A highly efficient regeneration system *via* somatic embryogenesis from immature embryos of Egyptian wheat cultivars (*Triticum aestivum* L.) using different growth regulators

(Received: 10.12.2003; Accepted: 28.12.2003)

Ashraf H. Fahmy*, Yehia H. El-Shafy, Osama M. El-Shihy** and M.A. Madkour***
* Agricultural Genetic Engineering Research Institute, ARC., Giza, Egypt
** Physiology Department, Faculty of Agriculture, Cairo University, Egypt

ABSTRACT

Two wheat cultivars (Giza 163 and Giza 164) were used to study the effect of three different growth regulators i.e. Thidiazuron (TDZ), Zeatin riboside (ZR) and Dicamba on regeneration efficiency. In comparing results of regeneration efficiency for the three growth regulators across the two cultivars, it is concluded that the highest regeneration efficiency was observed by using the highest TDZ concentration (0.2 mg/l) as compared with other used concentrations of the two other growth regulators. On the other hand, it was clear that ZR had higher influence on regeneration efficiency than Dicamba. Moreover, Giza 164 was better than Giza 163 in regeneration efficiency across all growth regulators and all concentrations. This highly efficient regeneration system is considered a new addition that will open the door for improving wheat crop by in vitro techniques.

***Key words**: Regeneration, immature embryo, wheat cultivars.*

INTRODUCTION

Wheat is the most critical agricultural crop worldwide, where the stability of the community and regimes depends mainly on the availability of the strategic commodities. The efficient regeneration of normal and fertile plants from single cells, a basic prerequisite for molecular genetic improvement of plants, proved to be rather difficult for different wheat varieties because of their extreme recalcitrance to manipulation *in vitro*. These constraints were overcome by the culture of immature embryos at defined stage of development onto a defined nutrient medium supplemented with defined concentrations of hormones. Establishment of a highly efficient regeneration system for the input use of efficient varieties with unique quality profiles is, however, a prerequisite. Wheat regeneration *via* tissue culture varies with the genotype (Machii *et al.*, 1998). Immature zygotic embryos of two wheat (*Triticum aestivum* L.) genotypes, known for their different ability to generate embryogenic callus, were used as initial explants to establish callus cultures (Jimenez, 2001). Thidiazuron was first reported to have cytokinin activity in 1982. It has been used successfully *in vitro* to induce shoot formation and to promote auxillary shoot proliferation. Thidiazuron is especially effective with recalcitrant woody

species. Shoot number produced on medium containing Thidiazuron is equivalent to or greater than the number initiated on medium with purine type cytokinins and low concentration of Thidiazuron is too effective for micropropagation (Chin, 1993), and for induced tobacco plant regeneration (Thomas and Katterman, 1986). Dicamba also, induced callus formation in dark grown wheat embryos cultivated on modified MS medium (Papenfuss and Carman, 1987). Dong and Jia (1991) reported that high frequency shoot regeneration was induced from water melon cotyledons cultured on MS medium containing Zeatin riboside and it was significantly more efficient than 2ip and Kinetin. Mee-Sook *et al.* (1997) stated that stable auxiliary shoot establishment was achieved on MSB5 medium containing a combination of 5μM Thidiazuron (TDZ), 5μM (BA) and 1μM (IBA). The objective of this research is to establish an efficient regeneration system which is a basic prerequisite for the molecular genetic improvement of plants, through studying the effects of different hormones, i.e., Thidiazuron, Zeatin riboside and Dicamba on regeneration frequency of two local wheat cultivars (Giza 163 and Giza 164).

