Improvement of plant regeneration from long-term callus cultures of two Egyptian wheat cultivars

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ABSTRACT

An efficient protocol was developed for regeneration of plants from long-term cultured calluses, originated from immature embryos of two Egyptian wheat (*Triticum aestivum* L.) *cultivars (Sids 1 and Giza 168). For callus induction and long-term maintenance, calluses were subcultured onto Murashige and Skoog's medium containing 2.0 mg/l 2, 4-D for eight months. The calli were then cultured onto a regeneration medium supplemented with Zeatin ribozide (0, 0.5 and 1.0 mg/l) or Thidiazuron (0, 0.1 and 0.2 mg/l) or Dicamba (0, 0.25 and 0.5 mg/l). Among the two cultivars, Sids1 produced the highest number of shoots per shooted callus (4.5) in TDZ containing medium (0.2mg/l). Important differences in regeneration characteristics were observed between the two genotypes. The developed in vitro system for maintaining of embryogenic calli for prolonged period is essential for conducting mutation, selection and genetic modifications for improvement of wheat cultivars.*

Keywords: *wheat, T. aestivum, immature embryo, long-term, regeneration.*

INTRODUCTION

More than 50% of the food used by man is provided by a single group of crop plants, the cereals, of which wheat is the most important species. A major problem of wheat cell culture is the establishment of long-term embryogenic cultures. Biotechnological methods, which provide new and unique opportunities for the genetic improvement of this crop, require efficient *in vitro* plant regeneration and the recovery of transgenic plants following delivery and integration of foreign genes into regenerable cells. Immature embryos have proven to be a good material for the production of callus and regeneration of plants in cereals (Kleijer *et al.*, 1996). Redway *et al.* (1990) reported that immature embryos from commercial cultivars of wheat (*Triticum aestivum* L.) gave the best embryogenic capacity and regeneration. Embryogenic calli with potential for plant regeneration in long-term cultures derived from high yielding cultivars of wheat (*Triticum aestivum* L.) plant regenerability and maintained on maintenance medium exhibited the capacity for plant regeneration even after 24 months of culture (Suprasanna *et al.*, 1997). Kothari *et al.* (1998) demonstrated that callus induced from immature embryos of wheat cv. Kharchia 65 on MS medium containing 2, 4-D was maintained in a regenerable state by subculturing every 5-6 weeks for about one year. Yang *et al.* (1999) indicated that manipulation of growth regulators leads to an

efficient plant regeneration in long-term callus cultures. Shan *et al*. (2000) investigated the effects of Thidiazuron (TDZ) on *in vitro* regeneration of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum* L.), and found that TDZ promoted shoot regeneration from callus in these two species. The addition of Zeatin to regeneration media had also a positive effect on regeneration (Barro *et al.*, 1999). Amirova *et al.* (2002) stated that a reproducible and genotype-independent system for the long-term regeneration in wheat tissue culture was developed. Vasil *et al.* (1992) obtained transgenic wheat plants resistant to the herbicide Basta (active ingredient phosphinothricin [glufosinate], PPT) by high velocity microprojectile bombardment and pBAR GUS plasmid from cells of long-term regenerable embryogenic callus. Zhang *et al.* (2000) co-bombarded morphogenic wheat calluses derived from scutellum tissue of immature embryos of *Triticum aestivum* cv. Bobwhite with separate plasmids carrying a selectable marker gene (bar) and a gene of interest. They mentioned that transformed wheat calluses with a vigorous growth retained morphogenic potential and were competent for plant regeneration for as long as 11 months. Bohorova *et al.* (2001) pointed out that successful regeneration of wheat and triticale varieties from long term cultures will be used as an integral part of the transformation process. Therefore, it is necessarily to optimize a maintenance protocol for embryogenic calli for prolonged periods in order to conduct mutation, selection and genetically modification for improvement of wheat cultivars.

