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Effects of ethylene inhibitors, silver nitrate ($AgNO_3$), cobalt chloride ($CoCl_2$) and aminooxyacetic acid (AOA), on *in vitro* shoot induction and rooting of banana (*Musa acuminata L.*)

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Significant increase in shoot regeneration, leaf chlorophyll content and rooting occurred when silver nitrate ($AgNO_3$), cobalt chloride ($CoCl_2$) or aminooxyacetic acid (AOA) were added to banana culture medium. The highest numbers of shoots per explants shoot length and leaf surface area was obtained when media were supplemented with 10 mgl^{-1} $AgNO_3$. Number of shoots per explants increased three fold, shoot length and leaf surface area increased by an average of 4.5 and 2 cm^2, respectively, in comparison to control. $CoCl_2$ and AOA had less promotive effects on shoot generation with maximum shoot number per explant and shoot length achieved at 15 mgl^{-1}. Rooting of banana shoots *in vitro* was enhanced by these compounds. The highest number of roots per shoot (21.7) and the longest roots (12.68 cm) were observed when rooting media was supplemented with 10 mgl^{-1} $AgNO_3$. For $CoCl_2$ and AOA the maximum rooting occurred in media supplemented with 15 mgl^{-1}, although roots number and root length were lower than those achieved by $AgNO_3$. Considerable increase in leaf total chlorophyll content occurred in shoots grown on media containing $AgNO_3$ and AOA. The largest increase in leaf chlorophyll content (120%) was noted when shoots were grown in the presence of 10 mgl^{-1} $AgNO_3$. This was followed by AOA which increased chlorophyll content by 35%. $CoCl_2$ had no significant effect on leaf chlorophyll content. These findings suggest that application of ethylene inhibitors, particularly $AgNO_3$, to culture media may be useful for improving *in vitro* growth performance of banana cultures.

Key words: Ethylene inhibitors, banana, *Musa acuminata* L, *in vitro* culture.

INTRODUCTION

In recent years, several studies have demonstrated that ethylene accumulates in vessels of *in vitro* plant culture systems (Biddington, 1992; Zobayed et al., 1999; Fuentes et al., 2000). Accumulation of ethylene in culture vessels

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Abbreviations: DMRT, Duncan's multiple range test; **AgNO3,** silver nitrate; **CoCl2,** cobalt chloride; **AOA,** aminooxyacetic acid; **AVG,** aminoethoxyvinylglycine; **BAP,** benzylaminopurine; **IAA,** indole-3-acetic acid; **2iP,** 2-isopentenyladenine; **IBA,** indole-3-butyric acid.

may induce growth abnormalities of *in vitro* generated plants including inhibition of growth, leaf epinasty, leaf senescence and diminution of foliar area (Jaksonet al., 1991; Joosten and Woltering, 1994; Kumar et al., 1998; Zobayedet al.,2001; Zhang et al., 2001;Turhan, 2004; Giridhar et al., 2003; Mullins et al., 2006; Hazarika, 2006; Kepczyn´ska et al., 2009; Dang and Wei, 2009; Steinitz et al., 2010). However, the influence of ethylene in plant cells and tissues grown *in vitro* is diverse and often controversial depending on plant species and even the cultivar (Hu et al., 2006; Santana-Buzzy et al., 2006; Jhaet al., 2007). For instance, ethylene was reported to be important for shoot morphogenesis in rice callus (Adkins et al., 1990) and embryogenesis from anther cultures of *Hordeumvulgare* (Cho and Kasha, 1989). In contrast, ethylene accumulation was found to inhibit *in vitro* regeneration of several plant species (Huxteret al., 1981; Purnhauseret al., 1987; Chi et al., 1991; Gong and Pua, 2004). In fact, addition of ethylene inhibitors such as silver nitrate ($AgNO_3$), cobalt chloride ($CoCl_2$), aminooxy-acetic acid (AOA) and aminoethoxyvinylglycine (AVG) to culture media have been demonstrated to improve regeneration and growth performance of both dicot and monocot plant tissue cultures (Beyer, 1976; Duncan et al., 1985; Davies, 1987; Songstad et al.,1988; Chi and Pua, 1989; Veen and Over Beek, 1989; Bais et al., 2000; Giridhar et al., 2003; Kumar et al., 2007; Abdellatef and Khalafalla, 2008; Osman and Kalafalla, 2010; Sandra and Maira, 2013).

Clonal propagation of banana (*Musa acuminata L*.) via *in vitro* culture techniques have been extensively studied using different explants sources, basal media components and phytohormones levels (Al-*amin* et al., 2009; Ngomuo et al., 2014; Devendrakumar et al., 2013). However, to the best of our knowledge, no information on the effect of ethylene or ethylene inhibitors on *in vitro* culture of banana is available despite the recognized positive effects of these inhibitors on plant regeneration and growth *in vitro* (Kumar et al., 1998). In the present investi-gation, we compare the efficacy of the ethylene inhibitors silver nitrate ($AgNO_3$), cobalt chloride ($CoCl_2$) and aminooxyacetic acid (AOA) to assess their effects on shoot and root development of *invitro* cultured banana plants.

