South African Journal of Botany 2005, 71(1): 110–113
Printed in South Africa — All rights reserved

SOUTH AFRICAN JOURNAL OF BOTANY
EISSN 1727–9321

Short Communication

In vitro high frequency direct root and shoot regeneration in sweet potato using the ethylene inhibitor silver nitrate

Y Gong[1,3], F Gao[2]* and K Tang[3]

[1] *Faculty of Life Science and Biotechnology, Ningbo University, Ningbo 315211, China*
[2] *College of Life Science, South China Normal University, Guangzhou 510631, China*
[3] *Plant Biotechnology Research Center, Fudan-SJTU-Nottingham Plant Biotechnology R&D Center, School of Agriculture and Biology, Shanghai Jiaotong University, Shanghai 200030, China*
* *Corresponding author, e-mail: peak0041@vip.sina.com*

Received 16 April 2004, accepted in revised form 14 July 2004

The role of silver nitrate in the regulation of direct root and shoot regeneration from various explants of sweet potato (*Ipomoea batatas* L.) was studied. A rapid and high frequency direct shoot regeneration procedure was achieved from stem and lamina explants. The magnitude of the response to silver nitrate varied among different explants and silver nitrate was found to have a significant effect on shoot regeneration. The best shoot regeneration was achieved from stem explants cultured on basal MS medium supplemented with 1.0mg l^{-1} NAA without silver nitrate, with the regeneration frequency reaching 86.3%. With the addition of 8.0mg l^{-1} silver nitrate on MS medium, 73.3% of lamina explants could directly regenerate shoots. Our study suggests that the addition of silver nitrate can promote shoot regeneration from various explants. The established procedure enables the production of a large number of plantlets within a short time, and thus can be used for assisting conventional breeding programmes and gene transfer studies.

Abbreviations: ANOVA = analysis of variance, BA = benzyladenine, MS = Murashige and Skoog (1962), NAA = 1-naphthaleneacetic acetic acid

Sweet potato (*Ipomoea batatas* L.) is a major food crop and an important source of starch, especially in the developing world. The establishment of an *in vitro* plant regeneration system in sweet potato is of potential importance to sweet potato quality improvement. However, successful transformation cannot be achieved unless efficient plant regeneration has been established. Some plant regeneration procedures through organogenesis and somatic embryogenesis have been described for sweet potato using different explants such as meristem tips, lamina segments, stems, petioles, storage roots, anthers and ovaries (Gong *et al.* 1998). Despite many efforts, the underlying problem in these procedures is a low frequency of regeneration, long periods of culture and frequent media changes (Gosukonda *et al.* 1995). The overall frequency of shoot regeneration in sweet potato was 2–20% (Lowe *et al.* 1992).

Ethylene is produced in *in vitro* plant culture systems (Biddington 1992) and is known to inhibit plant growth and morphogenesis depending upon the species and culture stage (Kumar *et al.* 1998). Consequently, inhibitors of ethylene action such as silver nitrate can induce variable *in vitro* responses. In some species, silver nitrate improved callus proliferation (Fei *et al.* 2000), enhanced shoot regeneration (Khan *et al.* 2003), promoted root formation (Khalafalla and Hattori 2000) and increased somatic embryogenesis (Mandal *et al.* 2001). In other species, silver nitrate inhibited shoot regeneration (Bandyopadhyay *et al.* 1999) and somatic embryogenesis (Al-khayri and Al-Bahrany 2001). These results indicate that the promotive function of silver nitrate on shoot regeneration is species-specific. Until now, the effect on shoot regeneration by silver nitrate in sweet potato has not been studied.

In our laboratory, *in vitro* propagation of sweet potato has been developed through organogenesis with different combinations of auxins and cytokinins (Gong *et al.* 2001). In the present study, we describe direct *in vitro* shoot induction and regeneration from different explants of sweet potato by using the ethylene inhibitor silver nitrate.

The superior sweet potato genotype, Gaozi No.1, was obtained from Chongqing Sweet Potato Research Center, Chongqing, China.

