In vitro regeneration of sugarbeet propagules and molecular analysis of the regenerants

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S.A. Bekheet ; H.S.Taha and M.A. Matter
Plant Biotechnology Dept., National Research Centre, Dokki, Cairo,Egypt.

ABSTRACT

An applicable regeneration protocol through in vitro direct and indirect organogenesis of sugarbeet was developed. Leaf and shoot base explants taken from in vitro grown seedlings were subjected to a medium containing different combinations of cytokinins (BA and Kin) and auxins (NAA and 2,4-D). The highest percentages of direct organogenesis were noticed with shoot base explants; however, leaf explant was more readily for indirect differentiation. Concerning growth regulators added to culture media, cytokinins (BA and kin) in combinations with NAA were more effective on direct regeneration since the best results of shoot emergence directly from shoot base explants (93%) were registered with a medium contained 0.5 mg/l BA + 0.5 mg/l NAA. However, the highest frequency of indirect regeneration (80%) was obtained when leaf explants were cultured on a medium containing 0.5 mg/l kin + 0.5 mg/l 2,4-D. For multiplication of sugarbeet propagules, the medium contained 1 mg/l BA+50 mg/l adenine sulfate gave the highest number of shoots and leaves. However, the best results of shoot length were observed when 1 mg/l kin was combined with 50 mg/l adenine sulfate. Among different types of auxins used for in vitro root formation, IBA in concentration of 2 mg/l was more effective than IAA and NAA. SDS-PAGE protein patterns did not give variation between the direct and indirect proliferated cultures of sugarbeet. RAPD analysis was used to identify the differences among regenerants from tissue cultures. The results of PCR products showed slight differences between cultures and demonstrated that direct organogenesis is the most effective way to produce true-type regenerants in sugarbeet. A successful acclimatization to the free conditions was obtained by transferring of plantlets into pots contained a mixture of peatmoss and vermiculite (1:1) under high relative humidity conditions. High percentages of survival and plants of normal appearance were obtained after five weeks of transplanting.

***Key words**: Sugarbeet, in vitro propagules, RAPD, acclimatization, regeneration.*

INTRODUCTION

Sugarbeet is the major sucrose-producing crop grown in temperate zones, and contributes approximately 37% of the world's supply, with the rest derived from sugarcane. In addition to sugar from sugarbeet, it is possible to obtain a considerable amount of leaves, slices from roots and molasses, which are valuable as animal feed. Sugarbeet has also attracted further attention, by virtue of the potential to accumulate novel or valuable specific metabolites in the storage tissues (green bioreactors). In Egypt, sugarbeet is considered one of the promising sucrose producing crops. About 26% of Egyptian sugar production comes from sugarbeet, which is grown mainly in North Delta. Cultivation of sugarbeet in Egypt faces several field problems. Beside the impossibility of

flowering and seed production in the free living conditions, crop productivity suffers extensively from the damage caused by pathogens and diseases. However, in view of the fact that sugarbeet is a biannual plant and the modern cultivars are highly heterozygous, being naturally cross-pollinated, the developing of new varieties by conventional breeding is difficult (Atanassov, 1986). Therefore a biotechnology strategy for sugarbeet may be expected to aid the breeders in introducing specific traits into commercially valuable genotypes. Development of an *in vitro* regeneration protocol and a micropropagation system for sugarbeet is considered a very important step for its genetic manipulation by modern biotechnology methods. In this respect, several attempts have been achieved to make progress in sugarbeet regeneration *in vitro*. Organogenesis in sugarbeet has been reported from callus (Ritchie *et al*., 1989; Tetu *et al*., 1987), leaf tissue (Ferytag *et al*., 1988), suspension culture cells (Van Geyt and Jacobs, 1985) and protoplast (Bhat *et al*., 1986). Direct differentiation of vegetative cultures was proliferated *in vitro* from many cultured tissues of sugarbeet such as axillary buds (Mezei *et al*., 1990; Mezei and Kovacev, 1991), apical meristems (Goska and Szota, 1992) and inflorescence pieces (Zhong *et al*., 1993). In addition, molecular techniques are increasingly used by plant breeders to develop improved cultivars. In this regard, using of SDS-PAGE protein pattern as a molecular marker are used to distinguish the differences between derived tissue culture plantlets (Roberts *et al*., 1989; Feirer and Simon, 1991). In recent years, there has been a great interest in new DNA-based marker techniques such as restriction fragment length polymorphism (RFLP) analysis, polymerase chain reaction, random amplification of polymorphic DNA (RAPD). RAPD technology has been successfully used for measuring genetic diversity within and between in plants. Patterns of variation observed are shown to closely resemble those obtained using more morphological characters (Howell *et al*., 1994; Virk *et al*., 1995). Moreover, RAPD analysis is sensitive enough to detect genetic variability in somaclones. The present study aims to develop an efficient protocol for *in vitro* direct and indirect regeneration of sugarbeet and carry out molecular characterizations of the regenerants, using SDS-PAGE protein patterns and RAPD techniques