MATERIALS AND METHODS

Shoot tip explants of banana (*Musa acuminata L*.) (cultivar Grand Nain) were excised from young suckers grown in pots. Explants were surface sterilized with 75% ethanol for 50 s followed by 30 min with 40% commercial bleach (Clorox 5.75% NaOCl) to which few drops of Tween-20 were added. After complete washing with sterile distilled water, explants were trimmed to final size of 10 to 15 mm in the laminar flow cabinet. For culture initiation, explants were cultured in screw-capped glass vessels containing 30 ml of initiation media composed of MS basal salts (Murashige and Skoog, 1962) supplemented with sucrose (40 g l^{-1}), thiamine (0.1 g l^{-1}), benzylaminopurine (BAP) (12 µM), indole-3-acetic acid (IAA) (3 µM) And cystein HCl (40 mg l^{-1}). Medium was solidified with 2 g l^{-1} gelrite (Sigma Chemical Co., St. Louis) and its pH was adjusted to 5.8 before autoclaving at 121°C for 15 min. All cultures were incubated at 25°C under 16 h photoperiod for 4 weeks. Light intensity was 35 µmol $s^{-1}m^{-2}$.To evaluate the influence of $AgNO_3$, $CoCl_2$ and AOA on shoot multiplication and growth, banana shoot tip explants from *in vitro* initiated cultures were transferred to multiplication media. Multiplication medium contained MS basal salts, sucrose (40 g l^{-1}), thiamine (0.1 g l^{-1}), BAP (20 µM) and cystein HCl (40 mg l^{-1}) supplemented with different concentrations (0 to 25 mg/l) of $AgNO_3$, $CoCl_2$ and AOA individually. Cultures were arranged in a randomized block design with 10 replicates per treatments (3 explants per culture bottle) and incubated at 25°C under16 h photoperiod for 4 weeks. Light intensity was 35 µmol $s^{-1}m^{-2}$. After 4 weeks of culture, the number of shoots formed per explant, shoot length(cm) and leaf surface area (cm^2) were determined.

For the determination of leaf chlorophyll content, 0.25 gfresh leaf material of individual treatments was extracted in 5ml 80% acetone (v/v) and total chlorophyll content was determined according to Lichtenthaler (1987).For evaluating the effect of $AgNO_3$, $CoCl_2$ and AOA on *in vitro* rooting, uniform banana shoots formed on multiplication media were excised and transferred to rooting medium. Rooting medium consisted of MS basal slats, sucrose (40 g l^{-1}), 2-isopentenyladenine (2iP) (5µM) and indole-3-butyric acid (IBA) (0.1 µM) supplemented with different concentrations (0 to 25 mg/l) of $AgNO_3$, $CoCl_2$ and AOA individually. Medium was solidified with 1.8 g l^{-1} gelrite and its pH was adjusted to 5.8. Cultures, consisting of 10 replicates per treatment, were incubated at 25°Cunder16 h photoperiod. After 4 weeks the number of roots formed per shoot and root lengths (cm) were estimated.All data were expressed as means of all replicates ± standard error. Means were separated by Duncan's multiple range test (DMRT) (Duncan, 1955) at 5% significance level.

RESULTS

The effect of the ethylene inhibitors $AgNO_3$, $CoCl_2$ and AOA on *in vitro* shoot regeneration of banana is presented in Table 1. In the control experiment low shoot regeneration (2.37 shoots/explant) with an average shoot length of 2.89 cm and mean leaf surface area of 3.74 cm^2 were observed. Presence of varying concentrations of $AgNO_3$ in the shoot multiplication medium had strong positive effect on shoot multiplicationand a maximum of 6.68 shoots/explant was achieved at10 mgl^{-1} $AgNO_3$ .This concentration was also the most effective in promoting shoot growth increasing shoot length and leaf surface area by 150 and 58%, respectively, compared to control. Addition of $CoCl_2$ or AOA to multiplication media was also beneficial to banana shoot multiplication although less effective than $AgNO_3$. The highest number of shoots/explant was observed in media supplemented with 15mgl^{-1} $CoCl_2$ or AOA giving an average of 4.59 and 5.12 shoots/explants, respectively. Among these treatments only $CoCl_2$ increased shoot length to a maximum of 85% relative to control when added to the medium at15mgl^{-1}. Application of 5 to 15 mgl^{-1} AOA to multiplication media did not, however, influence shoot length and higher concentrations inhibited shoot elongation. Leaf surface area on the other hand, was not significantly affected by all concentrations of $CoCl_2$ tested whereas considerablereduction in leaf surface area was

Table 1. Effects of various concentrations of $AgNO_3$, $CoCl_2$ and AOA on shoot regeneration from shoot tips of bananaafter 4 weeks of culture *in vitro.*

