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DIFFERENTIAL AXILLARY-BUD PROLIFERATION RESPONSES OF TWO SWEET POTATO CULTIVARS TO BENZYLADENINE AND THIDIAZURON

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ABSTRACT :

Plant growth regulator supplements into the nutrient medium is one of the most influential factors affecting efficiency of in vitro propagation. While benzyladenine (BA) has been extensively studied, no information is available on sweet potato (*Ipomoea batatas*, L.) responses to thidiazuron (TDZ). Current study used explants prepared from proliferating shoots of established axenic cultures of two cvs (11 and 44). Explants were incubated on agar solidified (0.7% g) Murashige and Skoog (MS) medium containing 3% sucrose and supplemented with 5µM BA or 0.5 µM TDZ or used free of BA and TDZ (MS-0, control). Data suggesting that BA and TDZ were indispensable for in vitro propagation of sweet potato since no excisable shoot were produced on MS-0 medium. BA was more effective than TDZ in inducing multiple shoot buds but only one shoot developed to well excisable shoot from such multiple shoot-buds. Responses to BA and TDZ were cultivar-dependent for percentage of explants excisable shoot which was higher on BA-medium in cv 11 while on TDZ-medium for cv 44. The harvested shoots from BA-medium had 13 leaves and those produced on TDZ-medium developed 10 leaves. In vitro rooting was not necessary since non-rooted shoots were capable to root while acclimatized to ex-vitro conditions. Cultivar 11 showed 100% survival after ex-vitro acclimatization whether on BA or TDZ medium. All plantlets of cv 44 produced on TDZ medium survived the ex-vitro acclimatized while those from BA medium showed 87% survival rate. It is proposed that culture of 12 single nodal axillary-buds prepared during September from growing sweet potato cv. 11 in the production field would produce transplants to grow one feddan (25,000) next season (April) after 4 sequential cycles of in vitro propagation on BA-medium. For cv. 44, starting with 25 axillary buds cultured on TDZ-medium would be needed. The described protocol may be useful to establish micropropagation industry of sweet potato that to help in overcoming difficulties of conventional vegetative propagation for this crop species.

INTRODUCTION:

Sweet potato (*Ipomoea batatas*) belongs to Family Convolvulaceae and it is a vegetable crop grown mainly for its storage roots. Apical shoots are used as leaf crop in some tropical regions (Hassan, 1991) and foliage can be used for animal feeding. Some varieties of *Ipomoea*

batatas **are used for starch extraction. Sweet potato is botanically perennial herb. As a crop plant, however, sweet potato cultivation is renewed annually through conventional vegetative propagation by stem cuttings. This method suffers several problems (Hassan, 1991) including loss of substantial tuber yield and reduction of land use efficiency when holding portion of previous crop area unplanted for 4 to 6 months as a source of stem cuttings and high opportunity for disease transfer and special intensive care and high costs when using plants from unmarketable tubers as cutting donors.**

Researchers, therefore, have shown a great interest in micropropagation (Abdalla, *et al*., 1995; Aboul-Nasr, 1990; Dagnino *et al*., 1991; Espinosa-Reyes *et al*., 2002; Gonzalez-Paneque *et al*., 2003; Kong *et al*., 1998; Love and Rhodes; 1985) as method to overcome abovementioned problems. All previous studies of sweet potato micropropagation used benzyladenine (BA) with or without auxin supplements. In past two decades, however, there has been an increasing number of research in several plant species suggesting that thidiazuron (TDZ, a cytokinin-like compound) is more effective than BA at much lower concentrations (Andrade *et al*., 1999; Babaoglu and Yorgancilar, 2000; Cuenca *et al*., 2000; Fellman *et al*., 1987; Fiola *et al*., 1990; Henny and Fooshee, 1990; Li *et al*., 2000; Mohamed *et al*., 1992a; Sriskandarajah *et al*., 2001; Thomas and Philip, 2005). The present study was implemented, therefore, to investigate response of axillary shoot-bud explants for two sweet potato cvs to BA vs. TDZ towards establishing an efficient in vitro protocol to propagate sweet potato.