MATERIALS AND METHODS

Two local wheat (*Triticum aestivum* L.) cultivars; Giza 163 and Giza 164 were tested for their performance during in vitro regeneration. Immature embryo was the system of tissue culture used in the present study. Immature caryopsis of the two cultivars were collected approximately two weeks post-anthesis. Seeds were surface sterilized with 20% commercial Clorox (5.25% Sodium hypochlorite) supplemented with few drops of Tween 20, then washed five times with sterile D.D.H2O. Immature embryos for each cultivar were aseptically isolated. Fifty immature embryos were cultured with the scutellum side up onto the callus induction medium modified, for wheat cell culture medium containing MS (Murashige and Skoog, 1962) salts (Sigma, M5524), supplemented with 2 mg/l 2,4-D as a source of auxin, 0.15 g/l of L-Asparagine, 0.1 mg/l of myo-inositol, 20 g/l sucrose and 2.5 g/l phytagel. Calli were maintained in dark at 25°C and subcultured two times onto a fresh medium at 2 weeks intervals. After four to six weeks from culturing, calli were transferred to a fresh medium at the rate of 10 calli per Magenta box (Sigma, GA7) containing 50 ml of Phytagel-solidified MST basal medium (Sigma, M5519) supplemented with 3% sucrose and different growth regulators with different concentrations i.e. Thidiazuron (TDZ) (0.1, 0.15 and 0.2 mg/l), Zeatin riboside (0.5, 1.0 and 1.5 mg/l) and Dicamba (0.05, 0.1 and 0.2 mg/l). Calli were maintained on MST for six weeks at 25°C temperature, 25-50 μl E/m^2 light intensity and 16 hr. photoperiod. Number of shoots containing at least one expanded leaf per calli, number of leaf like structures (not containing at least one expanded leaf) and number of regenerated calli containing at least one shoot were recorded six weeks post-culturing onto the medium, using dissecting microscope. Data obtained were exposed to the proper statiscal analysis of complete randomized design described by Snedecor and Cochran (1969), in three replicates. Differences between means were tested by using Duncan's new multiple range test as described by Duncan (1955).

RESULTS AND DISCUSSION

In the present study, we have made attempts to improve the regeneration efficiency of two local wheat cultivars i.e., Giza 163 and Giza 164. Different growth regulators with different concentrations were used to identify the best growth regulator and

its concentration that gives the highest regeneration frequency; to be utilized after then in transformation experiments for these two wheat cultivars. Regeneration efficiency is scored as the number of shoots/callus (Y1) and number of leaf-like structures/callus (Y2). Comparison between the two cultivars for their reaction to TDZ showed significant differences in their regeneration efficiencies. The mean Y1 of Giza 164 (10.7) was significantly higher than that of Giza 163 (8.21) across all treatments. As expected, opposite results were given for Y2 means, where Giza 163 showed higher number of leaf-like structures/callus than Giza 164 across all treatments as shown in Table (1) and Figures (1 and 2). Comparison among different concentrations of TDZ indicated that the higher the concentration of cytokinin, the higher the number of shoots/callus across the Means followed by different capital letters in columns and those followed by different small letters in rows are significantly different at P=0.05 according to Duncan's multiple range test two cultivars.

Table (1): Effect of different concentrations of TDZ on number of shoots (Y1) and leaf-like structures (Y2) per callus for two Egyptian wheat cultivars Giza 163 and Giza 164.

Trait	Cultivar	TDZ concentration mg/l				Cultivar mean	Trait mean
		Control	0.1	0.15	0.2		
	Giza163	2.50 D	6.75 bC	9.82 bB	13.76 bA	8.21 b	
Y1	Giza164	3.02 D	9.48 aC	13.19 aB	17.12 aA	10.70 a	
	Average	2.76 D	8.11 C	11.51 B	15.44 A		9.46
	Giza163	4.85 D	21.74 a A	18.88 aB	15.81aC	15.32 a	
Y2	Giza164	6.40 D	17.57 bA	14.50 bB	7.22 bC	11.42 b	
	Average	5.62 D	19.65 A	16.69 B	11.51 C		13.37

Table (2): Effect of different concentrations of Zeatin riboside on number of shoots (Y1) and leaf-like structures (Y2) per callus for two Egyptian wheat cultivars Giza 163 and Giza 164.

