Response of In Vitro Strawberry to Silver Nitrate (AgNO_3)

Yonghua Qin and Shanglong Zhang¹

Department of Horticulture, Zhejiang University, Key Laboratory of Horticultural Plant Growth, Development and Biotechnology, Ministry Agriculture, Hangzhou 310029, China

Lingxiao Zhang

Delta Research and Extension Center, Mississippi State University, P.O. Box 197 Stoneville, MS 38756

Daoyu Zhu

Department of Horticulture, Henan Agricultural University, Zhengzhou 450002 China

Asghar Syed

Department of Horticulture, Zhejiang University, Hangzhou 310029 China

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Abstract. Response of Toyonoka strawberry to AgNO_3 was studied. Types and combinations of plant growth regulators had significant effects on shoot regeneration efficiency. Explants cultured for 10 days in shoot regeneration medium in the presence of AgNO_3 not only enhanced shoot regeneration efficiency but also expedited the initiation of adventitious buds. Highest regeneration (87.38%) and number of shoots per explant (11.67) were achieved in shoot regeneration media containing $1.5 \text{ mg} \cdot \text{L}^{-1}$ TDZ, $0.4 \text{ mg} \cdot \text{L}^{-1}$ IBA and $1.0 \text{ mg} \cdot \text{L}^{-1}$ AgNO_3 . Half-strength MS containing $1.0 \text{ mg} \cdot \text{L}^{-1}$ AgNO_3 was an optimum medium for rooting. AgNO_3 advanced root emergence and increased percent rooting, root length, dry weight and activity. Lower concentrations of AgNO_3 inhibited ethylene production and promoted shoot regeneration and growth. It had a significant stimulatory effect on chlorophyll, soluble protein contents and antioxidant enzyme activities. Chlorophyll and soluble protein contents, superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) activities were increased in the presence of AgNO_3 and reached maximum at $1.0 \text{ mg} \cdot \text{L}^{-1}$ AgNO_3 . Root water content, superoxide free radicals (O_2^-), malondialdehyde (MDA) content, proline accumulation and IAA-oxidase activity in leaves were increased while (IAA) content was decreased in the presence of AgNO_3 . Chemical names used: indole-3-butyric acid (IBA); silver nitrate (AgNO_3); thidiazuron (TDZ); N⁶-benzyladenine (BA); 2,4-dichlorophenoxy acetic acid (2,4-D); indole-3-acetic acid (IAA); α -naphthalene acetic acid (NAA); gibberellic acid (GA_3); bovine serum albumin (BSA); 2,3,5-triphenyl-2H-tetrazolium chloride (TTC).

Protocols for strawberry shoot regeneration in vitro have been reported (Lis, 1993; Passey et al., 2003); however, regeneration efficiency is low and inconsistent. Highly efficient regeneration method is one of the most important factors in determining the success of genetic transformation (Bandyopadhyay et al., 1999), further improvement of strawberry plant regeneration and establishment of a high-efficiency regeneration system would make a significant contribution to strawberry genetic research.

Being ethylene inhibitor, AgNO_3 can markedly promote organogenesis, embryogenesis and shoot regeneration. It plays a significant role in somatic embryo production and shoot regeneration of durum wheat and maize (Carvalho et al.,

1997; Fernandez et al., 1999). With the addition of AgNO_3 in medium, shoot regeneration efficiency was enhanced in sunflower (Yordanov et al., 2002), *Arabidopsis* (Márton and Browne, 1991) and *brassica* (Pua et al., 1999). Organogenesis is a complex physiological and biochemical process associated with enzymatic, photosynthetic and endogenous growth regulators changes. Higher activities of antioxidant enzymes promote cell division and somatic embryogenesis (Cui et al., 1999; Tian et al., 2003). Relationship of antioxidant enzymes, MDA, chlorophyll and endogenous growth regulators to shoot regeneration is not well explored and little is known about the mechanism by which AgNO_3 promotes organogenesis.