MATERIALS AND METHODS

Two local wheat (*Triticum aestivum* L.) cultivars; Sids1 and Giza 168 were tested for their performance during *in vitro* long-term callus maintenance and regeneration. Immature embryo was the system of tissue culture used in the present study. Immature caryopses 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 three drops of Tween 20, and then washed five times with sterile d.d.H_2O. Immature embryos of each cultivar were aseptically isolated. Fifty immature embryos were cultured with the scutellum side up onto the callus induction medium, for wheat cell culture 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 onto a fresh medium at 4 weeks intervals and were maintained by subculture for at least 8 months. After thirty two weeks from culturing, the long-term maintained calli were transferred to a fresh medium supplemented with 3% sucrose and different growth regulators at different concentrations i.e. Thidiazuron (TDZ) (0, 0.1 and 0.2 mg/l) and Zeatin ribozide (0, 0.5, and 1.0 mg/l) and Dicamba (0, 0.25 and 0.5 mg/l); each treatment replication comprised three Magenta boxes (Sigma, GA7) at the rate of 5 calli per Magenta box containing 50 ml of Phytagel-solidified MSR basal medium (Sigma, M5519). Calli were maintained on MSR for six weeks at 16 hr. photoperiod of about 40-50 µE $m^{-2}s^{-1}$ provided by daylight cool fluorescent lamplight at 25°C temperature. Data obtained i.e. number of shooted calli, number of rooted calli, number of shoots, frequency of regeneration, percentage of rooted calli and number of shoots per shooted callus; were exposed to the proper statistical analysis of completely randomized design described by Sendecor and Cochran (1969), in

three replicates. Means were compared by using Duncan's new multiple range test as described by Duncan (1955).

RESULTS AND DISCUSSION

Calli initiation and maintenance

Cells or calli when subcultured over a long period, loose their regeneration ability, however, during *in vitro* selection and genetic transformation studies, regeneration ability from old callus is a prerequisite. The major problem of inducing and maintaining callus in wheat specifically has been resolved to an extent by using 2 mg/l 2,4-dichlorophenoxy acetic acid (2,4-D). Also, Kothari *et* ***al.*** (1998) induced callus from immature embryos of wheat cv. Kharchia 65 and maintained in a regenerable state by subculturing every 5-6 weeks on MS medium supplemented with 2,4-D.Shoot regeneration For regeneration, calli maintained on maintenance medium for 32 weeks were collected together and subcultured on Phytagel-solidified MSR basal medium (Sigma, M5519) supplemented with 3% sucrose and different growth regulators at different concentrations i.e. Thidiazuron (TDZ) (0, 0.1 and 0.2 mg/l) and Zeatin ribozide (0, 0.5, and 1.0 mg/l) and Dicamba (0, 0.25 and 0.5 mg/l).

Analysis of variance (not presented) showed significant differences among cultivars, growth regulator types and concentrations and significance for most of the two and three way interactions in regeneration characteristics.

Role of cultivar

Sids1 surpassed significantly Giza 168 in all studied regeneration characteristics i.e.; number of shooted and rooted calli, number of shoots, frequency of regeneration, percentage of rooted calli and number of shoots per shooted callus (Table 1). Genotypic differences were also reported by Henry *et al.* (1994). They tested *Triticum aestivum* stocks of Chinese spring wheats, for their ability to undergo somatic embryogenesis after 2 months of *in vitro* embryo culture. They reported that their regeneration capacity was observed after 4 months of *in vitro* cultures. Similar results were obtained by Furini and Jewell (1995); they evaluated five intergeneric crosses between 5 maize inbreds and *Tripsacum dactyloides* (clone 65-1234) for their ability to form long-term embryogenic and regenerable callus cultures. They obtained embryogenic cultures and plant regeneration from all five crosses tested. They classified maize inbred lines CML135 and Tzil8 as non-embryogenic. They recorded that frequency of embryogenic callus production ranged from 14.3% to 66.6%. Also, Kleijer *et al.* (1996), observed important differences in regeneration capacity between genotypes. Furthermore, Bronsema *et al.* (1997) compared differentiation of callus derived from immature maize embryos of inbred lines A188 and A632 by culture on regeneration medium. They found that callus of A188 was embryogenic and maintained its embryogenic capacity for at least one year and that immature embryos of A632 formed callus that was not embryogenic and produced only roots. Similar results were obtained by Jimenez and Bangerth (2001), who compared between two wheat cultivars i.e.; "Devon" and "Combi" for their differentiation ability and found that formation of non embryogenic callus of "Devon" cultivar began to increase without noticeable sign of differentiation; while higher rates of embryogenic callus formation for "Combi" cultivar were produced along the time of culture.

Table (1): Regeneration characteristics of two Egyptian wheat cultivars (Sids1 and Giza 168) across all studied growth regulator types and concentrations.