MATERIALS AND METHODS:

The present investigation for in vitro propagation was conducted during 2005 and 2006 in the tissue culture laboratory, Genetic Engineering and Molecular Biology Center, Assiut University, Assiut, Egypt. Two sweet Potato (*Ipomoea batatas*, L.) cultivars (cv. 11 and cv. 44) were used. These cultivars were obtained from Agriculture Genetic Engineering Research Institute (AGERI), Agriculture Research Center, Giza, Egypt. Cultivar 11 produces yellow–skin and white–flesh storage roots while those root tubers of cultivar 44 are red–skinned and with yellow–flesh.

1-Establishment of explant-donor axenic plantlets:

Apical cuttings (ca. 1 cm) were taken from the two sweet potato cultivars grown in the open production field at the Agricultural Experimental Farm (Elghoraib Farm), Assiut University, Assiut, during the summer season of 2005. The field grown plants received normal practices of cultivation, irrigation, fertilization and weed and pest control as recommended for sweet potato production. Cutting explants were left under running tap water for 30 min. These explants were surface sterilized by steering for 15 min in 1% sodium hypochlorite prepared using commercial bleach (Clorox) solution plus Tween-20 (3 drops/100 ml). Surface disinfested explants were then transferred through three rinses with sterile distilled water before being cultured in vitro. Three explants were vertically placed on 25 ml agar-solidified MS (Murashige and skoog, 1962) nutrient medium per each 150 ml glass jar. This medium composed of basal macro- and micro-elements, vitamins, 3% sucrose and 0.7% g agar. The pH of the medium was adjusted to 5.7±0.1 using 0.5M potassium hydroxide (KOH) or 0.5M hydrochloric acid (HCl) as needed. The medium was then autoclaved for 15 min at 120˚C under 1.2 Kg/cm². The cultures were incubated in a growth chamber at 25°C under 16 h/day illumination from cool white fluorescent tubes.

2-Establishment of cultures derived from donor axenic-plantlets:

Axillary shoot-bud (3 mm stem single-node microcuttings) explants were prepared from proliferating shoots of established contamination-free axenic cultures of the two sweet potato cultivars (cv 11 and cv 44). This was six to eight weeks after incubation of explant-donor axenic cultures. The nodal axillary-buds were then placed on the medium (one exlants/culture jar). Cultures were incubated in a growth chamber for 6-8 weeks under the same conditions as previously mentioned. Previous research (Kong *et al*., 2003; Love and Rhodes, 1985; Murata *et al*., 1989; Zhang *et al*., 1999) used Benzyladeniene (BA) in range of 0.3 mg/l to 2 mg/l, i.e., 1.3 µM to 8.8 µM with average of approximately 5 µM. Therefore, the MS medium in the present study was supplemented with 5µM BA. It is widely documented by other workers that thidiazuron (TDZ) is equal in effect to or more effective than BA at one tenth the optimum concentration of BA (Mohamed *et al*., 1992a; Tawfik and Mohamed, 2005). Therefore, a second MS medium containing 0.5 µM TDZ was additionally prepared. The medium without plant growth regulators was used as a control treatment. Twenty-five ml of the medium was poured into 150 ml glass jars. The experiment included 2 factors [two cultivars (cv. 11 and cv. 44) and three media (MS-0, MS plus 5µM BA and MS plus 0.5 µM TDZ)]. The 6 treatment combinations were arranged in randomized complete-blocks with 3 replicates. Each replicate contained 5 culture jars and each culture jars contained one explant.

The plantlets and shoots when taken out of culture jars for the 6 treatments, they were washed under running tap water and transferred to plastic pots containing peat moss and washed sand mixture (2:1, v/v). Every plastic pot was covered with a transparent plastic bag to maintain high relative humidity around the plants. The plastic bags were bored gradually starting from second day of transplanting in order to get rid of excess humidity as well as to expose the plantlets to normal atmosphere conditions. These plastic bags were removed completely after one week from transplanting. The plants were incubated indoor for 2 weeks at room temperature under indirect sunlight for the first 3 days. Then they were subjected to direct sun rays gradually starting from the fourth day of transplanting and were irrigated with tap water every 2 days.