Shoot regeneration capability of strawberry cultivars varies greatly and experiments need to be conducted to develop an optimized protocol for individual cultivars. In this study, *Fragaria × ananassa* Duch. 'Toyonoka' was used to define its regeneration capability and physiological responses to AgNO_3 under in vitro conditions. The objectives of this study were to establish an efficient shoot regeneration system in the shortest possible time without entering callus phase, and to study the physiological effects of AgNO_3 in strawberry. This highly efficient in vitro regeneration protocol will be a valuable tool in genetic transformation and other fundamental studies of strawberry.

Materials and Methods

Plant material and media. Runner tissues of strawberry were washed with tap water for 30 min and surface disinfected in 70% (v/v) ethanol for 10 s and 0.1% (w/v) HgCl_2 for 5 to 8 min, followed by four washes with sterile distilled water. Each time, tissues were soaked in water for 5 min. Shoot tips of 0.2 mm were aseptically dissected out under a stereomicroscope and cultured on trigger medium for 3 to 4 weeks. Microshoots were transferred to proliferation medium and cultured for 3 weeks. Healthy and fully expanded strawberry leaves from plantlets were used in all experiments. After discarding apices, margins and midribs, 4 × 4-mm explants were placed adaxially on the medium. MS medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose and 0.7% (w/v) agar with pH 5.8 was used throughout the course of study. Fifteen factorial combinations of TDZ (0.5, 1.0, 1.5, 2.0, 2.5 $\text{mg} \cdot \text{L}^{-1}$), IBA (0.2, 0.4, 0.6, 0.8, 1.0 $\text{mg} \cdot \text{L}^{-1}$) and 2,4-D (0.2, 0.4, 0.6, 0.8, 1.0 $\text{mg} \cdot \text{L}^{-1}$) were tested for their effects on shoot regeneration. After screening various combinations of growth regulators, media were named based on the best combinations and used in the preceding experiments (Table 1). Leaf discs were cultured on shoot regeneration medium with 0.5, 1.0, 2.0, 4.0 $\text{mg} \cdot \text{L}^{-1}$ AgNO_3 for 10 d and were transferred to the same medium without AgNO_3 . For rooting, shoots (2.0 cm in length) were separated and cultured on 1/2-strength MS medium supplemented with 0 to 4.0 $\text{mg} \cdot \text{L}^{-1}$ AgNO_3 .

Growth regulators and AgNO_3 solutions were filter sterilized and added to the autoclaved medium in the aseptic conditions. Cultures were incubated at $25 \pm 2^\circ\text{C}$ under 2000 Lx irradiance (provided by fluorescent lamp, 36-W, Philips Electronic N.V., Holland) with 16-h photoperiod.

Enzyme extraction. Fresh leaves (0.3 g) were homogenized in an ice bath containing 3 mL 50 mM potassium phosphate buffer

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¹Professor; to whom reprint requests should be addressed; e-mail shlzhang@zju.edu.cn.

Table 1. Medium and combinations of plant growth regulators.

Medium	Combinations of plant growth regulators ($\text{mg} \cdot \text{L}^{-1}$)	Explant type
Trigger medium	0.2 BA + 0.01 IBA + 0.1 GA_3	Shoot-tips
Proliferation medium	0.5 BA + 0.03 NAA	In vitro micro shoot-tips
Shoot regeneration	1.5 TDZ + 0.4 IBA	Leaf discs
Shoot regeneration plus AgNO_3	1.5 TDZ + 0.4 IBA + 1.0 AgNO_3	Leaf discs
Rooting medium	Half-strength MS medium + 1.0 AgNO_3	Shoots

(pH 7.8 + 0.1 mM EDTA + 2% insoluble polyvinylpyrrolidone). The homogenate was centrifuged at 15,000 g_n for 15 min at 4 °C. The supernatant was stored at -20 °C and used for assays of enzyme, O₂⁻ production and soluble protein.

