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Improvement of cassava shoot organogenesis by the use of silver nitrate *in vitro*

Peng Zhang[1], Salak Phansiri[2] & Johanna Puonti-Kaerlas[1,*]
[1]*Institute for Plant Sciences, ETH-Zentrum, CH-8092 Zürich, Switzerland;* [2]*Central Lab, Kasetsart University Research and Development Institute, Kasetsart University, Chatuchak, Bangkok 10900, Thailand (*requests for offprints and present address: European Patent Office, D-80298 Munich, Germany. E-mail: puontik@yahoo.com)*

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Abstract

To improve the regeneration efficiency of cassava (*Manihot esculenta* Crantz) *in vitro*, the effect of silver nitrate ($AgNO_3$) on shoot organogenesis from somatic cotyledons was assessed. Adding $AgNO_3$ to the regeneration medium improved the regeneration frequency and reduced callus formation in all tested cultivars. Both the extent of the response to and the optimum concentration of $AgNO_3$ were cultivar dependent. In the model cultivar MCol22, the use of $AgNO_3$ at concentrations between 4 and 12 mg l^{-1} increased shoot organogenesis frequency and the number of shoot primordia per explant, the maximum effect being observed on a medium containing 12 mg l^{-1} $AgNO_3$. At this concentration, the frequency of shoot organogenesis was enhanced from 60 to 90%, while callus formation decreased from 100 to 5%. The highest shoot organogenesis rates were obtained by supplementing the medium with 2 and 1 mg l^{-1} $AgNO_3$ in KU50 and Hanatee, respectively, while cultivar T5 showed an optimum response at 4 mg l^{-1}. The shoots regenerated from explants cultured on a medium containing $AgNO_3$ were more elongated than those cultured on a medium without $AgNO_3$. The application of $AgNO_3$ did not change the dose response of shoot organogenesis for the selective agents hygromycin and mannose.

Abbreviations: BA – 6-benzylaminopurine; CBM – cassava basic medium; CEM – cassava elongation medium; CMM – cassava embryo maturation medium; COM – cassava shoot organogenesis medium; IBA – indole-3-butyric acid

Introduction

Cassava (*Manihot esculenta* Crantz) is a perennial tropical root crop used as a staple by more than 500 million people world-wide (for a review see Puonti-Kaerlas, 1998). Genetic engineering of cassava has high potential as an efficient complement to traditional breeding in improving agriculturally valuable traits such as pest and virus resistance or improved root quality. However, the transformation efficacy of cassava, like many other crops, depends on plant regeneration efficiency. Direct shoot induction (shoot organogenesis) from cotyledons of somatic embryos (somatic cotyledons) of cassava is a rapid regeneration system minimising somaclonal variation (Li et al., 1998) and has already been demonstrated to be efficient for genetic transformation using particle bombardment (Zhang et al., 2000) as well as for transformation using *Agrobacterium* (Li et al., 1996; Puonti-Kaerlas et al., 1997). The regeneration efficiency is nonetheless genotype-dependent, varying between 5 and 70% (Puonti-Kaerlas, unpublished), which may constrain the use of genetic engineering in cultivars with low regeneration capacity.

Silver nitrate has been shown to be effective in improving somatic embryogenesis and plant regeneration in a number of crop species including *Brassica* spp. (Palmer, 1992; Zhang and Ling, 1995, 1996; Eapen and George, 1997; Kuvshinov et al., 1999), maize (Vain et al., 1989a,b; Carvalho et al., 1997), muskmelon (Yadav et al., 1996), cucumber (Roustan et al., 1992; Mohiuddin et al., 1997), cowpea (Brar

et al., 1999), peanut (Pestana et al., 1999), wheat (Lashermes, 1992), rice (Lentini et al., 1995) and barley (Castillo et al., 1998). As Ag^+ ions can prevent a wide variety of ethylene-induced plant responses, including growth inhibition and senescence, the effect is assumed to be mediated via the inhibition of the physiological action of ethylene (Beyer et al., 1984), a potential inhibitor of many plant regeneration systems (Vain et al., 1989a; Chraibi et al., 1991; Kong and Yeung, 1994). In this paper we present the results of the first study on the effect of $AgNO_3$ on cassava shoot organogenesis from somatic cotyledons. Our study shows that $AgNO_3$ can be used to improve the *in vitro* regeneration frequencies of cassava without affecting adversely the efficiency of selectable markers required for selection of transgenic plants.