Nodal segments of the above-mentioned superior sweet potato genotype, Gaozi No.1, was selected from field-grown plants and dipped in a solution of 2% (v/v) mild liquid detergent for 15min, then in 75% alcohol for 30sec, surface decontaminated in 0.1% (w/v) $HgCl_2$ solution with Tween-20 for 10min, and finally rinsed three times with sterile distilled water and used for culture. Nodal segments were subcultured on MS medium (Murashige and Skoog 1962) supplemented with 3% sucrose and 0.8% agar (Sigma # A 1296, Sigma Chemical Co., St Louis, Missouri), and all the media used in this study were adjusted to pH 5.8 and autoclaved at 121°C, 105KPa for 15min. All cultures were incubated in a growth chamber at 25 ± 2°C under a 16h/8h photoperiod with a light intensity (50µmol m^{-2} s^{-1}) provided by 'warm-white' tubular fluorescent lamps.

Various parts of three-week old shoots from nodal segments of Gaozi No.1 were used as explants. Stem (internode) and petiole sections, 3mm–5mm long, were cut and placed on root and shoot initiation medium. The stem and lamina explants used were devoid of a basal end to avoid having any kryptoblasts. Lamina of young leaves (about 25mm x 15mm) were cut into 3mm x 3mm sections before placing on the medium.

In an attempt to improve regeneration efficiency and development of regenerated buds and roots, an ethylene action inhibitor, silver nitrate, was tested. Explants were cultured on basal MS medium supplemented with 1.0mg l^{-1} 1-naphthaleneacetic acetic acid (NAA) and different concentrations (0, 2, 4, 6, 8, 10 and 12mg l^{-1}) of silver nitrate. Observations were made during incubation and the frequency and quantity of induced adventitious roots were recorded after 60 days of incubation. The frequency of regenerated shoots was recorded after 20, 40 and 60 days of incubation. At least eight explants were included in each Petri dish, which served as replications. All experiments were carried out with a minimum of three replicates per treatment.

The regenerated shoots (2–3cm long) could be easily rooted in all treatments or transferred onto fresh basal medium without growth regulators, and some regenerated plantlets were produced. Young *in vitro* regenerated plantlets were transferred to plastic containers containing a mixture of sterilised potting soil, vermiculite and sand (1:1:1), and cultured in a greenhouse with intermittent moisture to maintain 80% humidity, for two weeks.

Data were subjected to one-way analysis of variance (ANOVA). Mean separation was performed using the least significant difference (LSD) method at 5% significant level.

After 5 days of incubation, adventitious roots were directly derived from the cut surface of stem explants, with minimum time at the callus stage (Figure 1A). With various concentrations of silver nitrate, explants from stems, lamina and petioles could easily regenerate adventitious roots. Maximum rate of root induction (100%) and the number of