Data were recorded for survived explants (%), explants forming single-shoots (%), explants forming multiple-shoots, explants forming excisable shoots (>1 cm), explants totally forming non-excisable shoots (<1 cm), average number of leaves per shoot, rooting (%) and ex-vitro survived plantlets. All data were analyzed according to the procedure of analysis of variance (ANOVA) corresponding to factorial experiment in randomized complete-block design (Gomez and Gomez, 1984). As devised by coefficient of variation (C.V.), original data were used in the ANOVA. Appropriate mean comparisons were aided by the significance of the different components of total variance partitioning. All mean comparisons were made using the "Least Significant Differences" (LSD) test at 0.05 level of the probability.

RESULTS:

All studied parameters were influenced by the effect of both cultivar and medium except percentage of survived explants and average number of leaves per harvested shoot which were controlled only by medium. Percentage of survived explants was higher when cultured on

medium supplemented with BA or TDZ (Fig. 1 A). Higher percentage of explants formed single shoots (Fig. 1 B) were obtained on plant growth regulator-free medium (MS-0) and on TDZ medium than on BA medium in both cvs. There were no differences between the 2 cvs except on BA medium where cv 44 gave higher percentage of explants forming single shoots than cv 11.

Almost no multiple shoots buds were developed on MS-0 and TDZ media (Fig. 1 C). Cultivar 11 showed higher percentage of explants forming multiple shoots than cv 44. No excisable shoots were observed on MS-0 medium (Fig. 1 D). Higher percentage of explants forming excisable shoots were produced on BA medium in cv 11 and the vice versa for cv 44. All explants cultured on MS-0 medium totally forming non-excisable shoots (Fig. 2 A). None of the cultured explants totally formed non-excisable shoots on BA medium for cv 11 and on TDZ medium for cv 44.

The harvested shoots had the greatest number of leaves when grown on BA medium (≈ 13 leaves) (Fig. 2 B). Those ones excised from cultures on TDZ medium developed ≈ to leaves per shoot. In vitro rooting was the greatest on BA medium for cv 11 and on MS-0 medium for cv 44 (Fig. 2C). On TDZ medium, cv 11 showed higher percentage of rooting than cv 44. All shoots survived ex-vitro acclimatization in cv 11 whether produced on BA or TDZ media (Fig. 2 D). Same was for cv 44 when shoots were harvested form TDZ medium while lower percentage was survived of those shoot produced on BA medium.

Correlation coefficients (r) among the different micropropagation parameters are shown in Table (1). Percentage of survived explants showed a significant positive correlation coefficient with percentage of explants forming excisable shoots and average total number of leaves per plantlet but negatively associated with percentage of explants totally forming non-excisable shoots,. Percentage of explants forming single-shoot was negatively correlated with percentage of explants forming multiple-shoot. Percentage of explants forming excisable shoots had a negative r with percentage of explants forming totally non-excisable shoots while it was positively correlated with number of fully expanded leaves per plantlet, and percentage of ex-vitro survived plantlets. Percentage of explants totally forming non-excisable shoots had a negative r values with number of leaves per plantlet and percentage of ex-vitro survived plantlets. Average total number of leaves per plantlet was positively and significantly correlated with percentage of ex-vitro survived plantlets.

Table (1): Correlation coefficients among different studied parameters of sweet potato micropropagation

Parameter[(a)]	1	2	3	4	5	6	7	8
1	-	-0.462	0.462	0.914**	-0.914**	0.921**	-0.251	0.921**
2	-	-	-1.000**	-0.542	0.542	-0.673	-0.464	-0.483
3	-	-	-	0.542	-0.542	0.673	0.464	0.483
4	-	-	-	-	-1.000**	0.968**	-0.086	0.987**
5	-	-	-	-	-	-0.968**	0.086	-0.987**
6	-	-	-	-	-	-	-0.039	0.961**
7	-	-	-	-	-	-	-	-0.076
8	-	-	-	-	-	-	-	-

[(a)] (1) Percentage of survived explants, (2)Percentage of explants forming single-shoot, (3) Percentage of explants forming multiple-shoots, (4) Percentage of explants forming excisable shoots (> 1 cm), (5) Percentage of explants

totally forming non-excisable shoots(<1 cm), (6) Average total number of leaves per in vitro plantlet, (7) Percentage of explants forming roots and (8) Percentage of ex-vitro survived plantlets.