Trait	Cultivar	Zeatin riboside concentration mg/l				Cultivar mean	Trait mean
		Control	0.5	1	1.5		
	Giza163	2.50 D	9.18 bB	10.88 bA	4.96 bC	6.88 b	
Y1	Giza164	3.02 D	10.19 aB	14.40 aA	7.12 aC	8.68 a	
	Average	2.76 D	9.69 B	12.64 A	6.04 C		7.78
	Giza163	4.85 C	8.45 aA	7.32 aB	3.53 aD	6.04 a	
Y2	Giza164	6.40 C	7.03 bA	6.50 bC	3.20 bD	5.78 b	
	Average	6.63 C	7.88 A	6.91 B	3.37 D		5.91

Table (3): Effect of different concentrations of Dicamba on number of shoots (Y1) and leaf-like structures (Y2) per callus for two Egyptian wheat cultivars Giza 163 and Giza 164.

Trait	Cultivar	Dicamba concentration mg/l				Cultivar mean	Trait mean
		Control	0.05	0.1	0.2		
	Giza163	2.50 B	7.87bA	2.01 C	0.69 D	3.26 b	
Y1	Giza164	3.02 B	9.66 aA	2.30 C	0.80 D	3.95 a	
	Average	2.76 B	8.77 A	2.16 C	0.75 D		3.61
	Giza163	4.85 C	11.23 aA	7.68 aB	3.43 D	6.80 a	
Y2	Giza164	6.40 C	8.93 bA	6.97 bB	2.35 D	6.16 b	
	Average	5.62 C	10.08 A	7.32 B	2.89 D		6.48

As far as results of leaf-like structures/callus, it was shown that TDZ gave higher values than shoots/callus either within or across cultivars. In general, it can be concluded that the cytokinin activity of TDZ was highest, when using 0.2 mg/l, where the number of shoots/callus and consequently the no. of plantlets was highest either for each or across the two wheat cultivars. The ability of TDZ to stimulate cell division has also been demonstrated in many plant species, (Thomas and Katterman, 1986).

Apart from stimulating cell division, TDZ had also been shown to induce adventitious shoot formation from tobacco leaf discs (Thomas and Katterman, 1986). Also, it was reported that TDZ induced auxillary shoot proliferation in several plant species, i.e. carnation (Lu *et al.*, 1991; Nugent *et al.*, 1991), apple (Van Nieu Wkerk *et al.*, 1986), pear (Singha and Bhatia, 1988), peach (Zimmerman and Scorza, 1992) and azalea (Briggs *et al.*, 1988). Moreover, TDZ promotes conversion of cytokinin ribonucleotides to the biologically more active ribonucleotides (Capelle *et al.*, 1983). Furthermore, TDZ encourages the synthesis of endogenous purine cytokinins and inhibits their degradation (Thomas and Katterman, 1986). An advantage of using TDZ is the resistance to degradation by cytokinin oxidize (Mok *et al.*, 1982). Therefore, TDZ is considered as quite stable in tissue culture. Moreover, TDZ is more biologically active than BA or Zeatin since lower concentrations are sufficient in tissue culture, which agree with the present findings. In grain legumes, TDZ has also been used for shoot formation originated from multi-cellular explant (Malik and Saxena, 1993; Mohamed *et al.*, 1993) or from protoplast (Böhmer and Jacobsen, 1994). In comparing results of TDZ with those of 2,4-D or picloram, it was shown that TDZ induced higher somatic embryogenesis in protocalli (Lehminger-Mertens and Jacobsen, 1989). In studying the effects of TDZ against other hormones, Mee-Sook *et al.* (1997) revealed that shoots biomass, which is the determinant of both auxiliary shoot number and dry weight, is shown to be as a more accurate indicator of effective shoot proliferation than auxillary shoot number, only. However, the present finding disagrees with Mee-Sook *et al.* (1997), where shoot biomass takes into account the leaf-like structures, which results in no regenerated plants. Therefore, we think that number of auxillary shoots only is a better indicator for the regeneration efficiency of any given plant genotype.

