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An Efficient and Rapid Plant Regeneration System for Sweet Potato [*Ipomoea batatas* (L.) Lam.]

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Summary

An efficient and rapid system was established for direct shoot regeneration from leaf explants of sweet potato [*Ipomoea batatas* (L.) Lam.]. Adventitious shoots were induced from leaf explants of cvs. 'Tainong (T) 57', 'T 64' and 'T 66' of sweet potato on modified MS medium [MS inorganic salts (Murashige and Skoog 1962), 0.6 mM myo-inositol, 5.0 μM thiamine-HCl, 10.0 μM nicotinic acid, 5.0 μM pyridoxine-HCl (Zheng et al. 1996)] supplemented with benzyladenine (BA), thidiazuron (TDZ), 2-isopentenyladenine (2ip) alone as well as TDZ in combination with indole-3-acetic acid (IAA). Type and concentrations of cytokinins influenced adventitious shoot induction. Among BA, TDZ and 2ip, TDZ was the best for adventitious shoot induction with an optimal concentration of 5.0 μM (average of 73.6, 72 and 50.3 shoots were induced from 30 leaf explants of cvs. 'Tainong 66', 'T 64' and 'T 57', respectively). Adventitious shoot induction was not improved with the addition of IAA; however, it promoted elongation of shoots. *In vitro* grown shoots were rooted on MS medium alone and also on MS supplemented with IBA. The well developed plantlets were successfully acclimatized in growth chamber under controlled environmental condition. Hardened plants were transplanted to pots and maintained in green house. The survival rate of the plantlets was 86–95 %. This protocol of direct induction of adventitious shoots could be useful for the genetic transformation of sweet potato.

Key words. sweet potato – adventitious shoots – thidiazuron – regeneration

Introduction

Sweet potato [*Ipomoea batatas* (L.) Lam.] belongs to family *Convolvulaceae* and the sixth most important crop in the developing countries after wheat, rice, corn, potato and barley. The world production of sweet potato has been estimated at 115 million metric tons annually, and covered an area of 10 million hectares (Morán et al. 1998). In Taiwan, sweet potato is grown on 9.000 ha with a total production of 200.000 t year^{-1}. Increasing demand for this crop will necessitate improving the agronomic characteristics such as long keeping quality of tubers, increased biomass, resistance to diseases and pests, and also to saline and drought tolerance. Genetic improvement in sweet potato through conventional breeding has been slow due to male sterility, incompatibility and hexaploid nature (Dhir et al. 1998). This has led to more emphasis on the use of biotechnological methods for genetic improvement of particular traits in sweet potato. The development of a reliable plant regeneration protocol for this crop plant will form the basis for its genetic improvement program (Zheng et al. 1996). In sweet potato, plant regeneration via organogenesis and embryogenesis has been reported using different explants (Chée et al. 1990; Bieniek et al. 1995; Dessai et al. 1995; Gosukonda et al. 1995; Zheng et al. 1996; Sihachakr et al. 1997) and protoplasts (Ozias-Akins and Perera 1992; Belarmino et al. 1994; Dhir et al. 1998). However, none of the above reports provides promising response in *I. batatas* cvs. 'Tainong 57', 'T 64' and 'T 66', the three commercially important cultivars in Taiwan. The limited success in establishing highly morphogenic cultures has mostly been restricted to a few cultivars of sweet potato (Chée et al. 1990; Dessai et al. 1995; Zheng et al. 1996; Sihachakr et al. 1997). Recently, genetic transformation and subsequent plant regeneration has been reported in sweet potato (Otani et al. 1993; Newell et al. 1995; Gama et al. 1996; Morán et al. 1998; Okada et al. 2001; Berberich et al. 2005). However, the frequency of transformants was very low. Furthermore, there is a strong genotype dependency involved in the regeneration of transformants in sweet potato. Therefore, a highly efficient regeneration system is needed for the genetic transformation of current sweet potato cultivars.

In this study, we established an efficient plant regeneration protocol using the leaf explants of sweet potato. In addition, the effect of cytokinins at various levels and also combinations of TDZ and IAA on adventitious shoot induction from the leaf explants was investigated.