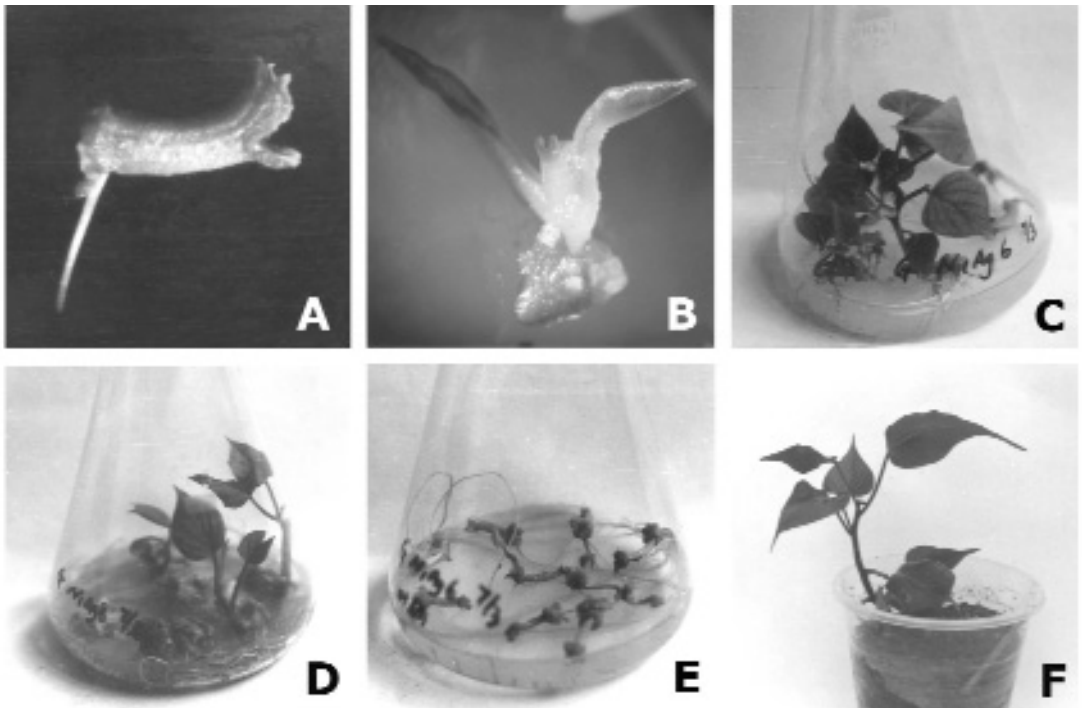

Figure 1: Effects of silver nitrate on direct shoot regeneration of sweet potato cv. Gaozi No.1. A: an adventitious root directly derived from the cut surface of stem explants; B: an adventitious bud directly derived from the cut surface of stem explants; C: some adventitious roots and buds were derived from the stem explants of sweet potato on MS medium + 1.0mg l^{-1} NAA + 6.0mg l^{-1} silver nitrate, and normal plantlets were regenerated; D: some adventitious roots and buds were derived from the leaf explants of sweet potato on a medium of MS + 1.0mg l^{-1} NAA + 8.0ml^{-1} silver nitrate, and normal plantlets were regenerated; E: some adventitious roots and an adventitious bud were derived from the petiole explants of sweet potato on a medium of MS + 1.0mg l^{-1} NAA + 6.0mg l^{-1} silver nitrate; F: the regenerated sweet potato plants were planted in soil

roots per explants were achieved with stem explants for all concentration of silver nitrate. As to lamina and petiole explants, the rate of root induction tended to decrease along with the increase of the silver nitrate concentration (from 2mg l^{-1} to 12mg l^{-1}) and the same trend was observed for the number of roots per explants in petiole explants. In contrast, lamina explants showed a higher number of roots per explant (6.4, 6.1 and 4.9) than that of the control (2.6), when concentrations of silver nitrate were at 8mg l^{-1}, 10mg l^{-1} and 12mg l^{-1} (Table 1).

Most adventitious shoots regenerated directly from the stem, lamina and petiole explants almost without an intervening callus stage. Shoot initiation started to form 15 days in stem and 30 days in lamina explants following initiation of cultures.

There was a significant effect of various explants and with different concentrations of silver nitrate on direct shoot regeneration (Table 1). A shoot regeneration response was obtained when stems were incubated on basal MS medium supplemented with 1.0mg l^{-1} NAA without silver nitrate (Figures 1B, 1C). An increase of silver nitrate concentration reduced the frequency of shoot regeneration of stem explants. Maximum shoot regeneration rate (86.3%) was obtained on MS medium supplemented with 1.0mg l^{-1} NAA devoid of silver nitrate (Table 1) and each explant produced only one shoot. The results in the present study suggest that silver nitrate inhibited direct shoot regeneration of stem explants of sweet potato. However, silver nitrate can obviously promote direct shoot regeneration of lamina explants and a high frequency of direct shoot regeneration (41.7% to 73.3%) was obtained when lamina explants were incubated on MS medium supplemented with 1.0mg l^{-1} NAA and different concentrations (2.0–12.0mg l^{-1}) of silver nitrate. Low shoot regeneration frequency (3.7%) was obtained without adding silver nitrate. Best direct shoot regeneration response (73.3%) of lamina explants was obtained with 8.0mg l^{-1} silver nitrate (Table 1, Figure 1D). Therefore, silver nitrate seems to act as a promoter of shoot regeneration in lamina explants above concentrations of 2–12mg l^{-1} silver nitrate.

It is very difficult to induce direct shoot regeneration from petiole explants. Only 3.1% of shoots' regeneration occurred on petiole explants with 6.0mg l^{-1} silver nitrate (Figure 1E), which was not significant. Our results showed that different explants had different responses to silver nitrate; silver nitrate was more suitable to lamina explants than stem and petiole explants. This suggests that the promotive function of silver nitrate on shoot regeneration has organ-specific sensitivity.