The influence of different concentrations of ZR on Y1 and Y2 of the two local wheat cultivars, Giza 164 and Giza 163, is presented in Table (2) and Figures (3 and 4). Comparison between the two cultivars showed significant differences in their number of shoots/callus, where the mean Y1 of Giza 164 (8.68) was higher than that of Giza 163 (6.88) across all ZR concentrations. Opposite results were given for Y2 means, where Giza 163 showed higher number of leaf-like structures/callus than Giza 164 across all treatments. The comparison among different concentrations of ZR for number of shoots/callus showed that 1 mg/l ZR gave the highest mean, while for leaf-like structures indicated that 0.5 mg/l ZR exhibited the highest means.

In general, it can be concluded that the cytokinin activity of Zeatin riboside was high, when using 1 mg/l, where the number of shoots/callus, and consequently the number of plantlets, was highest either for each or across the two wheat cultivars. Zeatin riboside, that has cytokinin activity, was used for many species to enhance the plant regeneration efficiency. Yadav and Sticklen (1995) reported that the culture of leaf explants in medium containing Zeatin or Zeatin riboside for six

days and then subcultured to medium containing Zeatin riboside (1mg/l) only caused shoot regeneration in high number.

Fig. (1): Effect of different concentrations of TDZ on regeneration efficiency of Giza 163 cultivar. A: control; B: 0.1 mg/l; C: 0.15 mg/l and D: 0.2 mg/l.

Fig. (2): Effect of different concentrations of TDZ on regeneration efficiency of Giza 164 cultivar. A: control; B: 0.1 mg/l; C: 0.15 mg/l and D: 0.2 mg/l.

Fig. (3): Effect of different concentrations of Zeatin riboside on regeneration efficiency of Giza 163 cultivar. A: control; B: 0.5 mg/l; C: 1.0 mg/l and D: 1.5 mg/l.

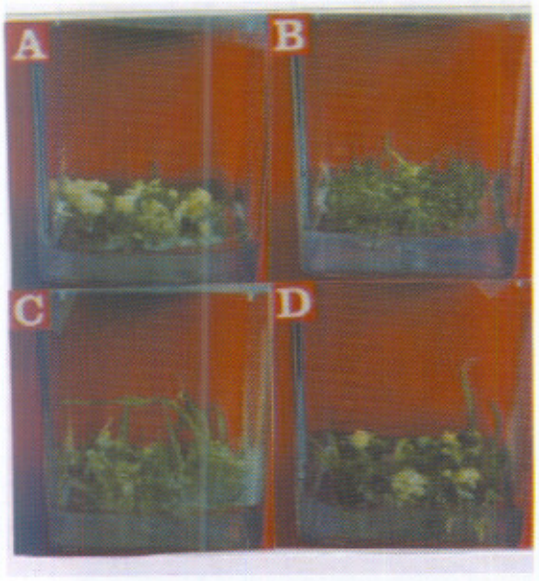
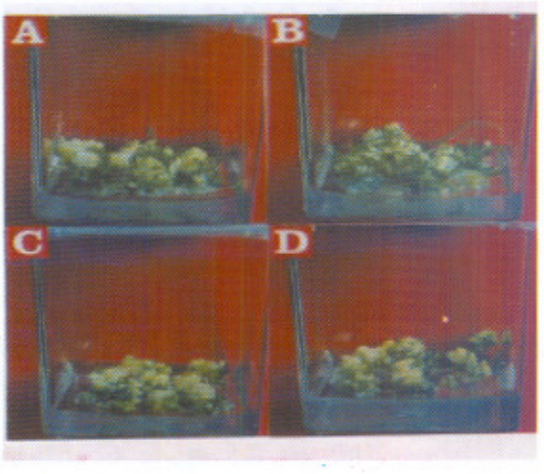


Fig. (4): Effect of different concentrations of Zeatin riboside on regeneration efficiency of Giza 164 cultivar. A: control; B: 0.5 mg/l; C: 1.0 mg/l and D: 1.5 mg/l.

Fig. (5): Effect of different concentrations of Dicamba on regeneration efficiency of Giza 163 cultivar. A: control; B: 0.05 mg/l; C: 0.1 mg/l and D: 0.2 mg/l.

Fig. (6): Effect of different concentrations of Dicamba on regeneration efficiency of Giza 164 cultivars. A: control; B: 0.05 mg/l; C: 0.1 mg/l and D: 0.2 mg/l.