Regeneration characteristics	Cultivar	
	Sids 1	**Giza 168**
Number of shooted calli	3.00 A	2.33 B
Number of rooted calli	8.67 A	7.07 B
Number of shoots	9.41A	5.22 B
Frequency of regeneration	20.00 A	15.56 B
Percentage of rooted calli	57.56 A	47.16 B
Number of shoots per shooted callus	2.23 A	1.29 B

Means followed by different capital letters in rows are significantly different at P=0.05 according to Duncan's multiple range test .

Role of growth regulator type

The effect of growth regulator type is summarized in Table (2). In comparing the influence of different growth regulator types, Zeatin ribozide was found to be optimum with a high significant increase than Thidiazuron in most of regeneration characteristics i.e.; number of shooted calli, number of shoots and frequency of regeneration. These data are reliable since cytokinen supplement enhances the promotion of shoot differentiation either *via* embryogenesis or organogenesis, while auxin promotes root formation. Moreover, it was reported that the culture of leaf explants in a medium containing Zeatin or Zeatin ribozide for six days and then subcultured to a medium containing Zeatin ribozide only caused shoot regeneration in a high number (Yadava and Sticklen, 1995). Furthermore, Kachhwaha and Kothari (1996) obtained plant regeneration from embryogenic callus induced from immature embryo explants of two species of barley, *Hordeum spontaneum* and *Hordeum vulgare*. The embryogenic callus initiated on 2,4-D was maintained for ten to twelve months, after which it lost its potential to regenerate plants. TDZ had also been shown to induce adventitious shoot formation from tobacco. Furthermore, TDZ encourages the synthesis of endogenous purine cytokinen and inhibits their degradation (Thomas and Katterman, 1986). 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 and Jacobsen, 1989). On the other hand, Dicamba has greater effect on other characteristics; number of rooted calli and percentage of rooted calli. Similar results were obtained earlier in many reports (Weeks *et al.,* 1993 and Fahmy *et al.*, 2004). Also, auxin activity of Dicamba was first reported by Keitt and Baker (1966). Dicamba was lately shown to be effective for plant regeneration from tissue cultures of *Zea mays* (Duncan *et al.,* 1985). Our findings are also supported by Yang *et al.* (1999) who developed an efficient protocol for regeneration of plants from long-term cultured calluses, which originated from mature seeds of a model rice variety "Taipei 309" and were maintained by subculturing for at least 6 months. They stated that their results indicate that manipulation of growth regulators leads to efficient plant regeneration in long-term rice callus cultures.

Role of growth regulator concentration

Data presented in Table (3) show the effect of growth regulator concentration on number of shooted calli, number of shoots and regeneration frequency which ranged from 0.50 to 3.78, from 0.50 to 11.72, and from

3.34 to 25.19; respectively. These results show the importance of exogenous supplementary of growth regulator to the regeneration medium and that was also in accordance with data presented by Yadava and Sticklen (1995). Moreover, it was reported that the role of growth regulators is critical on regenerative ability of old callus cultures of the high-yielding wheat cultivars CPAN3004 and PBW226 (Yadava and Chawla, 2002). Furthermore, Shrivastava and Chawla 2001 reported that callus was induced from immature embryo explants on MS basal medium supplemented with 2 mg/l 2,4-D and regeneration in old calluses (28-30 weeks old) was obtained only when the medium contained growth regulators. They indicated that increasing cytokinen concentration from 1 to 3 mg/l increased the regeneration frequency of old callus cultures.

Table (2): Effect of growth regulators type on regeneration characteristics across two Egyptian wheat cultivars (Sids1 and Giza 168).

Regeneration characteristics	Growth regulator types		
	Zeatin ribozide	Thidiazuron	Dicamba
Number of shooted callus	4.22 A	2.83 B	0.95 C
Number of rooted callus	7.33 B	6.50 B	9.78 A
Number of shoots	11.95 A	8.33 B	1.17 C
Frequency of regeneration	28.15 A	18.89 B	6.30 C
Percentage of rooted callus	48.89 B	43.33 B	65.19 A
Number of shoots per shooted callus	2.11 A	2.17 A	1.00 B

Means followed by different capital letters in rows are significantly different at P=0.05 according to Duncan's multiple range test

Table (3): Effect of growth regulator concentrations on regeneration characteristics across two Egyptian wheat cultivars (Sids1 and Giza 168).