Treatment	Concentration (mg/L)	Mean number of shoots/ explant ± SE	Mean shoot length ± SE (cm)	Mean leaf surface area ± SE (cm^2)
Control	0	2.37 ± 0.18[a]	2.89 ± 0.31[a]	3.74 ± 0.45[a]
Silver nitrate ($AgNO_3$)	5	3.24 ± 0.26[b]	4.62 ± 0.43[b]	4.21 ± 0.32[b]
	10	6.68 ± 0.38[c]	7.42 ± 0.25[c]	5.92 ± 0.58[c]
	15	4.54 ± 0.21[d]	5.87 ± 0.38[d]	4.86 ± 0.44[b]
	20	3.05 ± 0.19[b]	3.95 ± 0.26[e]	4.12 ± 0.36[b]
	25	2.76 ± 0.11[a]	2.85 ± 0.24[a]	3.68 ± 0.14[a])
Cobalt chloride ($CoCl_2$)	5	3.17 ± 0.42[b]	3.45 ± 0.36[e]	3.62 ± 0 .23[a]
	10	3.62 ± 0.38[b]	4.12 ± 0.24[b]	3.78 ± 0.36[a]
	15	4.59 ± 0.43[d]	5.35 ± 0.22[d]	3.96 ± 0.28[a]
	20	3.86 ± 0.35[b]	4.87 ± 0.44[b]	3.72 ± 0.39[a]
	25	3.21 ± 0.21[b]	3.68± 0.38[e])	3.72 ± 0.46[a])
Amino-oxyacetic acid (AOA)	5	2.89 ± 0.32[b]	2.72 ± 0.47[a]	3.07 ± 0.22[a]
	10	3.76 ± 0.38[b]	2.58 ± 0.14[a]	2.83 ± 0.15[a]
	15	5.12 ± 0.35[e]	2.94 ± 0.42[a]	2.62 ± 0.29[a]
	20	3.89 ± 0.46[b]	2.55 ± 0.24[a]	2.46 ± 0.10[b]
	25	2.16 ± 0.12[a]	2.10± 0.22[a]	1.95 ± 0.12[b]

Data are means of 10 replicates with 3 explants per replicate. Means followed by different alphabet denote significant differences within column based on DMRT (p = 0.05).

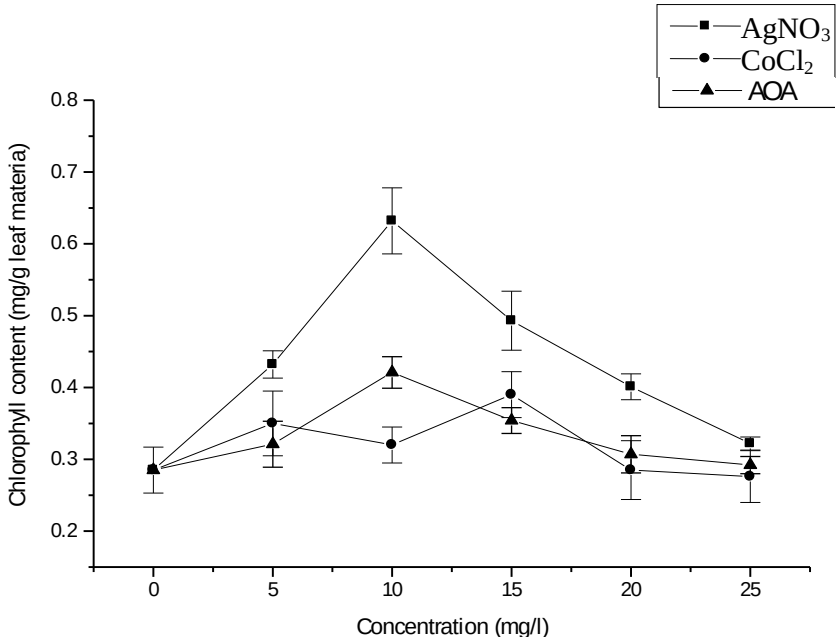

Figure 1. Effects of various concentrations of $AgNO_3$, $CoCl_2$ and AOA on total chlorophyll content in leaves of banana shoots cultivated *in vitro* for 4 weeks. Data are means of 10 replicates ± SE.

noticed in the presence of increasing concentrations of AOA in the medium.