MATERIALS AND METHODS

Establishment of aseptic cultures

Sugarbeet seeds cv. Pleno provided by Delta Sugar Company, were used as plant material for *in vitro* culture and subsequently for tissue culture experiments. To obtain aseptic seedlings of sugarbeet, the seeds were washed with distilled water and then immersed in 70 % ethanol for 1 min, followed by 30 % commercial Clorox (containing 5.25 % sodium hypochlorite) for 20 min and finally washed three times with distilled sterilized water. The disinfected seeds were placed in jars containing 50 ml of MS-basal medium (Murashige and Skoog, 1962). Forty-day old seedlings were taken and recultured aseptically on fresh MS-medium to improve their vegetative growth (Fig. 1). To get stock plant materials, the aseptic shoot tips were excised from the seedlings and cultured on MS medium supplemented with 1 mg/l BA. To develop a direct or indirect regeneration protocol of sugarbeet, true leaf segments and shoot bases were aseptically cultured on MS-medium supplemented with different combinations of cytokinins and auxins as follows:

1	MS + 0.5 mg/l BA + 0.1 mg/l NAA	2	MS + 0.5 mg/l BA + 0.5 mg/l NAA
3	MS + 0.5 mg/l BA + 0.1 mg/l 2,4-D	4	MS + 0.5 mg/l BA + 0.5 mg/l 2,4-D
5	MS + 0.5 mg/l kin + 0.1 mg/l NAA	6	MS + 0.5 mg/l kin + 0.5 mg/l NAA
7	MS + 0.5 mg/l kin + 0.1 mg/l 2,4-D	8	MS + 0.5 mg/l kin + 0.5 mg/l 2,4-D

Percentages of direct and indirect organogenesis (after callus initiation) were recorded from 15 replicates after six weeks of culturing.

In vitro multiplication of propagules

For multiplication of sugarbeet propagules, the proliferated shoots were taken and the leaves were excised and then cultured on media containing different types of cytokinins in addition to adenine sulfate as follows:

1. MS + 1 mg/l BA
2. MS + 1 mg/l kin
3. MS + 1 mg/l 2ip
4. MS + 1 mg/l BA+ 50 mg/l adenine sulfate
5. MS + 1 mg/l kin +50 mg/l adenine sulfate
6. MS + 1 mg/l 2ip +50 mg/l adenine sulfate

Number of proliferated shoot buds, shoot length (cm) and number of leaves/shoot were recorded after four weeks of culturing.

In vitro rooting and acclimatization

For rooting of plantlets, single dark green shoots (3 cm length) were cultured on MS-medium amended with 0.03 % of activated charcoal and different types of auxins as follows:

1	Basal MS-medium	2	MS + 1 mg/l IBA
3	MS + 2 mg/l IBA	4	MS + 1 mg/l NAA
5	MS + 2 mg/l NAA	6	MS + 1 mg/l IAA
7	MS + 2 mg/l IAA		

Root formation, number of roots/shoot and root length (cm) were recorded from 10 replicates after five weeks of culturing on rooting media. For acclimatization of *in vitro* rooted plantlets to the free-living conditions, the healthy complete plantlets (with good root system) were washed with tap water and disinfected by soaking in benlate solution (1g /l) for 20 min. Then plantlets were transplanted into plastic pots containing peatmoss and vermiculite (1:1). The pots were covered with clear polyethylene bags and sprayed with water to maintain a high relative humidity. Humidity was gradually reduced and covers were completely removed within four weeks.All tissue culture media were solidified with 0.7 % agar and adjusted to pH 5.8 before autoclaving at 121˚C and 1.5 Ib/M² for 25 min. Cultures were normally incubated at 25˚C and 16 hr photoperiod (white fluorescent tubes).