Regeneration characteristics	Growth regulator concentration		
	Control	Conc. 1	Conc. 2
Number of shooted calli	0.50 B	3.72 A	3.78 A
Number of rooted calli	3.34 B	10.45 A	9.83 A
Number of shoots	0.50 C	9.72 B	11.72 A
Frequency of regeneration	3.34 B	24.82 A	25.19 A
Percentage of rooted calli	22.22 B	69.63 A	65.56 A
Number of shoots per shooted callus	0.50 B	2.35 A	2.43 A

Means followed by different capital letters in rows are significantly different at P=0.05 according to Duncan's multiple range test

Table (4): Effect of cultivars and growth regulator type interaction on regeneration characteristics of two Egyptian wheat cultivars (Sids1 and Giza 168).

Regeneration characteristics and cultivar	Growth regulator type		
	Zeatin ribozide	**Thidiazuron**	**Dicamba**
Number of shooted calli			
Sids 1	4.40 Aa	3.56 Ba	1.00 Ca
Giza 168	4.00 Ab	2.11 Bb	0.90 Ca
Number of rooted calli			
Sids 1	7.89 Ba	8.56 Ba	9.56 Aa
Giza 168	6.78 Bb	4.45 Cb	10.00 Aa
Number of shoots			
Sids 1	13.56 Aa	13.44 Aa	1.22 Ba
Giza 168	10.33 Ab	4.22 Bb	1.11 Ca
Frequency of regeneration			
Sids 1	29.63 Aa	23.70 Ba	6.67 Ca
Giza 168	26.67 Ab	14.08 Bb	5.93 Ca
Percentage of rooted calli			
Sids 1	51.93 Ba	57.04 Ba	63.70 Aa
Giza 168	45.19 Bb	29.63 Cb	66.67 Aa
Number of shoots per shooted callus			
Sids 1	2.50 Ba	2.97 Aa	1.22 Ca
Giza 168	1.72 Ab	1.37 Ab	0.78 Bb

Means followed by different capital letters in rows and those followed by different small letters in columns are significantly different at P=0.05 according to Duncan's multiple range test

On the other hand, significant differences between the three concentrations tested were recorded on number of shoots which ranged from 0.50 to 11.72. These data exhibited that the second concentration of growth regulators was superior over the first one and that prolonged cultures need high cytokinen exogenous supplementation. Furthermore, Varshney *et al.* (1996) established tissue cultures of *Triticum durum* from immature embryos on Murashige and Skoog's (MS) medium supplemented with 2,4-D. They reported that both non-regenerative and the regenerative types of calluses were developed from long-term cultures of all genotypes tested. They added that callus was maintained on this medium by regular sub-culturing and plant regeneration occurred on MS medium supplemented with 0.2 mg/l IAA and 1.0 mg/l 6-benzyladenine.

Cultivar X growth regulator type interaction

In studying the growth regulator type X cultivar interactions, it can be concluded that the highest number of shooted calli, number of shoots and frequency of regeneration response were recorded with Sids 1 cultivar and Zeatin ribozide, while the lowest was obtained by Giza 168 cultivar and Dicamba with a high significant difference (Table 4). Zeatin ribozide, which has cytokinen activity, was

used for plant regeneration for many species to enhance the regeneration efficiency. Moreover, Barro *et al.* (1999**)** mentioned that addition of Zeatin to regeneration media had a positive effect on regeneration. They compared morphogenetic capacities of 19 different cultivars of wheat, barley and tritordeum. However, frequencies of regeneration in wheat and barley varied widely among the cultivars tested and, in both species. On the other hand, Dicamba and Giza 168 cultivar showed significant increments in number and percentage of rooted calli than sids1 and other tested growth regulators (Table 4). This demonstrates the significant influence of cell endogenous hormonal balance and cell differentiation manner and growth regulator mode of action. On the contrary, we had found in a previous report (Fahmy *et al.,* 2004) that shoot regeneration was more potent with TDZ followed by Zeatin ribozide and Dicamba; it was concluded that this may be due to cultivar and long period culture interactions. Kachhwaha *et al.* (1997) reported that long-term plant regeneration was obtained from embryogenic calluses induced from immature embryo explants of *Hordeum vulgare* cv. RD57 callus transferred to hormone-free MSB medium.