Methods of determination. SOD analysis was conducted according to Giannopolitis and Ries (1997). POD and CAT activities were determined following Cakmak and Marschner (1991). MDA content was assayed using Popham and Novacky (1991) method. Soluble protein content was determined according to Bradford (1976) using BSA as a standard. Chlorophyll content was determined

according to Arnon (1949). Superoxide free radicals (O₂⁻) production rate was measured spectrophotometrically by using the method described by Wang and Luo (1990). Ethylene production was detected according to Chen et al (1991). For root activities, TTC reduction procedure by Jiang and Zhu (1999) was followed. IAA content was measured according to Sweetser and Swartzfager (1978). Proline content was assayed by the photochemical method described by Bates et al (1973). For IAA-oxidase activity Lee (1971) method was used with minor modifications as follows. Fresh leaves (0.5 g) were homogenized in an ice bath containing a buffer solution with 5 mL 20 mM potassium phosphate (pH 6.1). The homogenate was centrifuged at 4,000 rpm for 20 min at 4 °C and stored under -20 °C. To 1 mL of the crude enzyme extract, 2 mL 1 mM MnCl₂, 2 mL 1 mM 2,4-dichlorophenol, 2 mL 1 mM IAA, and 3 mL potassium phosphate buffer (20 mM, pH 6.1) were added. Solution was store at 25 °C in the dark for 30 min and after addition of 4 mL Salkowski reagent (Pilet and Lavanchy, 1969), solution was again further stored at 25 °C in the dark for 30 min. IAA-oxidase activity was determined by measuring the light spectrum at 530 nm.

Statistical analysis. Data on shoot and root length, number of roots, rooting percentage, roots and shoots dry weight were recorded on the 21st day of culture. Shoot regeneration efficiency was scored after 45 d in culture. Shoot regeneration experiment was repeated three times with 5 petri dishes and 60 explants per treatment and root induction experiment was repeated three times with 15 bottles consisting of 5 explants per bottle. Plant parts from root induction experiment were harvested for analysis of SOD, POD, CAT and IAA-oxidase activities, O₂⁻ production rate, contents of soluble protein, IAA, MDA, proline and chlorophyll, root activities and ethylene production on the 21st day of culture. Data were analyzed by SAS statistical package (SAS Inst., Cary, N.C.) and differences between treatment means were compared using LSD at $\alpha \leq 0.05$ probability level. Regression analysis was made for various concentrations of TDZ, with a constant level of TDZ (1.5 mg·L⁻¹) having various concentrations of IBA and with constant levels of TDZ and IBA (1.5 and 0.4 mg·L⁻¹) having various concentrations of 2,4-D respectively.

Results

Effects of plant growth regulators on shoot regeneration. MS media supplemented with fifteen factorial combinations of TDZ, IBA and 2,4-D was used to select suitable levels for shoot regeneration of strawberry. Types and combinations of plant growth regulators had significant effect on regeneration of adventitious bud. TDZ at higher concentration promoted shoot regeneration efficiency but it also resulted in extended rates of vitrification. Maximum regeneration rate (47.45%) was achieved with 2.5 mg·L⁻¹ TDZ (Table 2). TDZ concentrations above 1.5 mg·L⁻¹ had an adverse effect on shoot regeneration. TDZ at 1.5 mg·L⁻¹ in combination with 0.2–0.4 mg·L⁻¹ IBA could markedly enhance the regeneration of adventitious buds (Table 3). At constant TDZ and IBA concentrations, all levels of 2,4-D had a negative effect on regeneration (Table 4). Explants ceased to induce shoots at 1.0 mg·L⁻¹ 2,4-D. Highest percent of shoot regeneration (76.67%) and maximum number of shoots per explant (5.78) were achieved on MS medium supplemented with 1.5 mg·L⁻¹ TDZ and 0.4 mg·L⁻¹ IBA. All treatments induced calli during the first 2 weeks in culture.