The data presented in Figure 1 shows that application of 5 to 20 mgl^{-1} $AgNO_3$ to banana multiplication media resulted in a significant increase in total leaf chlorophyll content. The highest amount of chlorophyll was observed

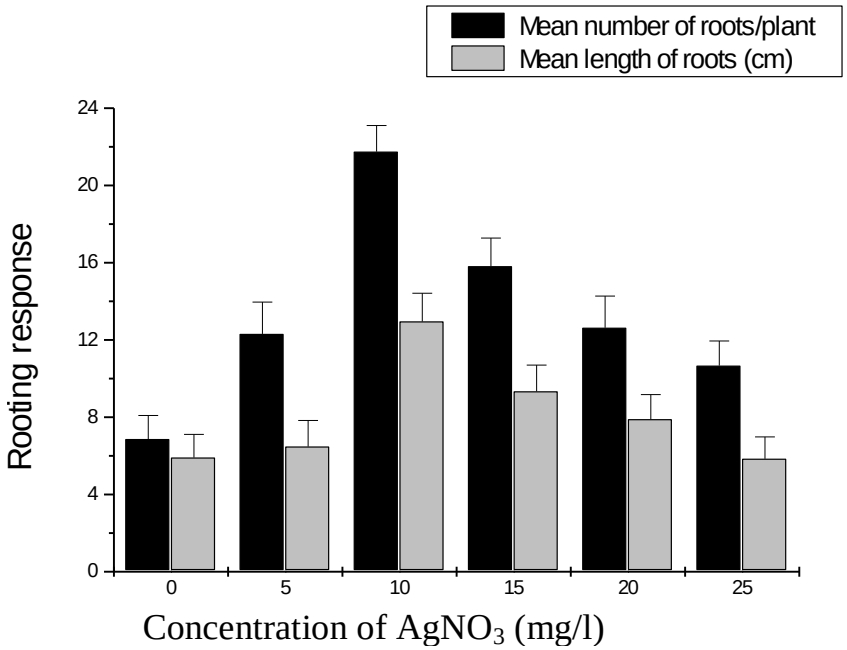

Figure 2. Effects of various concentrations of $AgNO_3$ (mg/L) on rooting of *in vitro* grown banana shoots after 4 weeks of culture. Data are means of 10 replicates± SE.

at 10 mgl^{-1} $AgNO_3$. At this concentration total leaf chlorophyll content increased by 120% compared to control. Treatment with AOA, however, resulted in a lower increase in total leaf chlorophyll content reaching a maximum of 35% over control at a concentration of 10 mgl^{-1}. On the contrary, $CoCl_2$ treatment had no significant effect on leaf chlorophyll content.The presence and concentration of $AgNO_3$ in the rooting medium had significant effect on rooting in banana (Figure 2). The highest number of roots/explant and the highest root growth were achieved on medium containing 10 mgl^{-1} $AgNO_3$ (Plate 1). At this concentration silver nitrate increased root formation and mean root length by 190 and 115%, respectively, relative to control. Incorporation of $CoCl_2$ and AOA in the rooting medium also stimulated rooting but proved to be less effective than $AgNO_3$ (Figures 3 and 4). The best concentrations of $CoCl_2$ and AOA for rooting (15 mgl^{-1}) produced 85 and 115% more roots/ explant, respectively, compared to control. Contrary to $CoCl_2$ treatment which had no significant effect on root length, treatment with 15 mgl^{-1} AOA resulted in 80% increase in root growth relative to control.

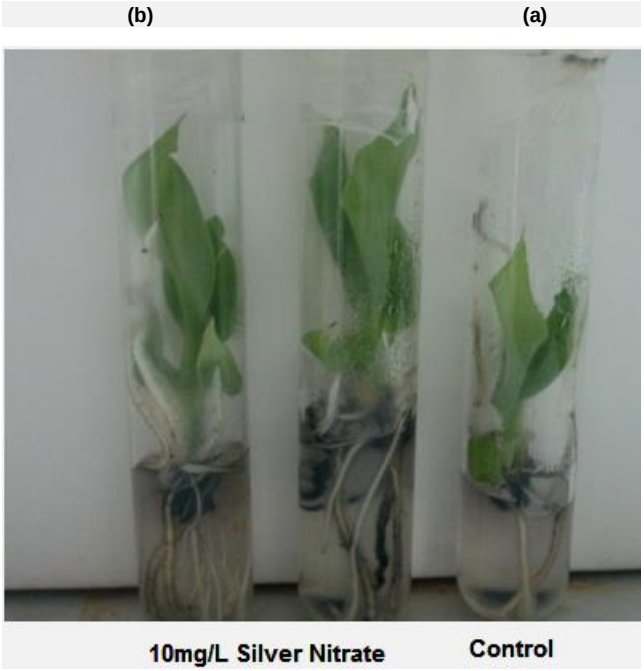

Plate 1. Rooting and shoot growth of *in vitro* grown banana shoots after 4 weeks of culture. **(a)** Control, **(b)** media supplemented with 10 mgl^{-1} $AgNO_3$.