Cultivars X growth regulator concentration interaction

A wide range of differences was observed among cultivar X growth regulator interactions for all tested regeneration characteristics (Table 5). Sids 1 scored the highest number of shooted calli, number of shoots and regeneration frequency with Zeatin ribozide, while Giza 168 scored the lowest estimates with Dicamba. In agreement with our results, Yadava and Sticklen (1995) regenerated shoots in a high number only on the medium containing Zeatin riboside. Also, Perl *et al.* (1992) reported that Zeatin ribozide promoted the shoot initials to plantlets in wheat. Bohorova *et al.* (2001) evaluated elite wheat advanced lines and released varieties, and five triticale varieties for their ability to produce embryogenic callus using three different media. After a 6-month period, genotypes lost their ability to regenerate plants and only 10 lines retained some plant regeneration potential, but regeneration was at reduced levels. On other hand, Navarrete *et al.* (1989) concluded that TDZ gave sufficient regeneration efficiency. Also, Thidiazuron (TDZ) was found to promote shoot regeneration from callus of barley (*Hordeum vulgare*) and wheat (*Triticum aestivum*) (Shan *et al.,* 2000). In contrast, Giza 168 showed the best response with Dicamba for number and percentage of rooted calli, but it gave the lowest with Thidiazuron.

These data are in agreement with our previous findings which observed that Dicamba gave lowest number of shoots within or across the two tested Egyptian wheat cultivars Giza 163 and Giza 164 (Fahmy *et al*., 2004). Growth regulator type X concentration interaction.

Data presented in Table (6) show significant differences for different growth regulators and their concentrations for most of regeneration characteristics. Zeatin ribozide at the high concentration (1mg/l) gave the highest values in number of shooted calli, number of shoots and frequency of regeneration with an increment reached to 6 fold in number of shooted calli and 18 fold in number of shoots and 6 fold in regeneration frequency.

Similar results were reported by Yadava and Sticklen (1995) who regenerated shoots in a high number only on the medium containing 1 mg/l Zeatin ribozide. Also, Perl *et al.* (1992) reported that Zeatin ribozide in the concentration of 1mg/l promoted the shoot initials to plantlets in wheat.

On the other hand, TDZ at the highest concentration surpassed in number of shoots per shooted callus both with Zeatin ribozide and Dicamba; respectively. Our data was in agreement with Navarrete *et al.* (1989) who concluded that TDZ when used at 0.2 mg/l, gave sufficient regeneration efficiency and with Shan *et al*., (2000) who found that shoot regeneration from barley calluses was the highest (38.3% for cv. Golden Promise) at 1 mg/l TDZ, while the optimal TDZ level for wheat regeneration seemed to be 0.2 mg/l (87% for cv. Bob White and 49.4% for cv. Hi-Line). In contrast the second concentration of Dicamba (0.5 mg/l) scored the highest values number and percentage of rooted calli, while hormone free medium scored the lowest values.

Cultivar X growth regulator type X growth regulator concentration interactions

In general, Figure (1) indicated that Sids 1 surpassed significantly Giza 168 in regeneration frequency. Moreover, Sids 1 gave highest regeneration frequency (42.22%) with 1 mg/l Zeatin ribozide while, Giza 168 scored 40% with 0.5 and 1 mg/l Zeatin riboside. On the other hand, considering number of shoots produced and number of shoots per shooted callus, Sids 1 gave the highest estimates using 0.2 mg/l thidiazuron (Fig.2). In contrast, Dicamba gave the highest rooted calli percentage but it gave the lowest regeneration frequency. The present results further revealed that the best cultivar was Sids 1. It was superior with Thidiazuron while, Giza 168 responded best with Zeatin ribozide.

Table (5): Effect of cultivars and growth regulator concentrations interaction on regeneration characteristics of two Egyptian wheat cultivars (Sids1 and Giza 168).