Effects of AgNO₃ on shoot regeneration. Effect of AgNO₃ on shoot regeneration of strawberry was compared using MS medium with 1.5 mg·L⁻¹ TDZ and 0.4 mg·L⁻¹ IBA. Significant difference was observed in percent shoot regeneration and number of shoots per explant when explants were exposed to AgNO₃ for 10 d (Table 5). Highest regeneration (87.38%) and number of shoots per explant (11.67) were achieved with shoot regeneration media containing 1.5 TDZ, 0.4 IBA and 1.0 mg·L⁻¹ AgNO₃. With AgNO₃ in the medium, an earlier (5 to 7 d) and stable shoot regeneration was observed, however higher concentrations (≥ 2.0 mg·L⁻¹) had negative effects on the general growth and development of explants associated with curly leaves, stunted growth and tissue browning. Explants did not produce shoots with AgNO₃ concentrations at 6.0 mg·L⁻¹ (Fig. 1f).

Table 2. Effects of TDZ on shoot regeneration of strawberry.^z

TDZ concn ^y (mg·L ⁻¹)	Shoot regeneration ^x (%)	Adventitious buds/explant ^v (no.)
0.5	25.45	1.29
1.0	36.84	1.71
1.5	45.45	2.10
2.0	46.61	2.38
2.5	47.45	2.68

^zLinear regression of shoot regeneration and linear regression of number of adventitious buds per explant on TDZ concentration was significant at $\alpha \leq 0.05$.

^yMurashige-Skoog medium with TDZ.

^xThe linear regression formula is $y = 10.754x + 24.229$.

^vThe linear regression formula is $y = 0.69x + 0.997$.

Table 3. Effects of various IBA concentrations with constant TDZ concentrations on strawberry shoot regeneration efficiency.^z

Plant growth regulator ^y (mg·L ⁻¹)		Shoot regeneration ^x (%)	Adventitious buds/explant ^v (no.)
TDZ	IBA		
1.5	0.2	60.34	3.20
1.5	0.4	76.67	5.78
1.5	0.6	74.07	4.68
1.5	0.8	67.27	4.49
1.5	1.0	63.64	4.0

^zPolynomial regression of both shoot regeneration and number of adventitious buds per explant on IBA concentration was significant at $\alpha \leq 0.05$.

^yMurashige-Skoog medium with TDZ and IBA.

^xThe polynomial regression formula is $y = -78.79x^2 + 93.14x + 47.18$.

^vThe polynomial regression formula is $y = -9.34x^2 + 11.36x + 1.72$.

Table 4. Effects of various concentrations of 2,4-D with constant TDZ and IBA concentrations on strawberry shoot regeneration efficiency.^z

Plant growth regulator ^y (mg·L ⁻¹)			Shoot regeneration ^x (%)	Adventitious buds/explant ^v (no.)
TDZ	IBA	2,4-D		
1.5	0.4	0.2	27.45	1.86
1.5	0.4	0.4	17.54	1.30
1.5	0.4	0.6	11.32	1.17
1.5	0.4	0.8	6.78	1.0
1.5	0.4	1.0	0	0

^zLinear regression of both shoot regeneration and number of adventitious buds per explant on 2,4-D concentration was significant at $\alpha \leq 0.05$.

^yMurashige-Skoog medium with TDZ, IBA and 2,4-D.

^xThe linear regression formula is $y = -32.83x + 32.316$.

^vThe linear regression formula is $y = -2.01x + 2.272$.

Table 5. Regeneration capacity of adventitious buds of strawberry after preculturing in shoot regeneration medium with AgNO₃ for 10 d.^z

AgNO ₃ ^y (mg·L ⁻¹)	Shoot regeneration ^x (%)	Adventitious buds/explant ^v (no.)
0	74.32 c ^z	4.98 d
0.5	83.95 b	9.06 b
1.0	87.38 a	11.67 a
2.0	73.61 d	6.81 c
4.0	39.48 e	2.97 e
6.0	0 f	0 f

^zPolynomial regression of both "shoot regeneration" and "number of adventitious buds per explant" on AgNO₃ concentration was significant at $\alpha \leq 0.05$. Means within a column followed by the same letters were not significantly different by SAS analysis using LSD at $\alpha \leq 0.05$.

^yMurashige-Skoog medium with TDZ, IBA, and AgNO₃.

^xThe polynomial regression formula is $y = -2.49x^2 + 0.97x + 81.34$.

^vThe polynomial regression formula is $y = -0.34x^2 + 0.59x + 7.81$.