DISCUSSION

In the present study the influence of ethylene inhibitors $AgNO_3$, $CoCl_2$ and AOA on *in vitro* culture of banana (*Musa acuminata L)* was investigated. The results show that the use of ethylene inhibitors in culture media can enhance the ability of banana shoot tip culture to produce higher number of shoots per explant along with shoot

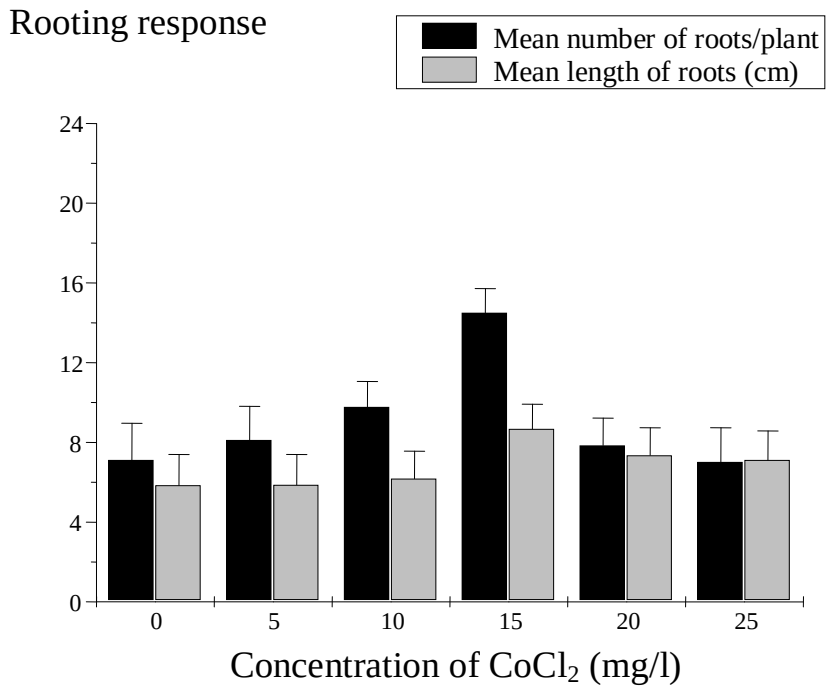

Figure 3. Effects of various concentrations of $CoCl_2$ (mg/L) on rooting of *in vitro* grown banana shoots after 4 weeks of culture. Data are means of 10 replicates± SE.

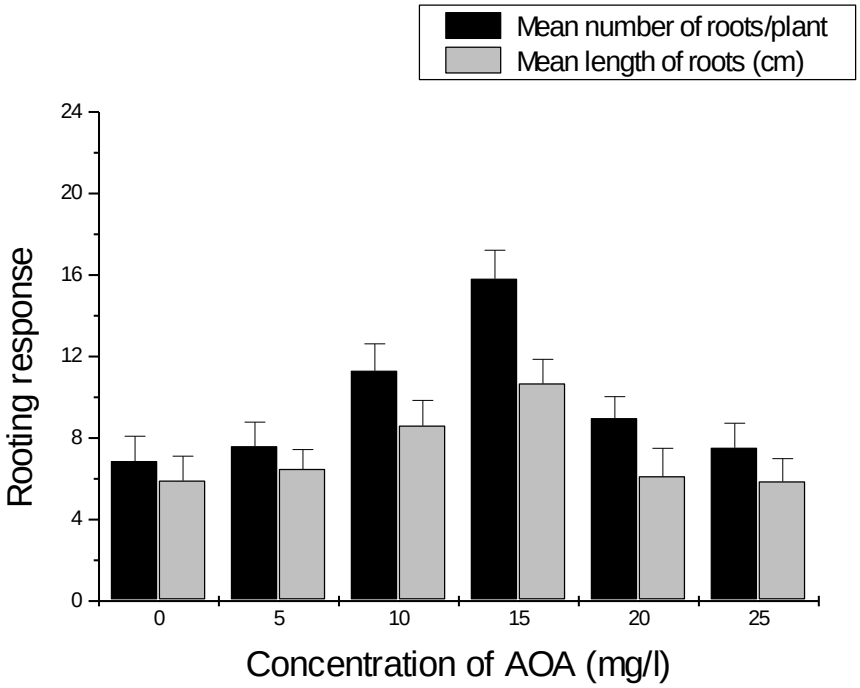

Figure 4. Effects of various concentrations of AOA (mg/L) on rooting of *in vitro* grown banana shoots after 4 weeks of culture. Data are means of 10 replicates± SE.

elongation and leaf expansion. The maximum number of shoots as well as shoot length and leaf surface area was achieved on media supplemented with $10mgl^{-1}$ $AgNO_3$; shoot number/expant were 3 times higher, shoots formed

were 4.5cm longer and leaf surface area were 2 cm^2 greater than those recorded on media without $AgNO_3$. This result agrees with previously reported findings demonstrating the stimulative role of $AgNO_3$ on shoot organogenesis in many plants such as coffee sp., (Giridhar et al., 2003), strawberry (Qin et al., 2005), sweet potato (Gong et al., 2005), sesame (Abdellatef et al., 2010), tomato (Osman and Khalafalla, 2010) and turmeric (Dikashet al.,2012). For $CoCl_2$ and AOA treatments, higher concentration (15 mgl^{-1}) were required to enhanced banana shoot development, although, lower number of shoots/explant were achieved compared to $AgNO_3$ treatment. While, shoot length was improved by $CoCl_2$ treatment, no positive effect on leaf surface area was noted by either of these compounds.