Materials and methods

Somatic embryogenesis

One Colombian (MCol22) and three Thai cultivars (T5, KU50 and Hanatee) obtained from CIAT, Colombia and Kasetsart University, Thailand, respectively, were used in this study. The plant material was maintained as shoot cultures on CBM (MS salts and vitamins [Murashige and Skoog, 1962] supplemented with 2% sucrose and 2 μM $CuSO_4$, solidified with 0.6% plant agar (Duchefa, pH 5.8) at 26°C with a 16-h photoperiod (fluorescent tubes, 200 μM m^{-2} s^{-1}) and subcultured at 4-week intervals. Apical shoot meristems and 2–5-mm long immature leaf lobes were isolated from shoot cultures and cultured on CIM medium (CBM supplemented with 12 mg l^{-1} picloram) at 26°C in the dark. After 2 weeks, the developing embryos were transferred to fresh CIM medium to initiate cycling secondary embryo cultures. After two to three cycles on CIM medium, the somatic embryos were harvested and cultured onto CMM medium (CBM with 0.1 mg l^{-1} BA) at 26°C with a 16-h photoperiod (90–110 μM $m^{-2}s^{-1}$) for production of maturing somatic embryos.

Shoot organogenesis

Green cotyledons were collected from somatic embryos cultured on maturation medium (CMM) for 10–15 days, cut into 5-mm^2 pieces and transferred to COM medium (CBM with 1 mg l^{-1} BA and 0.5 mg l^{-1} IBA) for shoot organogenesis at 26°C in the dark. Shoot primordia developing on the explants were transferred after 3 weeks to CEM (CBM supplemented with 0.4 mg l^{-1} BA) for shoot elongation.

Silver nitrate test

COM medium supplemented with $AgNO_3$ was used for assessing the effect of silver nitrate. An 8 mg ml^{-1} stock solution of $AgNO_3$ (Merck, Darmstadt) was filter sterilised, stored at 4°C and added into the autoclaved medium. The effect of various silver nitrate concentrations (0, 1, 2, 4, 8 and 12 mg l^{-1}) on callus formation and on shoot regeneration ability of cotyledon explants was tested. Fifty explants per cultivar were cultured per treatment. Each experiment had three replicates and was repeated 2 times. After 3 weeks, the status of shoot organogenesis and callus formation were recorded. Three different degrees of organogenesis and callus formation were used to evaluate the response of the cotyledon explants to silver nitrate. Organogenesis degree 1 (OD1) indicates less than five shoot primordia per explant, organogenesis degree 2 (OD2) between five and 10 shoot primordia per explant and organogenesis degree 3 (OD3) more than 10 shoot primordia per explant. In the evaluation of callus development, callus degree 1 (CD1) indicates callus size less than 0.25 cm in diameter, callus degree 2 (CD2) callus size between 0.25 and 0.5 cm in diameter and callus degree 3 (CD3) indicates the size of calli larger than 0.5 cm in diameter.

Plant regeneration

The developing shoot primordia and regenerating shoots were detached from the explants and transferred to CEM medium for shoot elongation. In one experiment, the effect of $AgNO_3$ on the competence of shoot primordia to produce elongating shoots was evaluated. Twenty clusters of shoot primordia per treatment were transferred to CEM for elongation. After 4 weeks, the number of elongated shoots was recorded. The shoots were transferred to CBM for rooting and further growth.