Materials and Methods

Plant material

In present investigation, we used three local cultivars viz., 'Tainong 57','T 64' and 'T 66' of sweet potato. We obtained *in vitro* cultures of these three cultivars from the Taiwan Agricultural Research Institute, Taichung, Tai-

wan. To get sufficient number of leaf explants, we subcultured nodal explants from these cultures onto modified MS basal medium consisting of MS inorganic salts (MURASHIGE and SKOOG 1962), 0.6 mM myo-inositol, 5.0 μM thiamine-HCl, 10.0 μM nicotinic acid, 5.0 μM pyridoxine-HCl and 0.09 M sucrose (ZHENG et al. 1996). After 4–5 weeks, the leaf explants were excised from axenic cultures in aseptic condition and cultured onto shoot induction medium according to DESSAI et al. (1995).

Shoot induction media

In experiment 1, the leaf explants were cultured on modified MS medium alone and also medium supplemented with cytokinins like BA, TDZ and 2ip (Sigma) individually at 0.5, 1.0, 2.0, 5.0, 10.0 or 20.0 μM. In experiment 2, the leaf explants were cultured on modified MS medium supplemented with combination of TDZ and IAA (1.0 or 2.0 μM) (Sigma). The pH of all media was adjusted to 5.8 using 0.1 N NaOH or HCl and media were solidified with 0.8 % agar (Duchefa Biochemic, The Netherlands). The medium was sterilized by autoclaving for 20 min at 121 °C, 1.01 x 10^{-5} Pa, and dispensed aseptically into sterile bottles (10 x 8 cm) or disposable Petri plates (2 x 9 cm). Thermolabile compounds, such as vitamins, amino acids, TDZ, and IAA were filter sterilized through 0.22 μm membranes (Millipore) and added to the autoclaved medium.

Root induction media

The elongated and well developed individual shoots of about 4–5 cm long and each with 5–6 leaves were excised from mother explants and cultured onto modified MS basal medium [medium consisting of MS inorganic salts, 0.6 mM myo-inositol, 5.0 μM thiamine-HCl, 10.0 μM nicotinic acid, 5.0 μM pyridoxine-HCl and 0.09 M sucrose (ZHENG et al. 1996)] alone or medium supplemented with IBA (1.0 or 2.0 μM).

Culture conditions

For shoot induction, the cultures were incubated at 24±2 °C under a 16 h photoperiod with a light intensity of 40–60 μMol m^{-2} s^{-1} provided by cool-white fluorescent lamps. For root induction, the cultures of *in vitro* grown shoots were maintained at 24±2 °C and16 h photoperiod (light intensity of 60 μMol m^{-2} s^{-1}).

Acclimatization of in vitro grown plants

Well developed in vitro plantlets were removed from culture vessels and washed in sterilized distilled water to remove the traces of media. These plantlets were transplanted to plastic pots (7 x 9 cm) containing a mixture of autoclaved peat and commercial compost (1:1), and were maintained in growth chamber at 24±2 °C, 80 % relative humidity under 16 h photoperiod with a light intensity of 80 μMol m^{-2} s^{-1}. After 4 weeks, the hardened plants were transferred to pots containing soil and commercial compost (1:1) and maintained in green house.

Histological studies

The responding explants were periodically collected at different intervals and were fixed in FAA (Formalin, glacial acetic acid, 70 % ethanol, 10:5:85) for 12 h at room temperature, dehydrated through a ethanol-butyl alcohol series and embedded in paraffin wax (FOWKE and RENNIE 1996). The tissues were sectioned at a thickness of 7 μm and stained with Safranin and Anilin blue, and examined under compound microscope (Nikon, Japan).

Data collection and analysis

All the experiments were arranged in a complete randomised design with 6 replicates in each experiment and each replicate consisting of 5 leaf explants. Each experiment was repeated three times. The cultures were observed periodically and morphological changes were recorded at weekly intervals. The number of responding explants per treatment and shoots per 30 explants were counted and the results were expressed as percentage of responding explants and number of shoots per 30 cultured explants per treatment. The data of all the experiments were subjected to analysis of variance (ANOVA), and mean values were separated based on Duncan's multiple range test.