Fig. 1. Shoot regeneration of strawberry cultured for 10 d in shoot regeneration medium with AgNO_3 . (a) MS + 1.5 $\text{mg}\cdot\text{L}^{-1}$ TDZ + 0.4 $\text{mg}\cdot\text{L}^{-1}$ IBA, (b) MS + 1.5 $\text{mg}\cdot\text{L}^{-1}$ TDZ + 0.4 $\text{mg}\cdot\text{L}^{-1}$ IBA + 0.5 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 , (c) MS + 1.5 $\text{mg}\cdot\text{L}^{-1}$ TDZ + 0.4 $\text{mg}\cdot\text{L}^{-1}$ IBA + 1.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 , (d) MS + 1.5 $\text{mg}\cdot\text{L}^{-1}$ TDZ + 0.4 $\text{mg}\cdot\text{L}^{-1}$ IBA + 2.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 , (e) MS + 1.5 $\text{mg}\cdot\text{L}^{-1}$ TDZ + 0.4 $\text{mg}\cdot\text{L}^{-1}$ IBA + 4.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 , (f) MS + 1.5 $\text{mg}\cdot\text{L}^{-1}$ TDZ + 0.4 $\text{mg}\cdot\text{L}^{-1}$ IBA + 6.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 .

Table 6. Rooting capacity and efficiency of strawberry as affected by AgNO_3 .

AgNO_3 ($\text{mg}\cdot\text{L}^{-1}$)	Root emergence peak (d)	Rooting (%)	Root length (cm)	Primary roots (no.)	Root dry wt (g)	Root water content (%)	Root activity [TTF $\mu\text{g}\cdot\text{g}^{-1}$ (fresh wt) $\cdot\text{h}^{-1}$]
0	9	91.10 ab ²	2.24 d	10.42 a	0.007 d	86.77 a	0.23 c
0.5	6	95.80 a	9.29 b	9.83 a	0.026 c	86.24 ab	0.42 b
1.0	6	93.68 a	14.32 a	10.08 a	0.046 a	85.58 ab	0.49 a
2.0	7	86.29 b	9.21 b	7.17 b	0.035 b	84.96 b	0.27 c
4.0	8	79.77 c	7.18 c	7.0 b	0.027 c	82.50 c	0.216 c

²Means within a column followed by the same letters were not significantly different by SAS analysis using LSD at $\alpha \leq 0.05$.

Number of days was recorded when most of the shoots induced roots.

Effects of AgNO_3 on rooting. Root primordia were observed after 5 to 6 d on rooting medium with optimum levels of AgNO_3 . Rooting was advanced 1 to 3 d as of the control (Table 6). Lower levels of AgNO_3 markedly improved

root length, dry weight and root activities (Fig. 2). AgNO_3 at 4.0 $\text{mg}\cdot\text{L}^{-1}$ significantly reduced root length and dry weight, however, they were higher than that of the control. At 1.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 , root length, dry weight and root ac-

tivity were increased by 6.39, 6.57, and 2.13 times respectively. In contrast to the control, no significant effect of AgNO_3 was observed on number of primary roots, root water content and rooting percentage below 1.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 . Higher concentrations of AgNO_3 inhibited rooting efficiency and primary roots formation. Root water content reduced progressively with increasing AgNO_3 concentration. It implied that AgNO_3 could promote the development of root lignification.

Effects of AgNO_3 on shoot growth. Stunted growth associated with shorter petioles and smaller leaves were observed in strawberry plants grown for 21 d in rooting medium with AgNO_3 . Compared with the control, no significant effect on shoot height was observed at ≤ 1.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 . Dry weights were gradually decreased in the presence of AgNO_3 (Table 7). Highest chlorophyll (4.58 $\text{mg}\cdot\text{g}^{-1}$ fresh weight) and soluble protein contents (521.80 $\mu\text{g}\cdot\text{g}^{-1}$ fresh weight) were recorded for 1.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 .