Effect of silver nitrate on selection agents

In order to test whether $AgNO_3$ influences the efficiency of hygromycin and mannose used for selection of transgenic cassava shoots, cotyledon explants were cultured on COM supplemented with 8 mg l^{-1} $AgNO_3$ and either with 20 mg l^{-1} hygromycin or 20 g l^{-1} mannose. Fifty explants of MCol22 were used in each

Table 1. The effect of silver nitrate on the frequency of shoot organogenesis (SO)

Cultivars		Concentration of $AgNO_3$ (mg l^{-1})					
		0	1	2	4	8	12
MCol22	SO	63.3 ± 5.0^{b}	63.3 ± 8.1^{b}	66.0 ± 4.0^{b}	80.7 ± 5.0^{a}	85.3 ± 4.2^{a}	88.7 ± 4.2^{a}
	OD1	42.7 ± 2.3^{a}	30.7 ± 6.1^{bc}	20.7 ± 5.0^{d}	23.3 ± 5.0^{cd}	36.7 ± 4.2^{ab}	34.7 ± 7.0^{ab}
	OD2	20.0 ± 3.5^{b}	21.3 ± 6.1^{b}	24.7 ± 5.0^{ab}	32.7 ± 3.1^{a}	26.7 ± 5.8^{ab}	24.0 ± 6.0^{ab}
	OD3	0.70 ± 1.2^{d}	11.3 ± 4.2^{c}	20.7 ± 3.1^{b}	24.7 ± 6.1^{ab}	22.0 ± 2.0^{b}	30.0 ± 7.2^{a}
KU50	SO	47.3 ± 14.2^{d}	72.7 ± 3.1^{bc}	87.3 ± 3.1^{a}	80.7 ± 4.2^{ab}	62.7 ± 6.1^{c}	49.3 ± 5.0^{d}
	OD1	28.7 ± 4.2^{a}	25.0 ± 6.1^{a}	24.0 ± 4.0^{a}	$21.\pm4.2^{a}$	26.7 ± 5.0^{a}	20.7 ± 3.1^{a}
	OD2	14.7 ± 6.1^{c}	28.0 ± 4.0^{ab}	34.0 ± 3.5^{a}	32.0 ± 3.5^{a}	21.3 ± 6.1^{bc}	18.0 ± 6.0^{c}
	OD3	4.0 ± 4.0^{c}	19.3 ± 5.0^{ab}	26.0 ± 5.3^{a}	27.3 ± 7.0^{a}	14.7 ± 7.0^{b}	10.7 ± 2.3^{bc}
Hanatee	SO	50.0 ± 5.3^{c}	67.3 ± 4.2^{a}	60.7 ± 3.0^{ab}	52.0 ± 8.0^{bc}	43.3 ± 5.0^{c}	43.3 ± 4.2^{c}
	OD1	36.0 ± 5.3^{ab}	37.3 ± 4.2^{a}	26.7 ± 3.1^{c}	30.0 ± 3.5^{bc}	25.3 ± 4.2^{cd}	19.3 ± 3.1^{d}
	OD2	8.0 ± 2.0^{c}	17.3 ± 1.2^{a}	19.3 ± 2.3^{a}	10.7 ± 3.1^{bc}	10.0 ± 2.0^{bc}	13.3 ± 2.3^{b}
	OD3	6.0 ± 2.0^{c}	12.7 ± 2.3^{ab}	14.7 ± 2.3^{a}	11.3 ± 3.1^{ab}	8.0 ± 3.5^{bc}	10.7 ± 3.1^{abc}
T5	SO	25.3 ± 6.4^{c}	32.0 ± 8.0^{bc}	32.7 ± 4.2^{bc}	47.3 ± 5.0^{a}	40.7 ± 7.0^{ab}	38.0 ± 3.5^{ab}
	OD1	19.3 ± 2.3^{b}	24.7 ± 5.0^{ab}	25.3 ± 2.3^{ab}	28.7 ± 3.1^{a}	26.0 ± 5.3^{a}	23.3 ± 3.1^{ab}
	OD2	6.0 ± 5.3^{b}	7.3 ± 3.1^{ab}	8.7 ± 1.2^{ab}	12.0 ± 2.0^{a}	12.0 ± 2.1^{a}	12.0 ± 4.0^{a}
	OD3	0^{b}	0^{b}	2.0 ± 2.0^{b}	6.7 ± 1.2^{a}	2.7 ± 1.1^{b}	2.7 ± 3.0^{b}

The numbers indicate the mean number of responding explants (SO) as percent of all explants, and the distribution of the response between different organogenesis degrees (OD1, OD2 and OD3). Letters a–d indicate significant differences (p=0.05) within each row using an LSD test.

treatment with three replicas. After 4 weeks, the number of developing shoots was recorded. For statistical significance, the data were analysed by the LSD test at the 5% level.