It is well known that $AgNO_3$ is a potent inhibitor of ethylene action (Beyer, 1979; Veen and Overbeek, 1989; Pua and Chi, 1993) whereas $CoCl_2$ and AOA are known to inhibit the enzymes ACC synthase and ACC oxidase involved in ethylene biosynthesis (Satoh and Esashi, 1980; Yang and Hoffman, 1984; Abeles et al., 1992). There is also accumulating evidence suggesting that *in vitro* tissue cultures produce ethylene in sealed containers (Chi et al., 1991). In addition there have been several reports indicating that ethylene produced during *in vitro* culture impairs plant growth and development and could limit *in vitro* propagation of several plants (Biddington, 1992; Pua and Chi, 1993; Chraibi et al., 1991). Accordingly, the findings of this study may suggest that ethylene inhibitors, particularly $AgNO_3$ alleviated the negative effects of ethylene on the growth of banana culture *in vitro.* Support for this suggestion comes from the finding that these compounds are also capable of increasing chlorophyll content in banana leaves (Figure 1). The association of ethylene with senescence of plant parts is well known (Jona et al., 1997) and its negative effect on chlorophyll content of plants was documented. For example, Jakob-Will et al. (1999) reported that ethylene induced expression of chlorophyllase genes (*Chlase*) in citrus fruits. There are also some reports suggesting that inhibition of ethylene action by silver ions increased leaf chlorophyll content (Ehsanpour and Jones, 2001; Perl et al., 1988). Apparently, addition of $AgNO_3$ and to a lesser extent $CoCl_2$ and AOA to banana culture media could improve shoot multiplication and promote the maintenance of green healthy *in vitro* tissue for long time periods.

In addition to their positive effects on *in vitro* shoot growth and development, $AgNO_3$, $CoCl_2$ or AOA incorporated into banana rooting media also improved rooting of *in vitro* produced banana shoots. Among these compounds $AgNO_3$ resulted in the best rooting response. Shoots in rooting media supplemented with 10mgl^{-1} $AgNO_3$ produced 3 times more roots and increased root length by 7 to 8 cm. The optimal concentration of $CoCl_2$ and AOA for rooting was achieved at 15 mgl^{-1}. This concentration resulted in approximately two fold increase in the number of roots formed per shoot, compared to control with limited or no significant influence on root elongation. These observations are in accordance with previous findings demonstrating improvement of *in vitro* rooting of plants by ethylene inhibitors such as *Decalepis hamiltonii* (Bais et al.,2000; Reddy et al.,2001), coffee (Giridhar et al.,2003) and apple (Ma et al.,1998).

In conclusionthe findings of this study demonstrate that ethylene inhibitors particularly $AgNO_3$ and to a lesser extent $CoCl_2$ and AOA enhanced vigour of banana shoots proliferation *in vitro* along with leaf expansion and shoot elongation; possessed the potential to protect developing banana leaves from ethylene induced senescence by maintaining high leaf chlorophyll content and increased the rooting capacity of *in vitro* grown banana shoots. Taken together, this study suggests that $AgNO_3$, $CoCl_2$ and AOA may be used as important tools for improving protocols of banana cultures *in vitro* and for protecting *in vitro* cultured banana tissue from the possible negative effects of ethylene in culture vessels.

Conflict of interests

The author did not declare any conflict of interest.

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REFERENCES

Abdellatef E,Khalafalla MM (2008). Ethylene Inhibitors Promote *in vitro* Regeneration of Medium Staple Cotton (*Gossypiumhirsutum*L.) Cultivar Barac B- 67. Adv. Nat. Appl. Sci. 2(3):178-184.

Abdellatef E, Ahmed M,Daffall H,Khalafalla M (2010). Enhancement of adventitious shoot regeneration in sesame (*Sesamumindicum* L.) cultivar Promo ky using ethylene inhibitors. J. Phytol. 2(1): 61-67

Abeles FB, Morgan PW, Saltiveit ME (1992).Ethylene in Plant Biology. 2nd Ed, AcademicPress, San Diego.

Adkins SW, Shiraishi T,McComb JA (1990). Rice callus physiology-identification of volatile emissions and their effects on culture growth. Physiol. Plant. 78: 526-531

Al-amin MD, Karim MR, Amin MR, Rahman S,Mamun ANM (2009). *In vitro*micropropagation of banana *(Musa* spp.) Bangladesh J. Agric. Res. 34(4):645-659.

Bais HP,Sudha G, Suresh B, Ravishankar GA (2000). $AgNO_3$ influences *in vitro* root formation in *Decalepishamiltonii*Wight, Arn. Curr.Sci. 79: 894-898.

Beyer E (1976). Silver ion: a potent anti-ethylene agent in cucumber and tomato. Hortic. Sci.11: 195-196.

Beyer EM (1979).Effect of Silver Ion, Carbon Dioxide, and Oxygen on Ethylene Action and Metabolism. Plant Physiol. 63: 169-173.