Results and Discussion

Effect of cytokinins on adventitious shoot bud induction

The effect of different cytokinins and their concentrations on adventitious shoot induction is summarized in Table 1. Adventitious shoots were induced from leaf explants on medium containing 2–10 μM of cytokinins. However, the explants did not show any response on medium devoid of cytokinins and also medium supplemented with 0.5, 1.0 and 20.0 μM cytokinins. The base of the leaf had become swollen after one week of inoculation and subsequently, adventitious shoot buds were induced from swollen base of the leaf explants in another 3 weeks (Fig. 1A). *In vitro* adventitious shoot induction in plants occurs either directly or indirectly with an intermediate callus stage (FLICK et al. 1983). In this study, induction of adventitious shoots occurred directly from the base of the leaf explants without an intermediate callus stage. However, callus was also induced from explants along with shoots on medium containing higher concentrations (5.0 and10 μM) of cytokinins. Adventitious shoots were elongating on induction medium after 5–6 weeks of culture (Fig. 1B). In cultivars 'Tainong 64' and 'T 66', the shoot buds were brown in colour during induction and they became green in another 2 weeks. Adventitious shoot bud induction occurred mostly on the cut edges or in association with vascular tissue, and histology of responsive explants showed the direct induction of shoot buds from the explants (Fig. 1C).

In this study, the type of response was dependent on type and concentration of cytokinins in induction medium, and ANOVA revealed significant difference among type and concentration of cytokinins on adventitious shoot induction (Table 1). Medium supplemented with TDZ induced highest percentage of responding explants and maximum number of adventitious shoots followed by BA and 2ip (Table 1). Among different concentrations of BA studied, the medium containing 5.0 μM exhibited highest percentage of responding explants, and the percentage of responding explants was affected by the in-

Table 1. Effect of cytokinins on adventitious shoot induction from leaf explants of sweet potato cvs. 'Tainong 57', 'T 64' and 'T66'.

Cytokinin	Concentration (μM)	Organogenesis[a,b]					
		'Tainong 57'		'Tainong 64'		'Tainong 66'	
		Responding explants (%)	No. of shoots	Responding explants (%)	No. of shoots	Responding explants (%)	No. of shoots
BA	2.0	15.5 de	7.0 e	21.1 c	8.6 e	15.5 e	7.3 ef
	5.0	22.2 c	11.0 d	23.3 c	13.3 d	22.2 cd	9.0 e
	10.0	17.7 cd	14.3 c	18.8 cd	15.0 d	17.7 de	13.3 d
TDZ	2.0	26.6 b	10.6 d	28.8 b	20.3 c	26.6 c	21.0 c
	5.0	58.8 a	50.3 a	62.2 a	72.0 a	61.1 a	73.6 a
	10.0	24.4 b	21.0 b	27.7 b	40.3 b	34.4 b	58.0 b
2ip	2.0	0.0 f	0.0 g	15.5 d	5.6 f	4.4 f	1.6 g
	5.0	11.1 e	3.3 f	22.2 c	7.6 ef	12.2 e	5.3 f
	10.0	13.3 de	5.6 e	18.8 cd	7.3 ef	15.5 e	7.0 ef

[a]30 leaf explants were cultured per treatment.
[b]In each column, mean values followed by same letters are not significantly different according to DMRT at P=0.05
Note: Explants cultured on Modified MS alone and also modified MS supplemented with 0.5 or 1.0 μM cytokinins did not show any response, however, explants induced only callus on modified medium containing 20 μM cytokinins (data not shown).

crease in BA concentration to 10 μM (Table 1). However, the number of adventitious shoots per explant increased as the concentration of BA increased from 5 to 10 μM (Table 1), and a number of adventitious shoots induced from 30 explants of cvs. 'Tainong 57', 'T 64' and 'T 66' were 14.3, 15 and 13.3, respectively. Earlier workers incorporated BA either in induction or differentiation medium for callus mediated organogenesis or somatic embryogenesis in sweet potato (Belarmino et al. 1994; Desamero et al. 1994; Gama et al. 1996; Zheng et al. 1996; Dhir et al. 1998; Morán et al. 1998). On the contrary, in this study, the explants induced adventitious shoots directly on medium containing BA.