Regeneration characteristics And cultivar	**Growth regulator concentration**		
	Control	**Conc. 1**	**Conc. 2**
Number of shooted calli			
Sids 1	1.00 Ca	3.56 Bb	4.44 Aa
Giza 168	0.00 Cb	3.90 Aa	3.11 Bb
Number of rooted calli			
Sids 1	6.67 Ba	9.56 Ab	9.78 Aa
Giza 168	0.00 Cb	11.33 Aa	9.90 Ba
Number of shoots			
Sids 1	1.00 Ca	12.22 Ba	15.00 Aa
Giza 168	0.00 Cb	7.22 Bb	8.45 Ab
Frequency of regeneration			
Sids 1	6.67 Ca	23.71 Bb	29.63 Aa
Giza 168	0.00 Cb	25.93 Aa	20.74 Bb
Percentage of rooted calli			
Sids 1	43.78 Ba	63.70 Ab	65.19 Aa
Giza 168	0.00 Cb	75.56 Aa	65.93 Ba
Number of shoots per shooted callus			
Sids 1	1.00 Ba	2.86 Aa	2.83 Aa
Giza 168	0.00 Bb	1.83 Ab	2.04 Ab

Means followed by different capital letters in rows and those followed by different small letters in columns are significantly different at P=0.05 according to Duncan's multiple range test

Table (6): Effect of growth regulator type and concentration interaction on regeneration characteristics across two Egyptian wheat cultivars (Sids1 and Giza 168).

Regeneration characteristics And growth regulator type	Growth regulator concentration Control	Conc. 1	Conc. 2
Number of shooted calli			
Zeatin ribozide	0.50 Ba	6.00 Aa	6.17 Aa
Thidiazuron	0.50 Ba	3.83 Ab	4.17 Ab
Dicamba	0.50 Ca	1.34 Ac	1.00 Bc
Number of rooted calli			
Zeatin ribozide	3.34 Ca	10.50 Ab	8.17 Bb
Thidiazuron	3.34 Ca	9.00 Ac	7.17 Bc
Dicamba	3.34 Ca	11.84 Ba	14.17Aa
Number of shoots			
Zeatin ribozide	0.50 Ca	17.00 Ba	18.33 Aa
Thidiazuron	0.50 Ca	10.17 Bb	15.84Ab
Dicamba	0.50 Ca	2.00 Ac	1.00 Bc
Frequency of regeneration			
Zeatin ribozide	0.34 Ba	40.00 Aa	41.11 Aa
Thidiazuron	0.34 Ca	25.56 Bb	27.78 Ab
Dicamba	0.34 Ca	8.89 Ac	6.67 Bc
Percentage of rooted calli			
Zeatin ribozide	22.22 Ca	70.00 Aa	54.45 Bc
Thidiazuron	22.22 Ca	60.00 Ab	47.78 Bb
Dicamba	22.22 Ca	78.89 Ba	94.44 Aa
Number of shoots per shooted callus			
Zeatin ribozide	0.50 Ba	2.83 Aa	2.99 Aa
Thidiazuron	0.50 Ca	2.71 Ba	3.31 Aa
Dicamba	0.50 Ca	1.50 Ab	1.00 Bb

Means followed by different capital letters in rows and those followed by different small letters in columns are significantly different at P=0.05 according to Duncan's multiple range test .

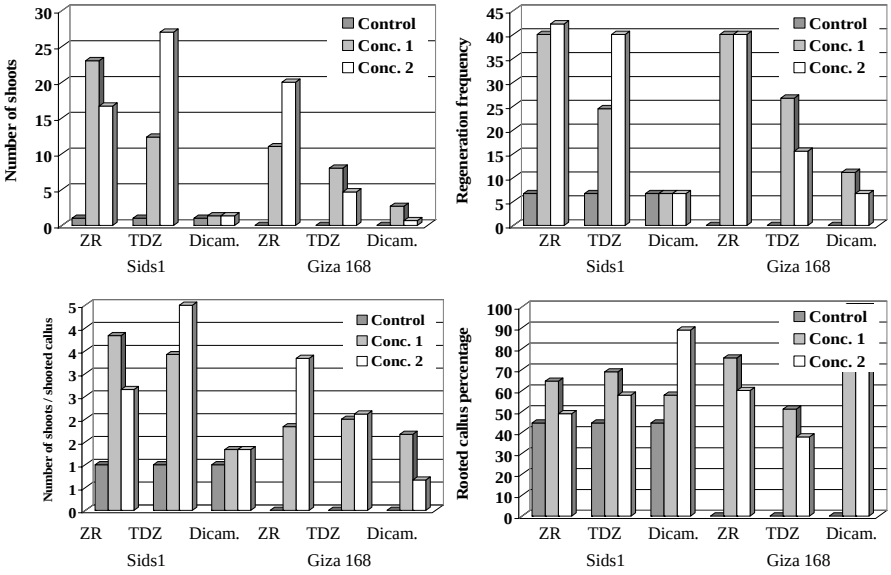

Fig. (1): Effect of cultivar, growth regulator type and concentration interaction on number of shoots, frequency of regeneration, number of shoots per shooted callus and rooted calli percentage of two Egyptian wheat cultivars (Sids 1 and Giza 168).