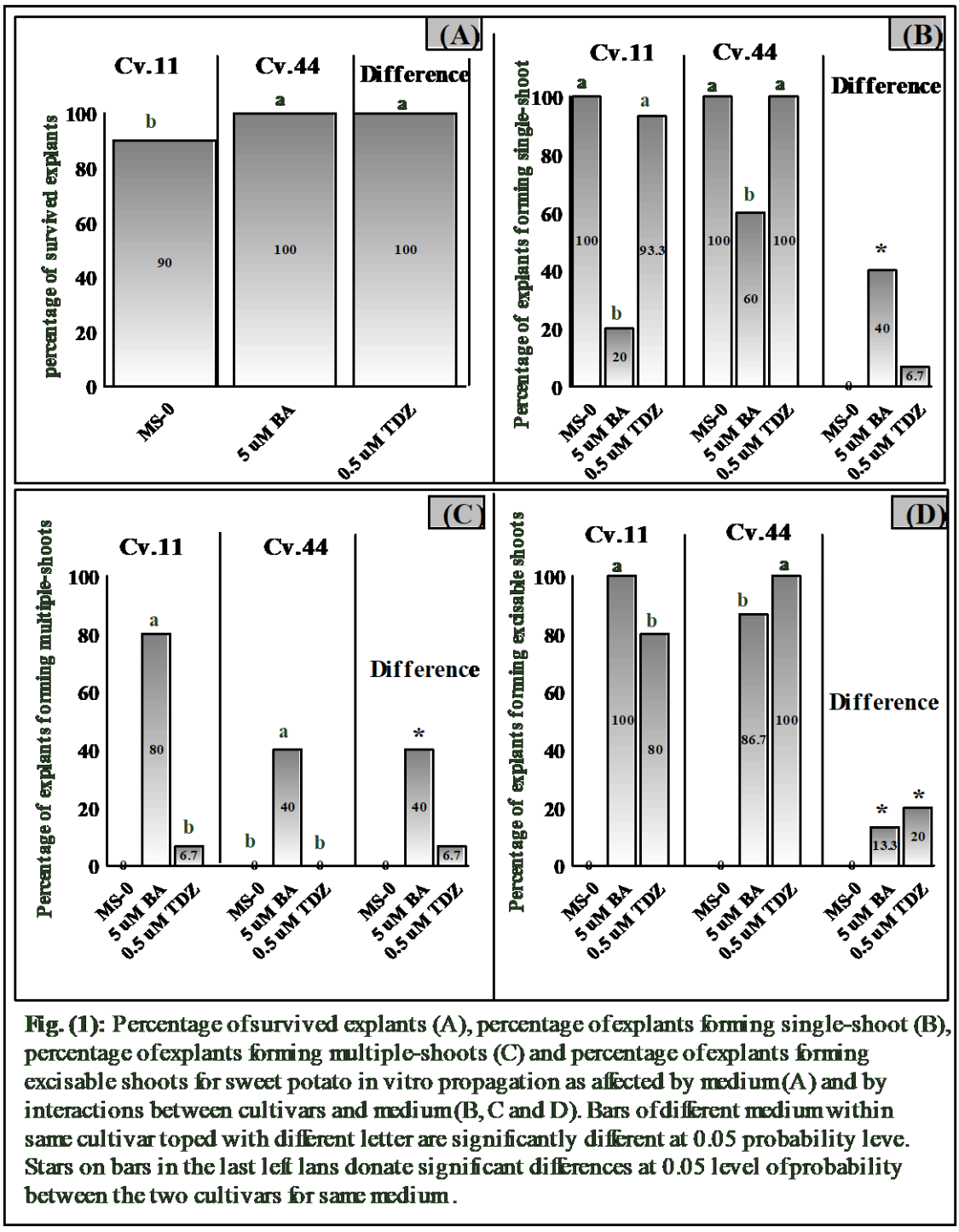

Fig. (1): Percentage ofsurvived explants (A), percentage ofexplants forming single-shoot (B), percentage ofexplants forming multiple-shoots (C) and percentage ofexplants forming excisable shoots for sweet potato in vitro propagation as affected by medium(A) and by interactions between cultivars and medium(B, C and D). Bars ofdifferent medium within same cultivar toped with different letter are significantly different at 0.05 probability leve. Stars on bars in the last left lans donate significant differences at 0.05 level ofprobability between the two cultivars for same medium.