Also, Perl *et al.* (1992) reported that Zeatin riboside in the concentration of 1mg/l promoted the shoot initials to plantlets in wheat. The influence of different concentrations of Dicamba as the only source of auxin on the regeneration efficiency of the two local wheat cultivars Giza 163 and Giza164 is shown in Table (3) and Figures (5 and 6). Comparison between the mean Y1 of the two cultivars showed significant differences, where it was higher for Giza 164 than Giza 163 across treatments. Comparison between the two wheat cultivars for Y2 indicated opposite results, where Giza 163

showed higher number of leaf-like structures/callus than Giza 164 across treatments. The lowest concentration (0.05 mg/l Dicamba) gave the highest Y1 and Y2 means for the two cultivars. The highest concentration of Dicamba (0.2 mg/l) was shown to give the lowest means for both studied traits for each or across the two cultivars. In other words, the lower the concentration of Dicamba, the higher the regeneration efficiency for both wheat cultivars. Auxin activity of Dicamba was first reported by Keitt and Baker (1966) and its use in plant tissue culture was first reported for wheat regeneration by Dudits *et al.* (1975). Dicamba was lately shown to be effective for plant regeneration from tissue cultures of *Dactylis glomerata* (Gary and Conger, 1985), *Poa pratensis* (McDonnell and Conger, 1984) and *Zea mays* (Duncan *et al.*, 1985). As a substitute to the cytokinin TDZ, the auxin Dicamba was used in many reports in the regeneration medium but with high concentrations. Weeks *et al.* (1993) reported that the concentration of 0.5 mg/l Dicamba improved wheat regeneration. Dicamba is successfully used for regeneration of Bobwhite wheat cultivar, which is considered as the model wheat cultivar worldwide. Also, Papenfuss and Carman (1987) reported an increased shoot formation rate from wheat callus cultures when incubated on medium containing Dicamba.

In comparing the results of the regeneration efficiency for the three growth regulators across the two cultivars, it can be shown that the influence of TDZ for the two characters, i.e., Y1 and Y2 was more potent when compared with the other two hormones. In comparing the results of the two hormones Zeatin riboside and Dicamba, ZR was much better than Dicamba because number of shoots/callus reflects number of plantlets developed through this regeneration regime, while number of leaf-like structures usually represent a dead-end plant regeneration system. A reason for the high efficiency of TDZ can be the potential of expressing a cytokinin activity as well as an auxin activity (Lu, 1993). In other reports, Huetteman and Preece (1993) indicated that TDZ shows a strong cytokinin-like activity and supposed to be the most effective plant growth regulator for apple tree (Coleman and Estabrooks, 1992), and in Geranium (Visser *et al.*, 1992). In this concern, chemical structure of TDZ was found to be different from common cytokinins (Huetteman and Preece, 1993). In general, TDZ was shown to satisfy both cytokinin as well as auxin requirements in many plant species (Visser *et al.*, 1992). However, it was reported that such a cytokinin was used in many reports in a very high concentration (up to 10mg/l) (Navarrete *et al.*, 1989). It is well known that the higher the regeneration efficiency for a given genotype, the higher the possibility of getting transgenic plants.

REFERENCES

Böhmer, P. and Jacobsen, H.L. (1994). Improved efficiency of protoplast regeneration in pea suitable for transformation. Congress of Plant Tissue and Cell Culture. Abstracts p 39.

Briggs, B.A., McCulloch, S.M. and Edick, L.A. (1988). Micropropagation of azaleas using Thidiazuron. *Acta Hort.*, 226: 205-208.

Capelle, S.C., Mok, D.W. and Kirchner, S.C. (1983). Effects of thidiazuron on cytokinin autonomy and the metabolism of N6-(2-isopentenyl) 18-C 14 adenosine in callus tissues of *Phaseolus lunatus* L. Plant Phys., 37: 796-802.

Chin, Y.L. (1993). The use of Thidiazuron in tissue culture. In Vitro Cell. Dev. Biol.,29 (7) 92-96.