Biddington NL (1992). The influence of ethylene in plant tissue culture. Plant Growth Regul. 11:173–178.

Chi GL, Pua EC (1989). Ethylene inhibitors enhanced *de novo* shoot regeneration from cotyledons of *Brassica campestris* spp *in vitro*. Plant Sci. 64:243-250.

Chi GL , Pua EC, Goh C J (1991). Role of ethylene on *de novo* shoot regeneration from cotyledonary explants of *Brassica campestris*ssp.

Pekinensis(Lour) olsson*in vitro*. Plant Physiol. 96:178-183.
Cho U, Kasha KJ (1989). Ethylene production and embryogenesis from anther cultures of Barley (*Hordeumvulgare*). Plant Cell Rep. 8:415-417.
Chraibi BKM, Latche A, Raustan P,Fallot J (1991). Stimulation of shoot regeneration from cotyledons of *Helianthus annuus*by ethylene inhibitors silver and cobalt. Plant Cell Rep. 10: 204-207
Dang W, Wei ZM (2009).High frequency plant regeneration from the cotyledonary node of common bean.Biol. Plant.53(2):312-316.
Davies PJ (1987). The plant hormones: their nature, occurrence, and functions. In: Davies PJ (Ed.). Plant Hormones and Their role in Plant Growth and Development. Ch. Al. Boston: MartinusNijhoff.
Devendrakumar D, Anbazhagan M,Rajendran R (2013). *In Vitro* propagation of Banana *(Musa acuminate* L.) cv.Cavandish Dwarf. Int. J. Res.Biomed.Biotechnol. 3(3):44-46.
Dikash S, Devala D, Punyarani K, Henary S, Brojendro SS, Brajakishor S,Sunitibala HD (2012). Silver nitrate and different culture vessels influence high frequency microrhizome induction *in vitro* and enhancement growth of turmeric plantlet during *ex vitro* acclimatization. Not. Sci. Biol. 4(4):67-78.
Duncan DB (1955). Multiple range and multiple F test. Biometrics 11:1-42
Duncan DR, Williams ME, Zehr B, Widholm JM (1985). The production of callus capable of plant regeneration from immature embryos of numerous *Zea mays* genotypes.Planta 165 (3): 322-332.
Ehsanpour AA, Jones MGK (2001). Plant regeneration from mesophyll protoplasts of potato (*Solanumtuberosum*L.) cultivar Delaware using silver thiosulfate (STS).J.Sci. Islamic Repub. Iran 12 (2): 103-110.
Fuentes SRL, Calheiros MB, Manetti-Filho, Vieira LGE (2000). The effects of silver nitrate and different carbohydrate sources on somatic embryogenesis in *Coffeacanephora*.Plant Cell Tissue Organ Cult.60 (1):5-13.
Giridhar P, Indu E P, RamuVD,Ravishankar GA (2003). Effect of silver nitrate on *in vitro* shoot growth of Coffee. Trop. Sci. 43:144-146.
Gong H, Pua E (2004). Identification and expression of genes associated with shoot regeneration from leaf disc explants of mustard (*Brassica juncea*) *in vitro*. Plant Sci. 167(6):1191-1201.
Gong Y, Gao F, Tang K (2005). *In vitro* high frequency direct root and shoot regeneration in sweet potato using the ethylene inhibitor silver nitrate. South Afr. J. Bot. 71:110-113.
Hazarika BN (2006). Morpho-physiological disorders in *in vitro* culture of plants.SciHortic.108 (2): 105-120.
Hu W-W, Gong H,Pua EC (2006). Modulation of SAMDC expression in *Arabidopsis thaliana* alters in vitro shoot organogenesis. Physiol. Plant. 128:740-750
Huxter JT, Thorpe TA, Reid DM (1981). Shoot initiation in light and dark grown tobacco callus; the role of ethylene. Physiol. Plant. 53: 319–326.
Jakob-Will D, Holland D, Goldschmidt E E, Riov J,Eyal Y (1999). Chlorophyll breakdown by chlorophyllase: isolation and functional expression of the *Chlase 1* gene from ethylene-treated *Citrus* fruit and its regulation during development. Plant J. 20:653-661.
JaksonMB, Abbott AJ, Belcher AR, Hall KC, Butler R, Cameron J (1991). Ventilation in plant tissue culture causes dioxide accumulation oxygen depletion and explants development. Ann. Bot. 67(3):229-237.
Jha AK, Dahleen LS, Suttle JC (2007). Ethylene influences green plant regeneration from barley callus. Plant Cell Rep. 26: 285-290
JonaR, Cattro A,Travaglio D (1997). Chlorophyll content as index of ethylene inside culture vessels.ActaHortic. 447:229-230.
Joosten -BuddendorfJMC,WolteringEJ (1994).Components of the gaseous environment and their effects on plant growth and development *in vitro*. Plant Growth Regul.15(1):1-16 .