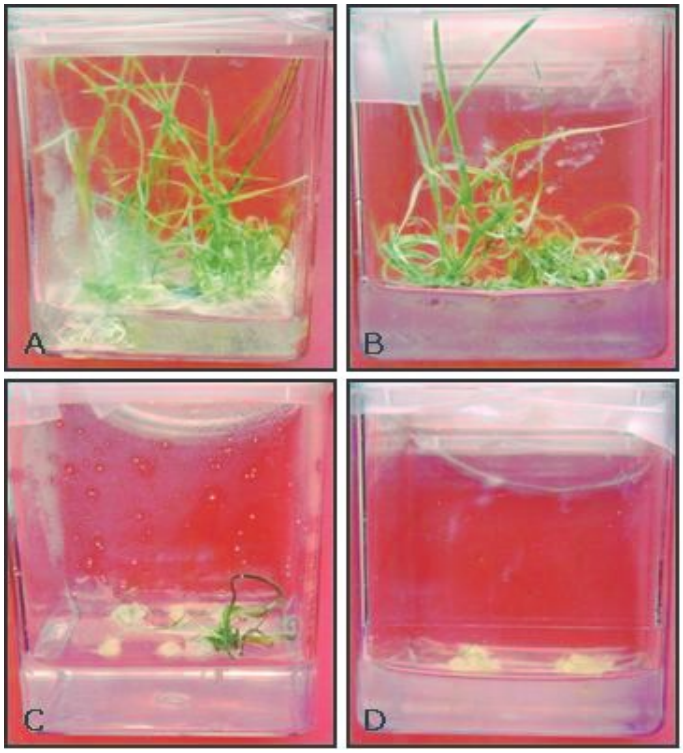

Fig. (2): Plant regeneration from immature long-term derived callus of wheat

A) Regenerated plantlets from Sids 1 cultivar on 0.2 mg/l thidiazuron.

B) Regenerated plantlets from Giza 168 cultivar on 1.0 mg/l zeatin ribozide.

C) Regenerated plantlets from Sids 1 cultivar on hormone free medium.

D) Regenerated plantlets from Giza 168 cultivar on hormone free medium.

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الملخص العربي

تحسين عملية إعاده الإستيلاد من الكالس المسن لصنفين من القمح المصرى

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لقد تم تطوير نظام كفؤ لإعادة إستيلاد النباتات من الكالس المسن الناشىء من الاجنة غير الناضجة لصنفين من اصناف القمح المصرى (سدس١ وجيزة ١٦٨). فقد تم إعادة زراعة الكالس الناشىء و المحافظة عليه لفترة طويلة على بيئة زراعة MS المضاف إليها ٢ مجم/لتر من منظم النمو 2,4-D وذلك لمدة ثمانية أشهر. ثم تم اعادة زراعتها على بيئة لإعادة الإستيلاد المضاف اليها عدة منظمات نمو بتركيزات مختلفة و هى منظم النمو zeatin riboside (صفر؛ ٠,٥٠ و ١,٠ مجم/لتر) أو منظم النمو thidiazuron (صفر؛ ٠,١٠ و ٠,٢٠ مجم/ لتر) أو منظم النمو Dicamba (صفر؛ ٠,٢٥ و ٠,٥٠ مجم/ لتر) وقد اظهرت النتائج تفوق صنف سدس١ حيث اعطى اعلى نسبة نباتات لكل كالس (٤,٥) و ذلك على بيئة تحتوى على منظم النمو thidiazuron بتركيز ٠,٢ مجم / لتر. وقد اظهرت الدراسة العديد من الفروق المعنوية الهامة فى صفات إعادة الإستيلاد عبر الصنفين محل الدراسة. و يعتبر هذا النظام المطور للمحافظة على الكالس الجنينى لفترات طويلة هاماً وذلك إما للحصول على طفرات أو للإنتخاب أو لإستخدامه فى عملية التعديل الوراثى لأصناف القمح المصرى ذات الأهمية الأقتصادية .