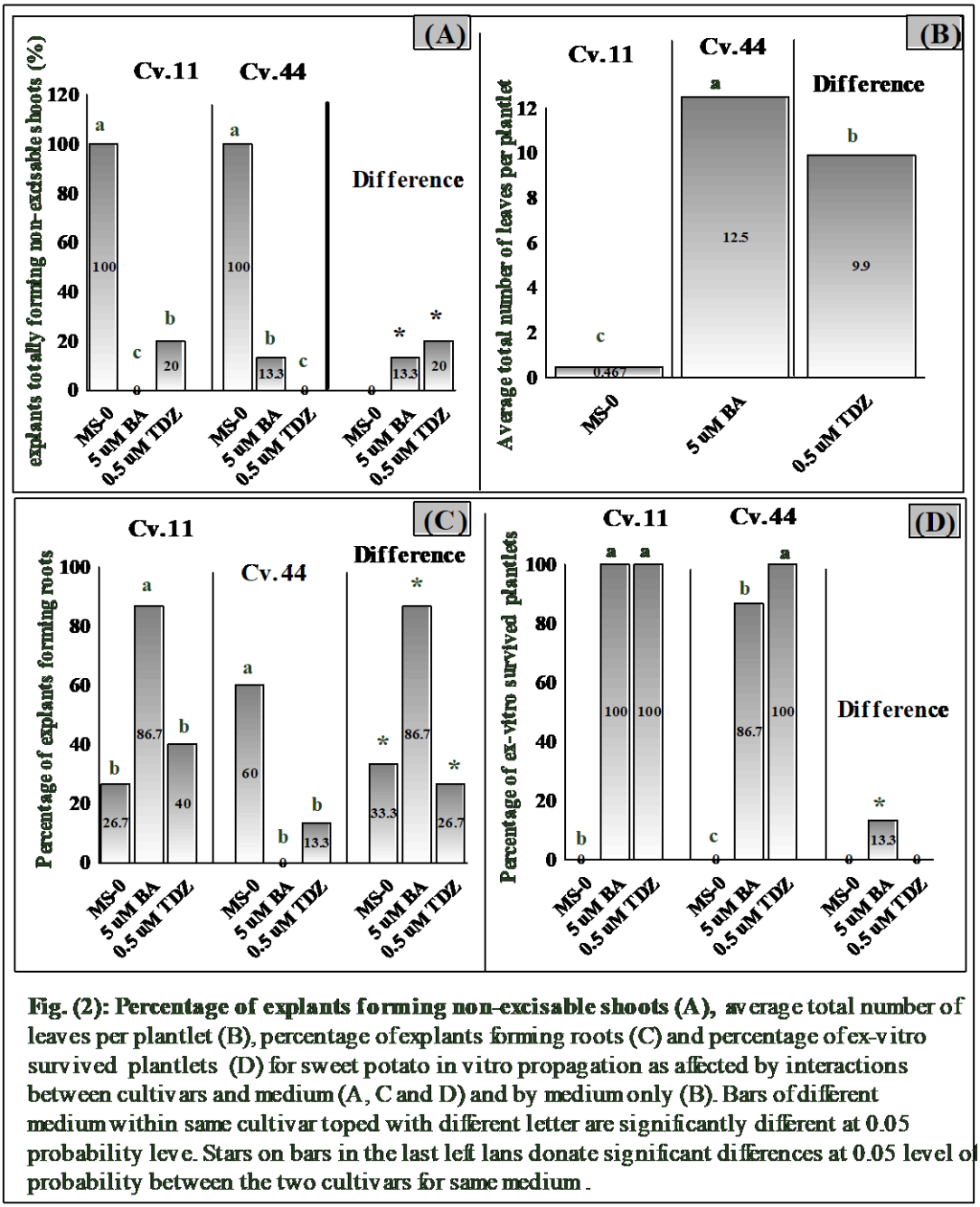

Fig. (2): Percentage of explants forming non-excisable shoots (A), average total number of leaves per plantlet (B), percentage ofexplants forming roots (C) and percentage ofex-vitro survived plantlets (D) for sweet potato in vitro propagation as affected by interactions between cultivars and medium (A, C and D) and by medium only (B). Bars of different medium within same cultivar toped with different letter are significantly different at 0.05 probability leve. Stars on bars in the last left lans donate significant differences at 0.05 level of probability between the two cultivars for same medium .

DISCUSSION:

Conditions for useful micropropagation include high rate of shoot multiplication and excisable shoots in short time, minimal concentrations use of plant growth regulators and high rate of rooting and ex-vitro plantlet survival (Hartmann *et al.*, 2002; Mohamed *et al.*, 1991). The present results demonstrated that medium without plant growth regulators (MS-0), in contrast to medium with PGRs added, significantly reduced percentage of survived axillary-bud explants. All survived explants developed single non excisable shoots (< 1 cm in length). No multiple shoots were produced on this medium indicating that benzyladenine (BA) and thidiazuron (TDZ) is indispensable for in vitro shoot multiplication (Mohamed *et al.*, 1991 and 1992a). Cytokinin (BA) and cytokinin-like compound (TDZ) break apical dominance (Mohamed *et al.*, 1991 and 1992; Tawfik and Mohamed, 2006). Thus several shoot can proliferate from a preexisting initial shoot meristem/bud of the explants. Beside their effect on dominance break, cytokinin (BA) as well as cytokinin-like compound (TDZ) affects cell division. It is reasonable as shown here that existence of BA or TDZ would enhance both the survival of the explants and the growth of their proliferated shoots.

Most researchers who investigated effects of BA vs. TDZ have shown that TDZ is more effective at much lower concentrations in inducing shoot multiplication than BA in many plant species (Andrade *et al.*, 1999; Babaoglu and Yorgancilor, 2000; Cuenca *et al.*, 2000; Fellman *et al.*, 1987; Fiola *et al.*, 1990; Henny and Fooshee, 1990; Li *et al.*, 2000; Mohamed *et al.*, 1992a; Sriskandarajah *et al.