Protein electrophoresis (SDS-PAGE)

Proteins were extracted from leaf tissues (0.5 g) of plantlets derived from proliferation of shoot tips, by direct and indirect organogenesis in addition to plants grown *in vivo,* by homogenizing in (25 mM Tris pH 9, 0.5 % SDS and 10% glycerol) buffer. A 30 µg sample of protein was separated in 10 % SDS-PAGE according to Laemmli (1970). The separation was carried out using EC mini gel unit at 60 volt for four hr. Gels were stained with Coomassie brilliant blue (R-250), distained with methanol solution (40 % methanol in 10 % acetic acid), photographed and the molecular weights of the polypeptide bands were estimated from a low molecular weight marker standards (Pharmacia.).

Randomly amplified polymorphic DNA (RAPD) analysis

For RAPD analysis, DNA isolation was performed using the Cetyl Trimethyl Ammonium Bromide (CTAB) method of Doyle and Doyle (1987). Half gram of fresh sample of leaf tissues derived from proliferated shoots, by direct and indirect organogenesis, were ground to a powder in liquid nitrogen with a prechilled pestle and mortar, suspended in 5 ml preheated CTAB

buffer, and incubated at 65°C for 1 hr with occasional shaking. The suspension was then mixed with 1/3 volume of chloroform, mixed gently, centrifuged and the upper phase was transferred to a new sterilized tube. Extraction was repeated with an equal volume of chloroform. The aqueous layer was transferred to a new tube, 2/3 volume of isopropanol was added and nucleic acids were either spooled using a Pasteur pipette or sedimentated by centrifugation. The pellet was washed carefully twice with 70 % ethanol, dried at room temperature and resuspended in 0.5 ml TE buffer. The enzyme, RNAse A (20 µg) was added to the resuspended mixture to digest any contaminating RNA and the tube was incubated at 37 ˚C for 30 min. To remove the enzyme and other contaminating protein; phenol/chloroform extraction was performed.

The polymerase chain reaction (PCR) mixture (25 µl) consisted of 0.8 units of Taq DNA polymerase, 25 pmol dNTPs, and 25 pmol of a random primer, and 50 ng of genomic DNA. The reaction mixture was placed on a DNA thermal cycler. The PCR program included an initial denaturation step at 94°C for 2 min followed by 45 cycles with 94°C for 1 min for DNA denaturation, RPPD 36°C standard as mentioned with each primer. Extension at 72°C for 30 sec and final extension at 72°C for 10 min were carried out. The amplified DNA fragments were separated on 2% agarose gel and stained with ethidium bromide. Five 10-mer primers (Operon technologies Inc., Alameda, California) were used in RAPD analysis .A 100 bp DNA ladder (Promga) was used as a marker with molecular sizes of 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. The amplified fragments were visualized on UV trans illuminator and photographed.

RESULTS AND DISCUSSION

Regeneration and differentiation

Re-differentiation of shoots and roots from aseptic grown tissues of sugarbeet is considered one of the important aspects in genetic manipulation by gene transfer. In this work, two types of explants and different combinations of growth regulators added to culture media were examined for direct and indirect regeneration of sugarbeet. Results obtained reveal that the highest percentages of direct organogenesis were noticed with shoot base explants (Fig 2). However, leaf explants were more ready for indirect differentiation, since shoots were formed after the phase of callus initiation (Fig. 3). Concerning growth regulators added to the culture media, cytokinins (BA and kin) in combinations with NAA were more effective for direct regeneration. However, 2,4-D was more suitable for indirect organogenesis. The best results of direct shoot emergence from shoot base explants (93 %) were recorded with MS-medium containing 0.5 mg/l BA + 0.5 mg/l NAA. On the other hand, the highest frequency of indirect regeneration (80 %) was obtained when leaf explants were cultured on MS-medium supplemented with 0.5 mg/l kin + 0.5 mg/l 2,4-D (Table 1).