There was a significant effect of culture time on direct shoot regeneration on basal MS medium supplemented with 1.0mg l^{-1} NAA and different concentrations of silver nitrate (Table 1). With the culture time increasing, the frequency of shoot regeneration from various explants of sweet potato also increased. Of all the explants tested, stem explants produced high frequency shoot regeneration and were the first to show shoot initiation. Direct shoot regeneration from stems occurred after 20 days of incubation, after 40 days from leaf explants and 60 days from petiole explants.

The *in vitro* regeneration of adventitious shoots is an essential base for most methods of genetic transformation (Torregrosa and Bouquet 1996). Therefore, a protocol to maximise the regeneration of adventitious shoots must be developed before attempting biological transformation using *Agrobacterium* as a vector (Marcotrigiano *et al.* 1996). It is important that direct regeneration without an intervening callus stage should be of single cell origin, avoiding possibility of chimeras after genetic transformation. Many

Table 1: Effect of silver nitrate on direct root induction and shoot regeneration of sweet potato cv. Gaozi No.1

Explant source	Silver nitrate (mg l^{-1})	Rate of root induction (%)	No. of roots per explants	Rate of shoot regeneration (%)		
				20d	40d	60d
Stem	0	100	7.8	40.9	82.8	86.3 ± 0.6a
	2	100	5.4	20.8	66.7	66.7 ± 4.2b
	4	100	4.8	20.7	44.8	55.2 ± 2.9c
	6	100	5.2	42.9	61.2	65.3 ± 1.8b
	8	100	5.4	8.3	16.7	50.0c
	10	100	5.6	12.5	35.3	37.8 ± 2.2d
	12	100	7.9	0	18.2	32.8 ± 4.3d
Lamina	0	85.2	2.6	0	3.9	3.7 ± 3.7d
	2	72.0	1.7	0	40.0	44.0 ± 3.6c
	4	75.0	2.3	0	14.8	42.9 ± 1.5c
	6	70.8	3.1	0	42.6	62.5 ± 3.6b
	8	81.3	6.4	0	43.8	73.3 ± 6.7a
	10	75.0	6.1	0	16.7	41.7 ± 8.3c
	12	66.7	4.9	0	22.2	55.6 ± 5.6b
Petiole	0	95.2	2.8	0	0	0
	2	87.0	1.8	0	0	0
	4	87.0	2.3	0	0	0
	6	78.1	1.3	0	0	3.1
	8	50.0	1.7	0	0	0
	10	62.5	1.1	0	0	0
	12	44.4	1.0	0	0	0

Means within columns having different letters are significantly different at $P \leq 0.05$

reports in other species have been published on direct regeneration protocols from lamina explants, which are compatible with gene transfer methods (Bobak *et al.* 1995, Marcotrigiano *et al.* 1996, Torregrosa and Bouquet 1996). However, such protocols in sweet potato have not been applied.

There are several reports of *in vitro* regeneration of sweet potato plants via organogenesis. However, most of these protocols are limited to a few genotypes and must undergo a callus stage (Porobodessai *et al.* 1995). In our preliminary studies, it was demonstrated that the callus was easily induced and proliferated, but the shoot was very difficult to regenerate from callus. In the present study, a rapid and high frequency direct shoot regeneration procedure was achieved from stem and lamina explants. Meanwhile, the adventitious shoot was derived from *de novo* induced meristems rather than a proliferation of a pre-existing meristem, because the basal ends of the stem and lamina explants of sweet potato were removed to avoid the possibility of any kryptoblasts at the stem and lamina base. Such regeneration could provide a route for genetic transformation (Horsch *et al*. 1986).

Ethylene produced in plant cell tissues and whole plants has been shown to be involved in plant cell differentiation (Pua and Chi 1993). In the present study, silver nitrate, a potent inhibitor of ethylene action with NAA, had a synergistic effect on enhanced shoot regeneration in sweet potato. When NAA was used alone, only 3.9% of the shoots developed from lamina explants whereas when it was applied in combination with silver nitrate, 73.3% of the shoots developed.

In conclusion, this study has demonstrated the effectiveness of silver nitrate in promoting shoot regeneration of explants of sweet potato. The most significant application of this effect would be to improve the efficiency of sweet potato micropropagation systems and to assist with the transfer of transgenic sweet potato from tissue culture to the greenhouse.