*, 2001; Thomas and Philip, 2005). This study used TDZ at 1/10 the concentration of BA as recommended by the previous studies (Mohamed *et al.*, 1992a). However, sweet potato produced no shoot multiplication on medium with TDZ. There were no differences in the percentage of single shoots formed whether the medium lacked (MS-0) or contained TDZ. Interestingly, almost all single-shoots formed on medium lacking TDZ were excisable while those initiated on MS-0 failed to develop into excisable size. This supports the suggestion mentioned elsewhere above concerning the possible role of cytokinin (BA) and cytokinin-like compound (TDZ) in affecting cell division and consequent enhancement of the shoot growth and development.

Growth of one shoot was observed to dominate over the others in the multiple shoots formed on BA-medium and develop into excisable size at the end of the primary culture passage. The harvested plantlets had, on average, 13 leaves. Since TDZ-medium produced plantlets of comparable size, it is suggested that most likely a higher concentration of TDZ would not be beneficial in increasing yield of harvested shoots. As far as micropropagation is concerned, harvested shoots simply can be used to prepare 8 to 10 nodal cultures of axillary buds for next propagation cycle. Interestingly, harvested shoots were capable to rooting while grown for ex-vitro acclimatization. The rate of ex-vitro survival and shoots grown into well developed plants was 100% for both cultivars. It is suggested that culture of 12 single nodal axillary-buds prepared during September from growing sweet potato cv. 11 in the production field would expected to produce transplants to grow one feddan (25,000) next season (April) after 4 sequential cycles of in vitro propagation on BA-medium. For cv. 44, starting with 25 axillary buds cultured on TDZ-medium would be needed. Correlation analysis proposed that percent of in vitro survived explants may be

useful to aid the production (r = 0.921) of ex-vitro acclimatized transplants.**

In conclusion, the described protocol is a step towards establishing national micro-propagation industry of sweet potato. This method would help to overcome difficulties facing the conventional vegetative propagation of this crop species.