A B C

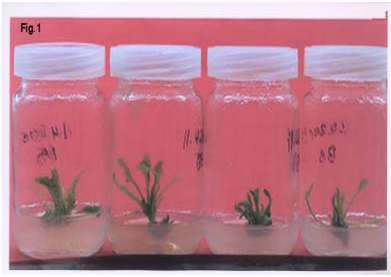

Fig.1

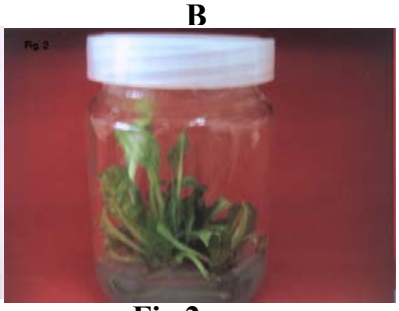

Fig.2

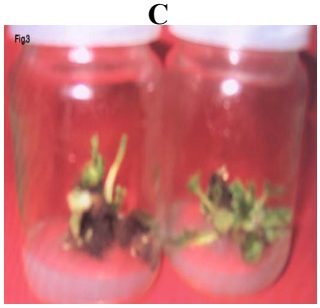

Fig.3

Fig.4

Fig.5

Fig.6

Fig.(1): In vitro grown seedlings of sugarbeet after three weeks of reculturing on fresh medium.

Fig. (2): Direct shoot formation on shoot base explants using MS-medium containing 0.5 mg/l BA + 0.5 mg/l NAA.

Fig. (3): Indirect shoot formation using MS-medium containing 0.5 mg/l kin+0.5 mg/l 2,4-D.

Fig. (4): The highest number of shoots obtained from MS-medium containing 1 mg/l kin + 50mg/l adenine sulfate.

Fig. (5): Root formation on MS-medium containing 2 mg/l of either IBA, NAA or IAA from left to right.

Fig. (6): Adapted plantlets of sugarbeet to free environmental conditions.

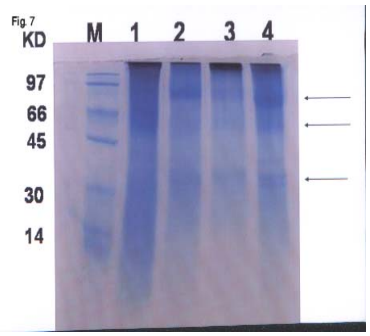

Fig. 7

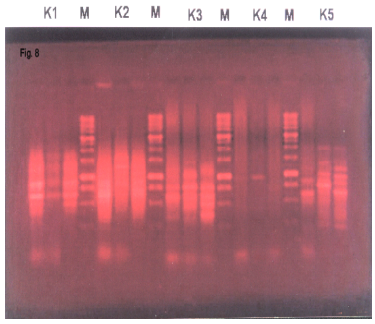

Fig.8

Fig. (7): SDS-PAGE of total protein extracted from in vivo grown plants (lane 1), plantlets derived from proliferation of shoot tips (lane 2), indirect organogenesis (lane 3) and direct organogenesis (lane 4).

Fig. (8): RAPD profile of proliferated shoots, direct and indirect organogenesis from left to right respectively, using primers (K1-K5) and DNA ladder marker of MW 1000, 900, 800, 700, 600, 500, 400, 300, 200 and 100 bp. (M).

Similar results were reported by several researchers. Direct shoot regeneration in sugarbeet was obtained from various explants including petiole (Freytag *et al.*, 1988; Krens and Jamar, 1989); leaf (Miedema, 1982) and shoot base (Rady, 1998). Indirect differentiaion was also achieved from callus derived from leaf explants (Doley and Saunders, 1989; Owens and Eberts, 1992).