Acknowledgements — This project was supported by the National Nature Science Foundation of China (No. 39670471).

References

Al-khayri JM, Al-Bahrany AM (2001) Silver nitrate and 2-isopentyladenine promote somatic embryogenesis in date palm (*Phoenix dactylifera* L.). Scientia Horticulturae **89**: 291–298

Bandyopadhyay S, Cane K, Rasmussen G, Hamill JD (1999) Efficient plant regeneration from seedling explants of two commercially important temperate eucalypt species — *Eucalyptus nitens* and *E. globules*. Plant Science **140**: 189–198

Biddington NL (1992) The influence of ethylene in plant tissue culture. Plant Growth Regulation **11**: 173–187

Bobak M, Blehova A, Kristin J, Ovecka M, Samaj J (1995) Direct plant regeneration from lamina explants of *Drosera rotundifolia* cultured *in vitro*. Plant Cell, Tissue and Organ Culture **43**: 43–49

Fei SZ, Read PE, Riordan TP (2000) Improvement of embryogenic callus induction and shoot regeneration of buffalograss by silver nitrate. Plant Cell, Tissue and Organ Culture **60**: 197–203

Gong YF, Gao F, Zhang PB (1998) Advances of *in vitro* culture of sweet potato in China. Crop Research **2**: 46–48

Gong YF, Huo J, Chen YW, Gao F (2001) Effect of NAA and BA on *in vitro* organogenesis of sweet potato. Journal of Southwest China Normal University **26**: 443–447

Gosukonda RM, Porobodessai A, Blay E, Prakash CS (1995) Thidiazuron-induced adventitious shoot regeneration of sweet potato (*Ipomoea batatas*). In Vitro Cellular and Developmental Biology **31**: 65–71

Horsch RB, Klee HJ, Winans SC, Nester EW, Rogers SG, Fraley RT (1986) Analysis of *Agrobacterium tumefaciens* virulence mutants in leaf discs. Proceedings of the National Academy of Sciences **83**: 2571–2575

Khalafalla MM, Hattori K (2000) Ethylene inhibitors enhance *in vitro* root formation on faba bean shoots regenerated on medium containing thidiazuron. Plant Growth Regulation **32**: 59–63

Khan MR, Rashid H, Ansar M, Chaudry Z (2003) High frequency shoot regeneration and *Agrobacterium*-mediated DNA transfer in Canola (*Brassica napus*). Plant Cell, Tissue and Organ Culture **75**: 223–231

Kumar PP, Dimps Rao C, Goh CJ (1998) Influence of petiole and lamina on adventitious shoot initiation from lamina explants of *Paulownia fortunei*. Plant Cell Reports **17**: 886–890

Lowe JM, Newel CA, Buitron F (1992) Development of plant regeneration and transformation systems in sweet potato (*Ipomoea batatas*). In Vitro Cellular and Developmental Biology **28**: 121A

Mandal AKA, Gupta SD, Chatterji AK (2001) Factors affecting somatic embryogenesis from cotyledonary explants of safflower. Biologia Plantarum **44**: 503–507

Marcotrigiano M, McGlew SP, Hackett G, Chawla B (1996) Shoot regeneration from tissue cultured leaves of American cranberry (*Vaccinium macrocarpon*). Plant Cell, Tissue and Organ Culture **44**: 195–199

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum **15**: 473–497

Porobodessai A, Gosukonda RM, Blay E, Dumenyo CK, Mrdina-Bolivar F, Prakash CS (1995) Plant regeneration of sweet potato (*Ipomoea batatas* L.) from lamina explants *in vitro* using a two-stage protocol. Scientia Horticulturae **62**: 217–224

Pua EC, Chi GL (1993) *De novo* shoot morphogenesis and plant growth of mustard (*Brassica juncea*) *in vitro* in relation to ethylene. Physiologia Plantarum **88**: 467–474

Torregrosa L, Bouquet A (1996) Adventitious bud formation and shoot development from *in vitro* leaves of *Vitis* x *Muscadinia* hybrids. Plant Cell, Tissue and Organ Culture **45**: 245–252

Edited by P Debergh