Thidiazuron is a potent growth regulator with both cytokinin and auxin like activity (Mok et al. 1982; Visser et al. 1992). Thidiazuron induced adventitious shoots from leaf explants of pear (Leblay et al. 1991), ohelo and bilberry (Shibli and Smith 1996), American cranberry (Marcotrigiano et al. 1996), and lingonberry (Debnath 2005). In this study, the highest percentage of responding explants for 'Tainong 57', 'T 64' and 'T66', 58.8, 62.2 and 61.1, respectively, was recorded on medium containing 5.0 μM TDZ. The percentage of responding explants was decreased as the concentration of TDZ increased from 5.0 to 10.0 μM (Table 1). Similarly, different frequencies of regeneration were correlated with the concentrations of TDZ in other species, such as 'McIntosh' apple (Sarwar and Skirvin 1997), cowpea (Le et al. 2002), and lingonberry (Debnath 2005). The induction medium containing 2.0 μM TDZ stimulated the formation of fewer adventitious shoots and the number of shoots increased significantly as the concentration of TDZ increased from 2.0 to 5.0 μM (Table 1). Whereas, supplementation of higher concentration (10.0 μM) of TDZ in induction medium was not beneficial for shoot induction (Table 1). The adventitious shoot induction capacity differed among genotypes; the maximum number of shoots for 'Tainong 64' and 'T 66', was 72 and 73.6, respectively, whereas 'Tainong 57' had a lower mean number of 50.3 shoots per 30 explants on medium containing 5.0 μM TDZ. Such genotypic difference for shoot regeneration has been reported for sweet potato (Dessai et al. 1995; Gosukonda et al. 1995). Gosukonda et al. (1995) reported that in sweet potato, the mean number shoots induced from leaf explants was one in two stage method, where the explants were incubated on medium containing 2,4-D for 3 days, and subsequently transferred to medium supplemented with TDZ. In this study, a maximum of 73.6 shoots per 30 explants was harvested and this is far superior compared to earlier reports on direct induction of adventitious shoots.

The effect of 2ip on shoot induction is varied with the concentration and genotype (Table 1). Among different

Table 2. Effect of TDZ alone or in combination with IAA on shoot length.

TDZ (μM)	IAA (μM)	Shoot length (cm)[a]		
		'Tainong 57'	'Tainong 64'	'Tainong 66'
5.0	–	1.5 c	1.4 c	1.7 c
10.0	–	1.1 d	1.1 d	1.2 d
5.0	1.0	2.2 a	2.3 a	2.5 a
10.0	1.0	1.6 bc	1.5 c	1.6 c
5.0	2.0	1.9 b	2.0 b	2.1 b
10.0	2.0	1.6 bc	1.5 c	1.7 c

[a]In each column, mean values followed by same letters are not significantly different according to DMRT at P=0.05.

concentrations of 2ip, adventitious shoot buds were induced on medium supplemented with 2.0–10.0 μM in case of 'Tainong 64' and 'T66', and 5.0 or 10.0 μM for 'Tainong 57'. The frequency of responding explants and number of shoot buds was increased as the concentration of 2ip increased from 5.0 to 10.0 μM, 2.0 to 10.0 μM in cvs. 'Tainong 57' and 'T66', respectively (Table 1). However, the percentage of responding explants of 'Tainong 64' was decreased as the concentration of 2ip increased from 5.0 to 10.0 μM, and there was no significant difference in shoot bud induction among different concentrations of 2ip (Table 1).

Effect of TDZ in combination with IAA

The efficiency of regeneration was improved with the addition of IAA to the induction medium containing TDZ in *Zizyphus jujuba* (Gu and Zhang 2005). In this study, the efficiency of adventitious shoot induction neither improved nor decreased with the addition of IAA (1.0 or 2.0 μM) to the induction medium containing 5.0 or 10.0 μM TDZ (data not shown). However, the shoots were elongated with the addition of IAA to the induction medium supplemented with TDZ, and medium containing 5.0 μM TDZ and 1.0 μM IAA found to be more effective for shoot elongation (Table 2).

Rooting and acclimatization

The roots were induced from cultured shoots on basal medium alone and also medium supplemented with 1.0 or 2.0 μM IBA after few days of culture. There was no significant difference in mean number of roots among different rooting media (data not shown). After two weeks, the plantlets with 5–6 fully expanded leaves and well developed root system (Fig. 1D) were removed from the culture bottles, and roots were washed with sterilized distilled water to avoid infection. Finally, the plantlets were transplanted into plastic pots containing commercial compost and Peat (1:1). *In vitro* grown plantlets were hardened in a growth chamber under controlled environmental conditions (24±2 °C temp; 80 % relative humidity; 80 μMol m^{-2} s^{-1} light intensity for 16 h photoperiod), which was necessary for the survival of the plantlets. In