Results

Differences were observed between the four cultivars in their competence for organogenesis under standard conditions (Table 1). MCol22 had the highest organogenesis frequency (63%); while in T5, only 25% of the explants were able to produce shoot primordia. The organogenesis frequency of KU50 and Hanatee was about 50%. In most of the explants organogenesis degree one (less than five shoot primordia per explant) was dominant. At the same time, large amounts of non-morphogenic callus were produced from the cut edges of the explants (Figure 1).

Enhancement of organogenesis frequency

After 4 weeks of culture on COM supplemented with different concentrations of $AgNO_3$, different responses could be observed among the tested cultivars both in shoot organogenesis frequencies and the numbers of developing shoot primordia per explant (Table 1). In MCol22, the frequency of shoot organogenesis and organogenesis degree increased with increasing $AgNO_3$ concentrations of up to 12 mg l^{-1}. On a medium containing 12 mg l^{-1} $AgNO_3$, 89% of the explants produced shoot primordia, and 30% of these had more than 10 shoot primordia per explant (organogenesis degree 3); whereas in the controls, the organogenesis frequency was 63%, and only 0.7% of the explants showed organogenesis degree 3. In KU50 and Hanatee, the highest increase in organogenesis rate could be obtained by supplementing the medium with 2 and 1 mg l^{-1} $AgNO_3$ respectively; while in T5, the best response was observed using 4 mg l^{-1} $AgNO_3$ in the medium. Concentrations higher than 8 mg l^{-1} had a slightly inhibitory effect on shoot organogenesis of the Thai cultivars.

Inhibition of callus formation

On COM, 100% of the somatic cotyledon explants of MCol22, KU50 and T5 formed callus, more than 70% of which was degree 3 (callus size larger than 0.5 cm in diameter) (Table 2). In Hanatee, 89% of the explants produced callus and 73% of this was degree 3. In all cultivars, increasing concentrations of $AgNO_3$ reduced callus formation, both in terms of callus frequency and of callus degree. In MCol22, callus frequency was decreased from 100% (control) to 5%

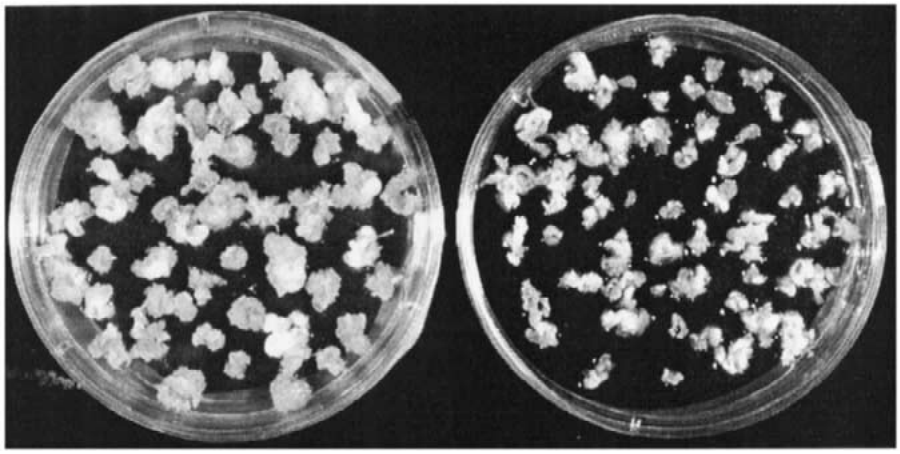

Figure 1. The effect of silver nitrate on shoot organogenesis and callus formation in MCol22; left, without silver nitrate; right, with 4 mg l^{-1} silver nitrate.