Effects of AgNO_3 on CAT-SOD-POD activities, O_2^- production rate and MDA contents. Significant differences of antioxidant enzyme activities, O_2^- production rate and MDA contents were observed with the addition of AgNO_3 in the medium. It had a significant stimula-

Fig. 2. Effect of AgNO_3 on growth of strawberry in vitro on half-strength MS medium supplemented with various concentrations of AgNO_3 . (a) 1/2MS, (b) 1/2MS + 0.5 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 , (c) 1/2MS + 1.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 , (d) 1/2MS + 2.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 , (e) 1/2MS + 4.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 , (f) 1/2MS + 0, 0.5, 1.0, 2.0, and 4.0 $\text{mg}\cdot\text{L}^{-1}$ AgNO_3 .

Table 7. Shoot growth of strawberry as affected by AgNO₃.

AgNO ₃ (mg·L ⁻¹)	Shoot ht (cm)	Shoot dry wt (g)	Chlorophyll content (mg·g ⁻¹ fresh wt)	Content of soluble protein (μg·g ⁻¹ fresh wt)
0	3.52 a ^z	0.097 a	4.286 ab	409.64 b
0.5	3.482 a	0.090 b	3.72 bc	486.34 a
1.0	3.62 a	0.0891 b	4.58 a	521.80 a
2.0	2.87 b	0.075 c	3.68 bc	346.86 b
4.0	2.45 c	0.060 d	3.48 c	254.67 c

^zMeans within a column followed by the same letters were not significantly different by SAS analysis using LSD at $\alpha \leq 0.05$.

Table 8. CAT, SOD, POD activities, O₂⁻ production rate and MDA content of strawberry as affected by AgNO₃.

AgNO ₃ (mg·L ⁻¹)	CAT activity (Unit/g fresh wt)	SOD activity (Unit/g fresh wt)	A-POD activity (Unit/g fresh wt)	G-POD activity (Unit/g fresh wt)	O ₂ ⁻ production rate (nmol·g ⁻¹ (fresh wt)·min ⁻¹)	MDA content (nmol/g fresh wt)
0	0.26 c ^z	290.51 c	0.22 e	0.74 d	5.85 c	4.43 d
0.5	0.39 bc	306.58 b	0.36 c	3.21 b	6.32 c	6.62 c
1.0	0.67 a	326.27 a	0.68 a	5.25 a	6.56 c	8.86 b
2.0	0.54 ab	294.94 c	0.55 b	3.40 b	10.17 b	9.54 b
4.0	0.45 abc	277.48 d	0.24 d	2.21 c	14.71 a	20.13 a

^zMeans within a column followed by the same letters were not significantly different by SAS analysis using LSD at $\alpha \leq 0.05$.

Table 9. Proline accumulation, IAA content, IAA-oxidase activity and ethylene production of strawberry as affected by AgNO₃.

AgNO ₃ (mg·L ⁻¹)	Proline content (μg·g ⁻¹ fresh wt)	IAA content (μg·g ⁻¹ fresh wt)	IAA-oxidase activity (μg IAA/g (FW)/h)	Ethylene production (nL·g ⁻¹ ·h ⁻¹)
0	26.88 e ^z	1.72 a	14.72 e	1.07 c
0.5	63.54 d	1.49 b	15.69 d	0.72 d
1.0	73.89 c	1.30 c	16.11 c	0.55 d
2.0	78.18 b	0.58 d	18.08 b	1.77 b
4.0	97.10 a	0.41 e	22.07 a	4.72 a

^zMeans within a column followed by the same letters were not significantly different by SAS analysis using LSD at $\alpha \leq 0.05$.

tory effect on antioxidant enzyme activities (Table 8). Maximum antioxidant enzyme activities were found in the presence of 1.0 mg·L⁻¹ AgNO₃, as compared to the minimum at 4.0 mg·L⁻¹ AgNO₃. It was observed that the toxic effect of higher concentrations of AgNO₃ could lead to necrotic leaves and darkened roots and poor plant growth. MDA content and O₂⁻ production rate gradually increased with the addition of 0 to 1.0 mg·L⁻¹ AgNO₃ and sharply increased thereafter.