Multiplication of propagules

With a view to optimize tissue culture medium for shoots proliferation and elongation of directly or indirectly regenerated propagules, were recultured on MS-medium supplemented with 1mg/l of each of cytokinins i.e., BA, Kin or 2iP alone, or in addition to 50 mg/l of adenine sulfate. Results presented in Table (2) indicate that BA was the most effective cytokinin for shoot multiplication of sugarbeet compared with kin or 2iP added separately to the culture medium. Moreover, addition of adenine sulfate to culture media significantly enhanced both the number of proliferated shoots and shoot length. MS-medium containing 1 mg/l BA+50 mg/l adenine sulfate gave the highest number of shoot as well as the highest number of leaves .However, the best results of shoot length (4 cm) was observed when 1 mg/l kin was combined with 50 mg/l adenine sulfate (Table 2 and Fig. 4). The problem of vitrification was not observed. From these results, it was concluded that BA is the most suitable cytokinin for shoot bud proliferation of sugarbeet *in vitro,* and kin can be used for shoot elongation. Moreover, adenine sulfate (50 mg/l) should be added to culture medium for enhancing both of the number of proliferated shoots and elongation. These results are in line with those reported by Rady (1998) who indicated that the highest number of shoots occurred when shoot tips of sugarbeet was grown on MS medium supplemented with 0.25 mg/l NAA and 1 mg/l BA. Mezei *et al*. (1990) added 0.5 mg/l BA to MS medium for plantlet formation from flower buds of sugarbeet. However, Goska and Szota (1993) stated that shoot proliferation was induced from apical meristems of different types of sugarbeet, when cultured on MS medium supplemented with 0.09 mg/l NAA and 0.22 mg/l BA. Moreover, the positive role of adenine sulfate as one of the important stimulating purines in tissue culture medium was reported by several investigators (Okasha *et al*., 1996; Bekheet, 1999).

***In vitro* root formation**

Root formation is an obligatory phase for micropropagation of plants reproduced *in vitro*. Some of them initiate roots without special treatments, while others require a medium supplemented with different growth regulators essentially of an auxin nature. Different plant species may vary in their requirement of auxin type for adventitious root formation. In this work, the effect of three types of auxins, i.e., IBA, IAA and NAA, added separately in two concentrations (1 and 2 mg/l) on *in vitro* rooting of sugarbeet shoots was investigated. Data in Table (3) indicate that increasing auxin concentration showed promoting effect on rooting parameters. IBA was the most suitable type of auxin for *in vitro* rooting of sugarbeet compared with IAA and NAA. The highest percentages of root formation (85 %) as well as number of roots (4.60) were noticed when 2 mg/l IBA and 0.03 % of activated charcoal were added to the culture medium However, the highest value of root length (3.10 cm) was obtained when 2 mg/l of NAA were added to the medium. (Table 3 and Fig.5). The present results are in accordance with those reported by Goska and Rogozinska (1990). Their results indicated that

MS medium with 2 mg/l IBA was the best for root development of sugarbeet *in vitro*. On the other hand, Mezei *et al*. (1990) mentioned that the best rooting of sugarbeet plantlets produced *in vitro* was achieved on an auxin-free medium.

Table (1): Effect of different combinations of cytokinins and auxins on organogenesis and proliferation of sugarbeet.

M-S-medium supp emoted with growth regulators (mg/l)	Direct organogenesis (%)		Indirect organogenesis (%)	
	Leaf explants	Shoot base explants	Leaf Explants	Shoot base explants
0.5 BA + 0.1 NAA	26	86	13	6
0.5 BA + 0.5 NAA	33	93	20	6
0.5 BA + 0.1 2,4- D	0	40	60	33
0.5 BA + 0.5 2,4- D	0	46	66	40
0.5 kin + 0.1 NAA	20	33	33	20
0.5 kin + 0.5 NAA	26	40	40	26
0.5 kin + 0.1 2,4-D	6	26	73	46
0.5 kin + 0.5 2,4-D	13	20	80	53

Table(2): Effect of growth regulators and adenine sulfate on multiplication of regenerated propagules of sugarbeet.

Culture medium	Proliferated shoots Mean No. ± SE	Shoot length (cm) Mean ± SE	Leaves/shoot Mean No. ±SE
MS + 1 mg/l BA	4.30 ± 0.25	1.90 ± 0.09	2.50 ± 0.21
MS + 1 mg/l kin	3.90 ± 0.20	3.10 ± 0.20	2.00 ± 0.10
MS + 1 mg/l 2ip	2.50 ± 0. 15	2.40 ± 0.11	2.00 ± 0.27
MS+1mg/l BA+50 mg/l AS	5.00 ± 0. 33	2.10 ± 0.17	3.00 ± 0.12
MS+1mg/l Kin+50 mg/l AS	4.30 ± 0.22	4.00 ± 0.30	2.75 ± 0.19
MS +1 mg/l 2iP+50 mg/l AS	3.00 ± 0.30	3.00 ± 0.20	2.50 ± 0.15

AS = Adenine sulfate SE = Standard Error

Table(3): Effect of different types of auxins on root formation of in vitro grown shoots of sugarbeet.