(12 mg l^{-1} $AgNO_3$) and the frequency of callus degree 3 was reduced from 98 to 0% (Figure 1). In Hanatee, callus formation was reduced to 25% by 4 mg l^{-1} and totally inhibited by 8 mg l^{-1} $AgNO_3$; while in KU50 and in T5, the effect was less pronounced, but still significantly different from the controls.

Improvement of shoot elongation

Shoot primordia of MCol22 induced on COM with or without 4 mg l^{-1} $AgNO_3$ were transferred individually onto CEM to test the effect of silver nitrate on shoot elongation. In all the tested cultivars, shoot primordia induced on COM with $AgNO_3$ produced more elongating shoots than those induced on a medium without $AgNO_3$ (Table 3).

Effect of silver nitrate on the dose response to selective agents

Hygromycin and mannose, used for production of transgenic cassava were tested for their efficacy in inhibiting shoot organogenesis of MCol22 cotyledon explants cultured on COM supplemented with $AgNO_3$. Addition of 8 mg l^{-1} silver nitrate did not affect the sensitivity of shoot primordia regeneration to hygromycin or mannose (Table 4).

Discussion

Many reports have demonstrated the positive effect of $AgNO_3$ on plant tissue culture, although in some plants and tissue types no positive or even an inhibitory effect has been reported (Puonti-Kaerlas, 1991; Palmer, 1992; Jun et al., 1997; Teo et al., 1997; Kumar et al., 1998; El Meskaoui and Tremblay, 1999). In the case of cassava, it has been shown that the maturation and regeneration of secondary somatic embryos of one cultivar could be improved by the use of 16 mg l^{-1} $AgNO_3$ and 0.25 mg l^{-1} ABA (Zhu et al., 1998). We show here that the capacity of shoot organogenesis in a number of cassava cultivars can be improved by supplementing the medium with $AgNO_3$. The optimum concentration of $AgNO_3$ was found to differ among the different cultivars. In MCol22, using 4–12 mg l^{-1} $AgNO_3$ increased the frequency of shoot organogenesis from 63% (control) to over 80%. At the same time, the number of regenerated shoots per explant increased as well, with concomitant inhibition of callus growth. The optimum $AgNO_3$ concentrations for shoot regeneration in the other cultivars were lower than in MCol22; 2 mg l^{-1} in KU50, 1 mg l^{-1} in Hanatee and 4 mg l^{-1} in T5. Hence, different cassava cultivars appear to have quantitatively different responses to $AgNO_3$ and the optimal concentration needs be determined separately for each cultivar. The genotype and the developmental stage of explants have been shown to affect the response of other plant species to silver nitrate as well (Palmer et al., 1992; Evans and Batty, 1994; Hyde and Phillips, 1996; Mohiuddin et al., 1997; Santos et al., 1997). Of the cassava lines tested in this study, MCol22 appears to have a broader optimum for $AgNO_3$ than the Thai cultivars. In contrast to MCol22, the most efficient concentrations for improving shoot regeneration in the Thai cultivars are lower than those required for maximal suppression of callus growth. The highest number of

Table 2. The effect of silver nitrate on the frequency of callus formation (CF) and the callus degrees (CD1, CD2 and CD3)