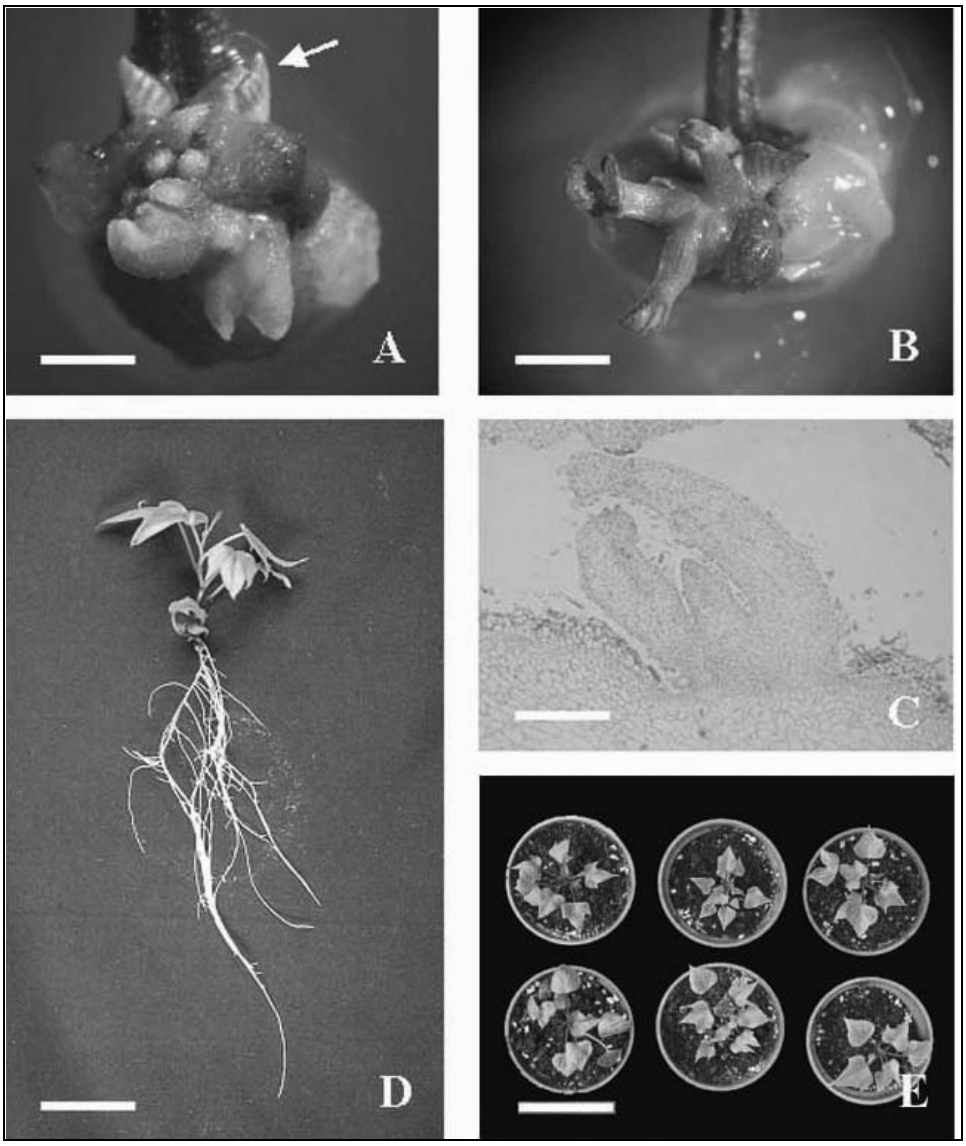


Fig. 1. Plant regeneration from leaf explants of sweet potato via organogenesis. (A) Induction of adventitious shoot buds from leaf explants (bar = 0.33 cm). (B) Elongation of adventitious shoots (bar = 0.5 cm). (C) Section of responding explant showing direct induction of adventitious shoot bud (bar = 0.1 cm). (D) *In vitro* rooted sweet potato plant (bar = 1.5 cm). (E) Acclimatized leaf-derived plants (bar = 5.0 cm).

each cultivar, 22 plantlets were transferred to green house, of which 19, 21 and 20 plantlets of 'Tainong 57', 'T64' and 'T66' respectively, survived. The acclimatized *in vitro* grown plants did not show any detectable morphological variation in compared with the donor plants (Fig. 1E).