Coleman, W.K. and Estabrooks, E.N.

(1992). Enhancement of cold hardiness in apple trees by paclobutrazol, thidiazuron and flurprimidol. Can. J. Plant Sci., 72, 1267-1274.

Dong, J.Z. and Jia, S.R. (1991). High efficiency plant regeneration from cotyledons of watermelon (*Citrullus vulgaris* Schrad). Plant Cell Rep., 9: 559-562.

Dudits, D., Nemet, G. and Haydu, Z. (1975). Study of callus growth and organ formation in wheat (*Triticum aestivum* L.) tissue cultures. Can. J. Bot., 53: 957-963.

Duncan, D.B. (1955). Multiple range and multiple "F-test". Biometrics, 11: 1-42.

Duncan, D.R., Williams, M.E., Zehr, B.E. and Widholm, J.M. (1985). The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes. Planta 165: 322-332.

Gary, D.J. and Conger, B.V. (1985). Influence of dicamba and casein hydrolysate on somatic embryo number and culture quality in cell suspension of *Dactyis glomerata* (*Gramineae*). Plant Cell, Tiss. Org. Cult., 4: 123-133.

Huetteman, C.A. and Preece, J.E. (1993). Thidiazuron a potent cytokinin for woody plant tissue culture. Plant Cell, Tiss. Org. Cult., 33: 105-119.

Jimenez, V.M. (2001). Endogenous hormone concentration and embryogenic callus development in wheat. Plant Cell, Tiss. Org. Cult., 67 (1): 37-46.

Keitt, G.W. and Baker, R.A. (1966). Auxin activity of substituted benzoic acids and their effect on polar auxin transport. Plant Phys., 41(10): 1561-1569.

Lehminger-Mertens, R. and Jacobson, H.J. (1989). Plant regeneration from pea protoplasts *via* somatic embryogenesis. Plant cell Rep., 8: 379-382.

Lu, C., Nugent, G., Wardley, T. (1991). Agrobacterium-mediated transformation of carnation (*Dianthus caryophyluss* L.). Bio-Tech., 9: 864-868.

Lu, C.Y. (1993). The use of Thidiazuron in tissue culture. In vitro Cell Dev. Bio. 29(2): 92-96.

Machii, H., Mizuno, H., Hirabayashi, T., Li, H. and Hagio, T. (1998). Screening wheat genotypes for high callus induction and regeneration capability from anther and immature embryo cultures. Plant Cell, Tiss. Org. Cult., 53 (1): 67-74.

Malik, M.C. and Saxena, P.K. (1992). Regeneration in *Phaseolus vulgaris* L. high frequency induction of direct shoot formation in intact seedlings by N-6-benzlaminopurine and Thidiazuron. Planta, 186: 384-389.

McDonnell, R.E. and Conger, B.V. (1984). Callus induction and plantlet formation from mature embryo explant of Kentucky bluegrass. Crop Sci., 24: 573-578.

Mee-Sook, K., Carol, M.S. and Ned B.K. (1997). Effects of Thidiazuron and benzyladenine on axillary shoot proliferation of three green ash (*Fraxinus pennsylvancia* Marsh.) clones. Plant Cell, Tiss. Organ Cult., 48: 45-52.

Mohamed, M.F, Coyne, D.P. and Read, P.E. (1993). Shoot organogenesis in callus induced from pedicel explants of common bean (*Phaseolus vulgaris* L.). J. Amer. Soc. Hort. Sci., 118: 158-162.

Mok, M.C., Moke, D., Armstrong, D.J., Shudo, K., Isogai, Y. and Okamoto, T. (1982). Cytokinin activity of N- Phenyl-N-1, 2 ,3- Thiadiazol- 5 -Ylurea (Thidiazuron). ,Phytochem., 21: 1509-1511.

Murashige, T. and Skoog, F. (1962). A revised medium for rapid growth and bioassays with tobacco tissue culture. Plant Phys., 15: 473-497.

Navarrete N.E., Van Sambeek, J.W., Preece, J.W. and Gaffeny, G.R. (1989). Improved micropropagation of white ash (*Fraxinus americana* L.). Proceedings of Conference of the Seventh Center Hardwood

3: 146-149.