Cultivars		Concentration of $AgNO_3$ (mg l^{-1})					
		0	1	2	4	8	12
MCol22	CF	100^{a}	92.0 ± 4.0^{a}	24.0 ± 8.0^{b}	20.7 ± 6.1^{b}	16.0 ± 4.0^{b}	4.7 ± 2.3^{c}
	CD1	0^{c}	26.6 ± 4.2^{a}	12.0 ± 8.0^{b}	13.3 ± 3.1^{b}	12.7 ± 1.2^{b}	4.0 ± 2.0^{c}
	CD2	2.0 ± 3.5^{cd}	36.7 ± 5.0^{a}	10.0 ± 2.0^{b}	7.3 ± 4.2^{bc}	3.3 ± 4.2^{cd}	0.7 ± 1.1^{d}
	CD3	98.0 ± 3.5^{a}	28.7 ± 3.1^{b}	2.0 ± 2.0^{c}	0^{c}	0^{c}	0^{c}
KU50	CF	100^{a}	98.7 ± 2.3^{a}	78.7 ± 4.2^{b}	56.0 ± 7.2^{c}	31.3 ± 10.1^{d}	32.0 ± 15.6^{d}
	CD1	4.0 ± 2.0^{c}	11.3 ± 3.0^{b}	16.6 ± 3.1^{b}	24.0 ± 4.0^{a}	15.3 ± 3.1^{b}	14.7 ± 4.2^{b}
	CD2	7.3 ± 1.2^{b}	14.0 ± 7.2^{ab}	17.3 ± 2.3^{ab}	19.3 ± 3.1^{a}	10.0 ± 2.0^{ab}	17.3 ± 12.9^{ab}
	CD3	88.7 ± 3.1^{a}	73.3 ± 6.1^{b}	44.7 ± 7.0^{c}	12.7 ± 7.0^{d}	6.0 ± 6.0^{de}	0^{e}
Hanatee	CF	89.3 ± 6.1^{a}	62.0 ± 5.3^{b}	40.7 ± 5.0^{c}	25.3 ± 3.1^{d}	0^{e}	0^{e}
	CD1	5.3 ± 2.3^{c}	34.0 ± 3.5^{a}	32.0 ± 2.0^{a}	20.0 ± 2.0^{b}	0^{d}	0^{d}
	CD2	11.3 ± 2.3^{b}	20.0 ± 4.0^{a}	7.3 ± 2.3^{c}	4.7 ± 1.2^{c}	0^{d}	0^{d}
	CD3	72.7 ± 10.1^{a}	8.0 ± 2.0^{b}	1.3 ± 1.2^{bc}	0.7 ± 1.1^{bc}	0^{c}	0^{c}
T5	CF	100^{a}	100^{a}	100^{a}	91.3 ± 3.0^{b}	90.7 ± 4.2^{b}	89.3 ± 5.0^{b}
	CD1	4.0 ± 4.0^{d}	18.0 ± 5.3^{c}	38.0 ± 6.0^{b}	37.3 ± 2.3^{b}	43.3 ± 5.0^{ab}	47.3 ± 4.2^{a}
	CD2	26.7 ± 6.1^{b}	38.7 ± 10.1^{a}	38.0 ± 5.3^{a}	30.0 ± 7.2^{a}	28.0 ± 3.5^{a}	29.3 ± 3.1^{a}
	CD3	69.3 ± 4.6^{a}	42.0 ± 8.7^{b}	24.0 ± 4.0^{c}	24 ± 5.3^{c}	19.3 ± 4.2^{cd}	12.7 ± 3.0^{d}

Letters a–e indicate significant differences (p=0.05) within each row using an LSD test.

responding explants, however, was obtained in all cultivars at the same concentration as the highest number of developing shoot primordia.

Histological studies in *Brassica parachinensis* showed that $AgNO_3$ influenced the mode of plant regeneration, allowing direct shoot primordia development without an intermediate callus phase (Zhang and Ling, 1995, 1996). Also in *Albizia procera* the use of silver nitrate has been reported to promote callus-free shoot regeneration (Kumar et al., 1998). In our experiments with cassava, supplementing COM with $AgNO_3$ altered the regeneration mode from organogenesis involving a callus phase to that without an intervening callus phase (Figure 1). Callus formation is often considered to inhibit plant regeneration and, in cassava, reproducible plant regeneration from callus has only been reported for one cultivar (Mussio et al., 1998). We assume therefore that the increased capacity of shoot organogenesis is at least partially related to the inhibition of callus formation. The correlation between organogenesis frequency and callus frequency in MCol22 is −0.82, indicating that organogenesis and callus formation are indeed inversely correlated.