Effects of AgNO₃ on proline accumulation, IAA content, IAA-oxidase activity and ethylene production. Significant differences in proline accumulation, IAA content and IAA-oxidase activity were observed during the growth of strawberry treated with AgNO₃. Higher proline accumulation and IAA-oxidase activities had a positive relationship with AgNO₃ concentration while IAA content had a negative relationship with AgNO₃ (Table 9). Compared to the control, AgNO₃ at 0.5 and 1.0 mg·L⁻¹ decreased ethylene production and promoted shoot regeneration and plant growth. There was no significant effect on ethylene production at ≤ 1.0 mg·L⁻¹ AgNO₃ concentration while ≥ 2.0 mg·L⁻¹ AgNO₃ significantly promoted ethylene production.

Discussion

Being a heavy ionic metal, AgNO₃ had dual effects on shoot regeneration and growth of strawberry. In this experiment, AgNO₃ at lower concentrations promoted shoot regeneration and plant growth. Lower concentrations of AgNO₃ inhibited ethylene production and promoted shoot regeneration and plant growth

while higher concentrations of AgNO₃ promoted ethylene production. Ethylene disrupts auxin translocation, induces hyperhydricity, reduces chlorophyll content and causes tissue mortality (Lentini et al., 1988). It has been reported that Ag⁺ interferes with the binding of ethylene receptor site and helps reduce ethylene production with promotion of polyamine biosynthesis (Roustan et al., 1990). The main function of AgNO₃ is to eliminate the potential danger to plant cells and tissues in liverwort caused by ethylene (Elmo and Beyer, 1979). In light of these reports, results in this study clearly indicated that specific binding of Ag⁺ to certain ethylene receptors was most likely responsible for its stimulating effect on shoot regeneration and rooting.

Root is the major organ that absorbs water and nutrients. Root activity is an indicator of growth and vigor of the plants (Jiang and Zhu, 1999). It has been reported that AgNO₃ at the appropriate concentrations enhanced in vitro faba bean root number, root growth rate and root length (Mutasim and Kazumi, 2000). In this study, it was observed that AgNO₃ at appropriate concentrations could promote rooting efficiency. Higher proline accumulation and IAA-oxidase activities had a positive relationship with AgNO₃. In our case proline accumulation, SOD, CAT and POD activities were promoted in the presence of AgNO₃. Antioxidant activities play a major role in plants scavenging system to get rid of O₂⁻, H₂O₂, ¹O₂ and ·OH during plant metabolism (Camp et al., 1997; Scandalios, 1993; Willekens and Inze, 1995). The O₂⁻ and MDA cause severe damage to plant cell. The former causes lipid peroxidation while the latter is a product of

membrane lipid peroxidation (Jiang et al., 1994). In our case, lower concentrations of AgNO₃ improved plant defense mechanism. O₂⁻ and MDA content significantly increased while antioxidant activities decreased in the presence of higher AgNO₃ concentrations. This may be due to the accumulation of H₂O₂ and high oxidation of membrane lipids.

In this experiment, IAA-oxidase activity and proline accumulation were increased with AgNO₃ while endogenous IAA content decreased. Reason for increasing rooting efficiency maybe attributed to the lower endogenous IAA content. Inverse-correlation between IAA content and IAA-oxidase activity was found in this study, which is in agreement with Jasdawala et al. (1977). IAA-oxidase is one of the key enzymes to regulate endogenous IAA level and interfere in auxin biosynthesis (Reinecke and Bandurski, 1988). Stresses induced proline accumulation in plants. Proline is involved in releasing stresses; protection of enzymes, organizing cellular structure and act as a free radical scavenger, but its specific role still remains a controversial subject (Delauney and Verma, 1993; Rensburg et al., 1993). In this study, proline content significantly increased in the presence of AgNO₃, which may have helped in protection of plant tissues from the toxic effects of AgNO₃. These findings confirm the previous reports stating that proline accumulation is helpful for plant to resist stresses (Delauney and Verma, 1993; Rensburg et al., 1993). The role of AgNO₃ in plant organogenesis has not yet been clearly understood and further research needs to be carried out.

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