In conclusion, we have developed an efficient and rapid plant regeneration system for sweet potato via organogenesis. Adventitious shoots were induced from leaf explants on medium containing cytokinins, and TDZ was the most effective cytokinin. In our protocol, the shoots induced directly from the explant, and this pathway appears to be effective for genetic transformation studies in sweet potato.

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References

BELARMINO, M.M., T. ABE and T. SASAHARA 1994: Plant regeneration from stem and petiole protoplasts of sweet potato (*Ipomoea batatas*) and its wild relative, *I. lacunosa*. Plant Cell Tiss. Org. Cult. **37**, 145–150.

BERBERICH, T., T. TAKAGI, A. MIYAZAKI, M. OTANI, T. SHIMADA and T KUSANO 2005: Production of mouse adiponectin, an antidiabetic protein, in transgenic sweet potato plants. J. Plant Physiol. **162**, 1169–1176.

BIENIEK, M.E., R.C. HARRELL and D.J. CANTLIFFE 1995: Enhancement of somatic embryogenesis of *Ipomoea batatas* in solid cultures and production of mature somatic embryos in liquid cultures for application to a bioreactor production system. Plant Cell Tiss. Org. Cult. **41**, 1–8.

CHÉE, R.P., J.R. SCHULTHEIS and D.J. CANTLIFFE 1990: Plant recovery from sweet potato somatic embryos. HortSci. **25**, 795–797.

DEBNATH, S.C. 2005: A two-step procedure for adventitious shoot regeneration from *in vitro*-derived lingonberry leaves: shoot induction with TDZ and shoot elongation using zeatin. HortSci. **40**, 189–192.

DESAMERO, N.V., B.B. RHODES, D.R. DECOTEAU and W.C. BRIDGES 1994: Picolinic acid-induced direct somatic embryogenesis in sweet potato. Plant Cell Tiss. Org. Cult. **37**, 103–111.

DESSAI, A.P., R.M. GOSUKONDA, E. BLAY, C.K. DUMENYO, F. MEDINA-BOLIVAR and C.S. PRAKASH 1995: Plant regeneration of sweet potato (*Ipomoea batatas* L.) from leaf explants *in vitro* using a two-stage protocol. Sci. Horti. **62**, 217–224.

DHIR, S.K., J. OGLESBY and A.S. BHAGSARI 1998: Plant regeneration via somatic embryogenesis, and transient gene expression in sweet potato protoplasts. Plant Cell Rep. **17**, 665–669.

FLICK, C.E., D.A. EVANS and W.R. SHARP 1983: Organogenesis. In: EVANS, D.A., W.R. SHARP, P.V. AMMIRATO and Y. YAMADA (eds): Handbook of plant cell culture, Vol 1. Techniques for propagation and breeding. Macmillan, New York, pp 13–81.

FOWKE, L.C. and P.J. RENNIE 1996: Botanical microtechnique for plant cultures. In: GAMBORG, O.L. and G.C. PHILLIPS (eds): Plant Cell, Tissue and Organ Culture. Fundamental methods. Narosa Publishing House, New Delhi, 217–228.

GAMA, M.I.C.S., R.P.Jr. LEITE, A.R. CORDEIRO and D.J. CANTLIFFE 1996: Transgenic sweet potato plants obtained by *Agrobacterium tumefaciens*-mediated transformation. Plant Cell Tiss. Org. Cult. **46**, 237–244.

GOSUKONDA, R.M., A.P. DESSAI, E. BLAY, C.S. PRAKASH and C.M. PETERSON 1995: Thidiazuron-induced adventitious shoot regeneration of sweet potato (*Ipomoea batatas*). In Vitro Cell Dev. Biol. **31**, 65–71.

GU, X.F. and J.R. ZHANG 2005: An efficient adventitious shoot regeneration system for Zhanhua winter jujube (*Zizyphus jujuba* Mill.) using leaf explants. Plant Cell Rep. **23**, 775–779.

LE, B.V., M.H. CRUZ DE CARVALHO, Y. ZUILY-FODIL, A.T.P. THI and K.T.T. VAN 2002: Direct whole plant regeneration of cowpea [*Vigna unguiculata* (L.) Walp] from cotyledonary node thin cell layer explants. J. Plant Physiol. **159**, 1255–1258.