The elongation of shoots was more efficient from primordia induced on a regeneration medium containing $AgNO_3$ than from those cultured on a medium without $AgNO_3$ (Table 3). Thus, the use of $AgNO_3$ during shoot primordia induction also has a positive effect on later plant development at the shoot elongation step. Silver nitrate has been shown in *Brassica rapa* to improve the frequency of shoot organogenesis from seedling explants pre-treated with $AgNO_3$ during seed germination (Burneet et al., 1994). In some cases, the use of $AgNO_3$ has reduced or inhibited rooting of the regenerated shoots (Castillo et al., 1998; Madsen et al., 1998) but in cassava no adverse effects on rooting ability could be observed.

The mode of action of $AgNO_3$ in plant tissue culture is assumed to be associated with the physiological effects of ethylene, silver ions acting as a competitive inhibitor of ethylene action rather than inhibiting ethylene synthesis per se. Ethylene production may in fact increase in plant cultures treated with silver nitrate (Pua et al., 1993, 1999; Lee et al., 1997; Zhang et al., 1998). Until now, the mechanism of ethylene action on plant tissue culture has not been elucidated. While ethylene may act as an inhibitor in some plant regeneration systems (Vain et al., 1989b; Chraibi et al., 1991; Kong and Yeung, 1994), it may on the other hand function as stimulator in others (Hatanaka et al., 1995, Nissen, 1994). Biochemical studies suggested that a transition metal is involved in binding of ethylene to receptors, since addition of $CuSO_4$ to

Table 3. The frequency of shoot elongation from four cassava cultivars on CEM from shoot primordia induced either on COM or AgCOM (COM with 4 mg l^{-1} silver nitrate)

Cultivar	Medium used for shoot primordia induction	No. of shoot primordia clusters	No. of elongating shoots	Shoot elongation frequency (%)
MCol22	COM	20	8	40
	AgCOM	20	12	60
T5	COM	20	4	20
	AgCOM	20	5	25
KU50	COM	20	8	40
	AgCOM	20	11	55
Hanatee	COM	20	3	15
	AgCOM	20	9	45

Table 4. The influence of silver nitrate (Ag) on inhibition of shoot primordia development by hygromycin (Hm) and mannose (Man) in MCol22

Type of medium	Shoot organogenesis frequency (%)
COM	63.0 ± 4.0^a
COM+Hm	0.7 ± 1.2^b
AgCOM+Hm	1.3 ± 1.2^b
COM+Man	2.5 ± 2.3^b
AgCOM+Man	1.0 ± 1.7^b

Letters a–b indicate significant differences (p=0.05) using an LSD test.

membranes isolated from ETR1-expressing yeast resulted in up to a 20-fold increase in ethylene binding (Bleecker, 1997). Very interestingly and surprisingly, Ag^+ ions also increased receptor affinity for ethylene in the yeast system, suggesting that silver ions act by interfering with intramolecular signal transduction rather than by ligand binding (Bleecker, 1997). Other studies showed that silver ions may interact with polyamines, which have been shown to promote organogenesis and embryogenesis (Feirer et al., 1984; Meijer and Simmonds, 1988; Pua et al., 1999) since ethylene and polyamines compete for the same precursor, SAM (*S*-adenosyl methionine). Few studies have addressed the interaction between silver ions, ethylene and polyamines (Pua et al., 1999), and so far the nature of these interactions is not known.

The enhancement of *in vitro* shoot organogenesis by $AgNO_3$ should not interfere with transformation or selection efficiencies, so as not to prevent the use of $AgNO_3$ in transgenic plant production. Our results show that silver nitrate did not influence the dose response of cassava tissues to the selective agents hygromycin and mannose. Shoot organogenesis is inhibited by 20 mg l^{-1}hygromycin or 20 g l^{-1}mannose both in the presence and absence of $AgNO_3$. The addition of $AgNO_3$ in shoot regeneration medium has been shown to be essential for regeneration of transformed tissues in *Brassica napus* and *Brassica oleracea* (DeBlock et al., 1989), *Brassica campestris* (Mukhopadhyay et al., 1992; Kuvshinov et al., 1999) and *Brassica rapa* (Radke et al., 1992). The present study shows that it is also possible to improve the frequencies of shoot organogenesis in cassava by supplementing the regeneration medium with $AgNO_3$. This should allow higher transformation frequencies and more efficient regeneration of transgenic cassava plants in the future.

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