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تباين نمو البراعم الجانبية لصنفين بطاطا استجابة للبنزيل ادنين والثيديزرون

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أجريت هذه الدراسة للإكثار الدقيق للبطاطا خلال عامين ٢٠٠٥ و٢٠٠٦ بمعمل زراعة الأنسجة بمركز الهندسة الوراثية والبيولوجيا الجزيئية بجامعه أسيوط. وقد استخدم صنفان من البطاطا (صنف ١١)، وهو صنف لون الجلد الخارجى له أصفر واللحم أبيض، صنف ٤٤ وهو صنف لون الجلد الخارجى له أحمر واللحم أصفر من مركز بحوث الهندسة الوراثية الزراعية التابع لمركز البحوث الزراعية, الجيزة, مصر. استخدمت تلك الدراسة أجزاء نباتية معقمة منفصلة من السيقان (براعم جانبية) زرعت تلك الأجزاء على بيئة موراشيجى وسكوج بها ٠.٧% أجار. وقد كان تركيز السكر بها ٣%، أما بالنسبة لمنظم النمو البنزيل أدينين فقد أضيف بتركيز ٥ ميكرو مول وأضيف الثيديزرون بتركيز ٠.٥ ميكرومول، كما استخدمت بيئة خالية من أى منظمات نمو (كنترول)، وقد أوضحت البيانات أن البنزيل أدينين أو الثيديزرون هام جداً وضرورى للإكثار الدقيق فى البطاطا حيث أن البيئة الخالية من منظمات النمو لم تنتج أى سيقان قابله للحصاد، وقد كان البنزيل أدينين أفضل من الثيديزرون فى تضاعف السيقان، ولكن فى كلا الحالتين كانت هناك ساق واحدة فقط قابلة للحصاد والباقى أقل من ١ سم، وقد كانت النسبة المئوية لاستجابة تلك البراعم الجانبية لتكوين سيقان قابلة للحصاد تتوقف على صنف البطاطا فقد كانت بيئة البنزيل ادينين أفضل عند استخدامها للصنف ١١ وبيئة الثيديزرون كانت أفضل عند استخدامها للصنف ٤٤، وقد كانت السيقان القابلة للحصاد للصنف ١١ على بيئة البنزيل أدينين تحتوى على متوسط عام ١٣ ورقة أما بالنسبة للصنف ٤٤ المنزرع على بيئة الثيديزرون فقد كان على الساق متوسط ١٠ أوراق، وبالنسبة لنقل تلك النبيتات على بيئة تجذير لم يكن ضرورى حيث أن النبيتات كانت لها القدرة على التجذير تحت ظروف الأقلمة، وقد كانت النسبة المئوية لنجاح الأقلمة للصنف ١١ (١٠٠%) سواء كانت على بيئة البنزيل ادينين أو بيئة الثيديزرون، أما الصنف ٤٤ فقد كانت النسبة المئوية لنجاح الأقلمة (١٠٠%) عند استخدام بيئة الثيديزرون و (٨٧ %) عند استخدام بيئة البنزيل ادينين. بالتالى فإنه من المقترح أن نبدأ بعدد ١٢ برعماً جانبياً من الصنف ١١ من حقل البطاطا السابق فى شهر سبتمبر لكى نحصل على ما يقرب من ٢٥.٠٠٠ شتلة (كافيه لزراعة فدان) فى شهر أبريل التالى، وذلك من خلال أربعة دورات متتالية من الأكثار الدقيق على بيئة البنزيل أدينين، أما الصنف ٤٤ فإنه من المقترح أن نبدأ بعدد ٢٥ برعماً جانبياً لأربعة دورات متتالية من الأكثار الدقيق على بيئة الثيديزرون، وهذا البرتوكول المقترح قد يفيد فى إدخال محصول البطاطا ضمن صناعة إنتاج شتلات محاصيل الخضر فى مصر باستخدام زراعة الأنسجة، وبالتالى نتلافى الكثير من عيوب وصعوبات الأكثار الخضرى التقليدى.