LEBLAY, C., R. CHEVREAU and L.M. RABOIN 1991: Adventitious shoot regeneration from in vitro leaves of several pear cultivars (*Pyrus communis* L.). Plant Cell Tiss. Org. Cult. **25**, 99–105.

MARCOTRIGIANO, M., S.P. MCGLEW, G. HACKETT and B. CHAWLA 1996: Shoot regeneration from tissue-cultured leaves of the American cranberry (*Vaccinium macrocarpon*). Plant Cell Tiss. Org. Cult. **44**, 195–199.

MOK, M.C., D.W.S. MOK, D.J. ARMSTRONG, K. SHUDO, Y. ISOGAI and T. OKAMOTO 1982: Cytokinin activity of *N*-phenyl-*N*-1,2,3-thiadizol-5-ylurea (thidiazuron). Phytochemistry **21**, 1509–1511.

MORÁN, R., R. GARCÍA, A. LÓPEZ, Z. ZALDÚA, J. MENA, M. GARCÍA, R. ARMAS, D. SOMONTE, J. RODRÍGUEZ, M. GÓMEZ and E PIMENTEL 1998: Transgenic sweet potato plants carrying the delta-endotoxin gene from *Bacillus thuringiensis* var. *tenebrionis*. Plant Sci. **139**, 175–184.

MURASHIGE, T. and F. SKOOG 1962: A revised medium for rapid growth and bioassays with tobacco tissues cultures. Physiol. Plant. **15**, 473–497.

NEWELL, C.A., J.M. LOWE, A. MERRYWEATHER, L.M. ROOKE and W.D.O. HAMILTON 1995: Transformation of sweet potato (*Ipomoea batatas* (L.) Lam.) with *Agrobacterium tumefaciens* and regeneration of plants expressing cowpea trypsin inhibitor and snowdrop lectin. Plant Sci. **107**, 215–227.

OKADA, Y., A. SAITO, M. NISHIGUCHI, T. KIMURA, M. MORI, K. HANADA, J. SAKAI, C. MIYAZAKI, Y. MATSUDA and T. MURATA 2001: Virus resistance in transgenic sweet potato [*Ipomoea batatas* L. (Lam)] expressing the coat protein gene of sweet potato feathery mottle virus. Theor. Appl. Genet. **103**, 743–751.

OTANI, M., M. MII, T. HANDA, T. KAMADA and T. SHIMADA 1993: Transformation of sweet potato (*Ipomoea batatas* (L.) Lam) plants by *Agrobacterium rhizogenes*. Plant Sci. **94**, 151–159.

OZIAS-AKINS, P. and S. PERERA 1992: Regeneration of sweet potato plants from protoplast-derived tissues. In: HILL, W.A. and P.A. BONSICK LORETAN (eds): Sweet potato- technology for the 21st century. Tuskegee University, Tuskegee, Ala, 61–66.

SARWAR, M. and R.M. SKIRVIN 1997: Effect of thidiazuron and 6-benzylaminopurine on adventitious shoot regeneration from leaves of three strains of 'McIntosh' apple (*Malus* x *domestica* Borkh.) *in vitro*. Sci. Horti. **68**, 95–100.

SHIBLI, R. and M.A.I. SMITH 1996: Direct shoot regeneration from *Vaccinium pahalae* (ohelo) and *V. myrtillus* (bilberry) leaf explants. HortSci. **31**, 1225–1228.

SIHACHAKR, D., R. HAÏCOUR, J.M.C. ALVES, I. D. UMBOH, Nzoghé, A. SERVAES and G. DUCREUX 1997: Plant regeneration in sweet potato (*Ipomoea batatas* L., *Convolvulaceae*) Euphytica **96**, 143–152.

VISSER, C., J.A. QURESHI, R. GILL and P.K. SAXENA 1992: Morphoregulatory role of thidiazuron: substitution of auxin and cytokinin requirement for the induction of somatic embryogenesis in geranium hypocotyls cultures. Plant Physiol. **99**, 1704–1707.

ZHENG, Q., A.P. DESSAI and C.S. PRAKASH 1996: Rapid and repetitive plant regeneration in sweet potato via somatic embryogenesis. Plant Cell Rep. **15**, 381–385.

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