Rooting media	Roots formation (%)	Number of roots Mean ± SE*	Root length(cm) Mean ± SE*
MS + 1 mg/l NAA	10	2.20 ±0.12	1.90 ±0.19
MS + 2 mg/l NAA	20	2.50 ±0.15	3.10 ±0.13
MS + 1 mg/l IBA	60	4.00 ±0.22	1.70 ±0.11
MS + 2 mg/l IBA	85	4.60 ±0.19	2.10 ±0.15
MS + 1 mg/l IAA	30	3.40 ±0.25	1.00 ±0.20
MS + 2 mg/l IAA	40	3.00 ±0.10	1.50 ±0.30

*SE = Standard Error

Table (4): The sequence of the selected random primers, total number of amplification products per primer, number of polymorphic bands and percentage of polymorphism.

Primer	Sequence ($5^{\backslash}\rightarrow3^{\backslash}$)	Total number of bands	No. of Polymorphic bands	Polymorphism (%)
K1	TGGCGACCTG	11	1	9.0
K2	GAGGCGTCGC	7	2	28.6
K3	CCCTACCGAC	12	2	16.7
K4	TCGTTCCGCC	4	1	25.0
K5	CACCTTTCCC	12	2	16.7

Acclimatization to free-living conditions

The success of *in vitro* methods in plant propagation depends, not only on the number of plantlets produced but also on their survival rate, upon transfer to nursery and field conditions. In our study, complete plantlets of sugarbeet with good root systems were easily adapted to the free environmental conditions. High percentage of survival was obtained after five weeks of transplanting. Three weeks after transfer, new leaves were produced. At the end of the fifth week, the plantlets grew into plants of normal appearance (Fig.6). As we observed, the most essential requirement for successful transplantation is to maintain the plants under a very high humidity specially in the first 15 days by covering them with transparent plastic bags. Small holes were pored in the bags for air circulation. Moreover, partial defoliation of plantlets at the time of transplantation is beneficial. The regenerated plants showed no morphological differences from those grown *in vivo*.

SDS-PAGE protein analysis

Total soluble proteins of three types of regenerants of sugarbeet in addition to their parents (growing *in vivo*) were extracted and subjected to protein electrophoresis to detect their variation. The protein profiles were analyzed by SDS-PAGE under reducing conditions. The electrophoretic protein banding patterns of different regenerated cultures, in addition to *in vivo* grown plants (control) of sugarbeet illustrated in Fig.(7) showed three bands (35,60 and 80 KD), which were clearly expressed in different regenerated tissue cultures. Similar electrophoretic migration rate was observed with the three types of tissue cultures. From the results of the patterns we may conclude that, plantlets derived from shoot tips, *via* direct and indirect organogenesis are identical and they are similar to those *in vivo* grown plantlets. The present results are in accordance with those reported on broccoli by El-Kazzaz and Taha, (2002) and garlic by Bekheet (2004). On the other hand, Metry *et al*. (2003), in their study on genetic stability of transgenic potato expressing *cry1Aa7* gene reported that protein banding profiles were not sufficient to detect variations among transgenic and non-transgenic lines.

