

Ouyang J. W., S. M.Zhou and S. E. Jia (1983). The Response Of Anther Culture to Culture Temperature in *Triticum Aesitivum*. Thero. Appl. Genet., 66: 101-109.

Ram J. Singh (2002). Plant Cytogenetics *second edition* ISBN 0-8493-2388-6 (alk. Paper).

Redha A., T. Attia, B. Biiter, S. Saisingtong, P. Stamp, J. E. Schmid, (1998). Improved Production of Doubled Haploids by Colchicine Application to Wheat *(Triticum aestivum L.)* Anther Culture. Plant Cell Reports 17: 974-979

Saber M., (1991). Genetical Studies on Production of Wheat Haploids. B. Sc., Agric. Minia University

Schaeffer G. W. P. S. Aenziger and J. Worley, (1979). Haploid Plant Development From Anther and *in vitro* Embryo Culture Of Wheat. Crop Sci., 19: 697-702.

Trop A. M., A. L Hansen &. S. B. Andersen, (2001). Chromosomal Regions Associated With Green Plant Regeneration in Wheat (*Triticum aestivum L.)* Anther Culture. Euphitica 119: 377 – 387.

Tuvesson S., A. Ljungberg, N. Johansson, K.-E. Karlsson, L. W. Suns and. I.-P. Josset, (2000). Large-Scale Production of Wheat and Triticale Double Haploids Through The Use of a Single-Anther Culture Method. Plant Breeding 119, 455-459

Wang C. C., Chu C. C., Sun S. C., Wu S. H, Yin K. C. and Hsu C (1973) the androgenesis in wheat *(Triticum aestivum L.)* anther culture *in vitro*. Sci. Sin, 16:218-222.

Weiguo Liu, Y. Ming Zheng, E. A. Polle and C. F. Konzak, (2002). Highly Efficient Doubled-Haploid Production in Wheat *(Triticum aestivum L.) via* Induced Microspore Embryogenesis. Crop science 42: 686-692.

Zheng M. Y., (2003). Microspore Culture in Wheat *(Triticum aestivum)* - Doubled Haploid Production Via Induced Embryogenesis. Plain Cell, Tissue and Organ Culture. 203: 213-230

Zhuhang, J. J. and X. Jia, (1983). Increasing differentiation frequencies in wheat pollen callus. In: Cell and tissue callus for cereal crop improvement, Eds: H Hu and M R Vega, Science press, Beijing, p431.

زراعة المتك كطريقة لانتاج سلالات نقية من قمح الخبز

سامى رضا صابر صبرى[٢]، اسامة محمد الشيحى[١]، محمد رمضان ابو العلا نسيم[١]و ضياء الدين سيد دغمة[٣].

١- فرع الفسيولوجى، قسم النبات الزراعى، كلية الزراعة، جامعة القاهرة، جيزة.

٢- قسم بحوث القمح، معهد بحوث المحاصيل الحقلية، مركز البحوث الزراعية، الجيزة.

٣- البنك القومى للجينات، وزارة الزراعة واستصلاح الاراضى، الجيزة.

يهدف هذا البحث الى ايجاد طريقة جديدة تستخدم فى برنامج تربية قمح الخبز للحصول على سلالات نقية وراثيا فى وقت قصير مقارنة بطرق التربية التقليدية واستخدام السلالات الناتجة فى اغراض التربية المختلفة.

تم عمل تجربة لاختبار تحمل احد عشر تركيبا وراثيا من قمح الخبز هى جيزة ١٦٨، سدس ١، ياكورا، سخا ٨، سلالة ٢٥، سخا ٦١، جميزة ٧، توسون، جيزة ١٥٥، جيزة ١٥٧ وجميزة ٥ تحت مستويات ملوحة مختلفة وهى ٠، ٦٠٠٠، ٩٠٠٠، ١٠٠٠٠، ١١٠٠٠ و ١٢٠٠٠ جزء فى المليون. تم انتخاب سخا ٨ وسدس ١ كآباء متحملة للملوحة. كما تم اختيار سلالة ٢٥ كسلالة حساسة للملوحة. تم عمل ثلاث هجن وهى هجين ١ (سدس ١ X سخا ٨)، هجين ٢ (سخا ٨ X سلالة ٢٥) و هجين ٣ (سدس ١ X سلالة25) تم زراعة حبوب الجيل الاول للهجن الثلاث فى الحقل وجمع السنابل لاختبار اسجابتها لطريقة زراعة المتك.

تم زراعة المتك من الثلاث هجن على بيئة P4s. استجابت الهجن الثلاث لزراعة المتك وكانت نسب تكوين الكلس الجنيني ١٠,١، ٦١,٩، و٥١,٤ لكل من هجين ١، ٢، و٣ على التوالى. تم نقل الاجنة على على بيئة استيلاد وتم الحصول على نباتات خضراء وكانت النسب ١,٤، ٨,٥، و٩,٠ لكل من هجين ١، ٢، و٣ على التوالى.

تم فحص قمم الجزور للنباتات الخضراء تحت المجهر الضوئى لتحديد عدد الكروموسومات فى الخلايا الجسمية وكانت جميع النباتات التى فحصت احادية المجموعة الكرموسومية. تم معاملة النباتات الاحادية بمحلول الكولشيسين لأحداث تضاعف كرموسومي فى خلايا النباتات الاحادية وكانت نسب النباتات التى حدث بها تضاعف ٢٢,٣، ٢٤,٤، و٢٢,١ لكل من هجين ١، ٢، و٣ على التوالى. ويستخلص من تلك الدراسة ان استخدام زراعة المتك تختصر الزمن المطلوب لتأصيل الوراثى وخاصة فى برامج تربية للمحاصيل ذاتية الاخصاب كالقمح اذ يمكن الحصول على سلالات نقية تماما فى فترة من ٢٠-٢٤ شهرا فقط بينما يستغرق ذلك فى برامج التربية التقليدية من ٦ – ٨ سنوات مع وجود نسبة ضئيلة من التراكيب الخليطة.