Nugent, G., Wardley-Richardson, T. and Lu, C. (1991) Plant regeneration from stem and petal of carnation (*Dianthus caryophyllus* L.). Plant Cell. Rep., 10: 477-480.

Papenfuss, J.M. and Carman, J.G. (1987). Enhanced regeneration from wheat callus cultures using Dicamba and kinetin. Crop Sci., 27: 588-593.

Perl, A., Kless, H., Blumenthal, A., Galili, G. and Galun (1992). Improvement of plant regeneration and GUS expression in scutellar wheat calli by optimization of culture conditions and DNA-microprojectile delivery procedures. Mol. Genet., 235: 279-284.

Sendecor, G.W. and W.G. Cochran (1969). Statistical methods. 6th ed. Iowa State Univ., press, Ames, Iowa: USA., p.:593.

Singha, S. and Bhatia, S. (1988). Shoot proliferation of pear cultivars on medium containing Thidiazuron and benzylamino-purine. Hort. Sci., 23: 803.

Thomas, J.C. and Katterman, F.R. (1986). Cytokinin activity induced by Thidiazuron. Plant Phys., 81: 681- 683.

Van NieuWkerk, J.P., Zimmerman, R.H. and Fordlham, I. (1986). Thidiazuron stimulation of apple shoot proliferation *in vitro*. Hort. Sci., 12: 516-518.

Visser, C., Qureshi, J.A., Gill, R. and Saxena, P.K. (1992). Morphoregulatory role of Thidiazuron substitution of auxin and cytokinin requirement for induction of somatic embryogenesis in Geranium hypocotyl cultures. Plant Phys., 99: 1704-1707.

Weeks, J.T., Anderson, A.D. and Blechl, A.E. (1993). Rapid production of multiple independent lines of fertile transgenic wheat (*Triticum aestivum*). Plant Phys., 1077-1084.

Yadav, N.R. and Sticklen, M.B. (1995). Direct and efficient plant regeneration from leaf explants of *Solanum tubersum* L. cv. Bintje., Plant Cell. Rep., 14: 645-647.

Zimmerman, T.W. and Scorza, R. (1992). Growth and proliferation of peach under varying environmental regimes. Hort. Sci., 27: 696.

الملخص العربي

طريقة ذات كفاءة عالية لإعادة الإستيلاد من الأجنة غير الناضجة لأصناف من القمح المصرى بإستخدام منظمات نمو مختلفة

اشرف حسين فهمى* ،يحيى حسان الشافعى** ،أسامة محمد الشيحى** و مجدي أحمد مدكور*

*معهد بحوث الهندسة الوراثية ، مركز البحوث الزراعية

**قسم الفسيولوجى ، كلية الزراعة ، جامعة القاهرة

تم إستخدام صنفين من القمح هما جيزة 164 وجيزة 163 لدراسة تأثير ثلاثة منظمات نمو مختلفة TDZ و Zeatin riboside (ZR) والـ Dicamba على كفاءة عملية الإستيلاد. وبمقارنة نتائج كفاءة عملية الإستيلاد المستخدمة عبر الصنفين أمكن إستنتاج أن أعلى كفاءة للإستيلاد قد لوحظت عند إستخدام التركيز المرتفع للـ TDZ (0.2mg/L) وذلك بالمقارنة بمنظمى النمو الآخرين المستخدمين. وعلى جانب آخر كان من الواضح أن منظم النمو ZR له تأثير أعلى على كفاءة عملية الإستيلاد عن منظم النمو Dicamba . وقد وجد أن الصنف جيزة 164 أفضل من الصنف جيزة 163 عبر كل منظمات النمو المستخدمة بتركيزاتها المختلفة فى كفاءة عملية الإستيلاد . ويعتبر هذا النظام ذا الكفاءة العالية لعملية الإستيلاد إضافة جديدة والتى بدورها سوف تفتح الباب لتحسين محصول القمح عبر تقنيات البيوتكنولوجيا.