RAPD analysis

DNA isolated from proliferated shoots, *via* direct and indirect organogenesis was subjected to RAPD analysis. Five random primers (K1-K5) were used for RAPD analysis. The results are presented in Fig. (8). The number of fragments generated per primer varied between 4 to 12 (Table 4). The total number of bands was 46 and the average percentage of polymorphism was 19.2. The primers K3 and K5 gave the highest number of amplified bands (12). The highest percentage of polymorphism (28.0) was observed with primer K2. However, primer K1 gave the most similar patterns of bands. In general, the results of banding patterns reveal that there is a slight genetic variation between the three types of *in vitro* regenerants of sugarbeet. These findings may suggest that the protein banding profiles were not sufficient to detect variation among the *in vitro* regenerated plants of sugarbeet. The present results are in accordance with those reported by Toldi *et al*. (1996).They mentioned that shoot regeneration through direct organogenesis is the most efficient way to produce true-type regenerants in sugarbeet. Moreover, Jacq *et al*. (1992) found that organogenesis in sugarbeet is less genotype dependent and regenerates are genetically stable. In this respect, genetic marker analysis has been used to study the degree of genetic change in plants regenerated

in vitro such as pea (Cecchini *et al*., 1992), sugarbeet (Sabir *et al*., 1992) and wheat (Brown *et al*., 1993).

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الملخص العربي

الأستيلاد المعملى لنموات بنجر السكر والتحليل الجزيئى للمستولدات

شوقى عبد الحميد بخيت- حسين سيد طه- محمد أحمد مطر
قسم التكنولوجيا الحيوية النباتية- المركز القومى للبحوث- القاهرة- مصر.

تم تطوير بروتوكول تطبيقى لاستيلاد نموات نبات بنجر السكر في الانبوب (in vitro). أخذت مقاطع من الأوراق وقواعد السيقان من البادرات المستنبتة فى الأنابيب وزرعت على بيئات تحتوى على توافيق من السيتوكينينات (بنزيل أدينين وكينيتين) مع الأكسينات (نفثالين حمض الخليك و داى كلوروفينوكسى حمض الخليك). لوحظت أعلى نسبة للتكشف المباشر للأعضاء من اجزاء السيقان بينما أجزاء الأوراق كانت أكثر استعدادا للتكشف غير المباشر. فيما يختص بمنظمات النمو المضافة الى بيئة الزراعة، كانت السيتوكينينات عند اضافتها فى توافيق مع نفثالين حمض الخليك أكثر تاثيرا على التكشف المباشر حيث سجلت أفضل نتائج لانبثاق النموات الساقية من أجزاء قواعد السيقان (٩٣ ٪) مع بيئة احتوت على ٠,٥ ملجم/لتر بنزيل أدينين + ٠,٥ ملجم/ لتر نفثالين حمض الخليك. بينما أعلى معدل للاستيلاد غير المباشر (٨٠ ٪) قد تم الحصول عليها عندما زرعت مقاطع الأوراق على بيئة محتوية على ٠,٥ ملجم/لتر كينيتين + ٠,٥ ملجم/ لتر ٤،٢- داى كلورو فينوكسى حمض الخليك. أعطت البيئة المحتوية على ١ ملجم/ لتر بنزيل أدينين + ٥٠ ملجم/لتر كبريتات الأدينين أعلى عدد للنموات المفرخة. بينما لوحظت أفضل نتائج لارتفاع السيقان عندما استخدم ١ ملجم/ لتر كينتين + ٥٠ ملجم/ لتر كبريتات الأدينين. من بين مختلف أنواع الأكسينات التى استخدمت للتجذير، كان أندول حمض البيوتريك المضاف بتركيز ٢ ملجم/ لتر الأكثر تأثيرا بالمقارنة باندول حمض الخليك ونفثالين حمض الخليك. لم يعطى التفريد الكهربى للبروتين اختلافات بين زراعات البنجر المستولدة بطريقة مباشرة أو غير مباشرة. تم استخدام تحليل التكبيرالعشوائى لمقاطع الحمض النووى DNA لمقارنة الاختلافات بين مستولدات مزارع الأنسجة. نتائج تفاعل البلمرة المتسلسل أشارت الى اختلافات طفيفة بين المستولدات و أثبتت أن التكشف المباشر للأعضاء هو أكثر الطرق فاعلية لإنتاج مستولدات مطابقة للآباء. تم الحصول على أقلمة ناجحة للظروف البيئية الحرة عن طريق نقل النباتات فى أصص احتوت على خليط من البيت موس و الفيرموكيوليت (١:١) تحت ظروف رطوبة نسبية عالية.تم الحصول على نسبة عالية من النباتات الحية الباقية وذات مظهر طبيعي بعد خمسة أسابيع من النقل.