Kępczyńska E, Ruduś I,Kępczyński J(2009). Endogenous ethylene in indirect somatic embryogenesis of *Medicagosativa* L. Plant Growth Regul. 59:63-73.
Kumar PP, Lakshmanan P, Thorpe TA (1998). Regulation of morphogenesis in plant tissue culture by ethylene.*In Vitro* Cell.Dev. Biol. 34 (2): 94–103.
Kumar V, Ramakrishna A, Ravishankar GA (2007). Influence of different ethylene inhibitors on somatic embryogenesis and secondary embryogenesis from *Coffeacanephora*P ex Fr. Plant Cell Tissue Organ Cult. 43(6):602-607.
Lichtenthaler HK (1987). Chlorophylls and carotenoids: pigments of photosyntheticbiomembranes. Methods Enzymol. 148: 350-382.
Ma JH, Yao JL, Cohen D, Morris B (1998). Ethylene inhibitors enhance *in vitro* root formation from apple shoot cultures. Plant Cell Rep. 17:211-214.
Mullins E, Milbourne D, Petti C, Doyle-Prestwich BM, Meade C (2006).Potato in the age of biotechnology. Trends Plant Sci. 11(5): 254-260.
Murashige T,Skoog F (1962). A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15:473-497.
Ngomuo M, Mneney E,Ndakidemi PA (2014). Producing banana using shoot tip cultures.Am. J. Plant Sci. 5:1614-1622
Osman MG,Khalafalla M (2010). Promotion of in vitro shoot formation from shoot tip of tomato (*Lycopersiconesculentum* mill. cv. omdurman) by ethylene inhibitors. Int. J. Curr. Res. 4:82-86.
Perl A, Aviv D,Galun E (1988). Ethylene and *in vitro* culture of potato: suppression of ethylene generation vastly improves protoplast yield, plating efficiency and transient expression of an alien gene. Plant Cell Rep. 7(6): 403-406.
Pua EC, Chi GL (1993). *De novo* shoot morphogenesis and plant growth of mustard (*Brassica juncea*) *in vitro* in relation to ethylene. Physiol. Plant. 88:467-474.
Purnhauser L, Medgysey P, Czako M, Dix JP, Marton L (1987). Stimulation of shoot regeneration in *Triticum aestivum* and *Nicotiana plumbaginifolia* via Tissue cultures using the ethylene inhibitor silver nitrate. Plant Cell Rep. 6:1-4.
Qin Y, Zhang S, Zhang LX, Zhu D,Asghar S (2005). Response of strawberry cv. Toyonoka*in vitro* to silver nitrate ($AgNO_3$).Hortscience 40:747-751.
Reddy BO, Giridhar P, Ravishankar GA (2001). *In vitro* rooting of *Decalepishamiltonii* Wight and Arn., an endangered shrub by auxins and root-promoting agents. Curr.Sci. 81(11):1479-1481.
Sandra AT,Maira O *(*2013). Effect of culture medium consistence and silver nitrate on micropropagation of two potato (*Solanumtuberosum*) cultivars. Rev. Colomb. Biotecnol. 15(2):55-62
Santana-Buzzy N, Canto-Flick A, Iglesias-Andrue LG, Montalvo-Peniche MC, Lopez-Puc G,Barahona-Perez F (2006). Improvement of *in vitro* culturing of Habanero Pepper by inhibition of ethylene effects. Hort. Sci.42:405-409.
Satoh S,Esashi Y (1980). a-Aminoisobutyric acid: A probable competitive inhibitor of conversion of 1 -aminocyclo propane-1 -carboxylic acid to ethylene. Plant Cell Physiol. 21:939-949.
Songstad DD, Duncan DR, Widholm JM (1988). Effect of 1-aminocyclopropane-1carboxylic acid, silver nitrate and non-bornadiene on plant regeneration from maize callus cultures. Plant Cell Rep.7:262-265.
Steinitz B, Barr N, Tabib Y, Vaknin Y, Bernstein N (2010). Control of *in vitro* rooting and plant development in *Corymbia maculate* by silver nitrate, silver thiosulfate and thiosulfate ion. Plant Cell Rep. 29 (11) : 1315-1323.
Turhan H (2004). The effect of silver nitrate (ethylene inhibitor) on *in vitro* shoot development in potato (*Solanumtuberosum*L.). Biotechnology 3(1):72-74.
Veen H, Over Beek JHM (1989).The action of silver thiosulphate in carnation petals. In: Cligsters, H.D.; Proft, M.; Marcelle, R. and Van Poucke, M. eds. Biochemical and physiological aspects of ethylene production in lower and higher plants. Kluwer Academic publication Dordedrecht, the Netherlands. pp. 109-117.
Yang SF, Hoffman NE (1984).Ethylene biosynthesis and its regulation in higher plants.Annu. Rev. Plant Physiol. 35:155-189.
Zhang P, Phansiri S,Puonti KJ (2001). Improvements of Cassava shoot organogenesis by the use of silver nitrate in vitro. Plant Cell Tissue Organ Cult. 67:45-54.
ZobayedSMA, Armstrong J, Armstrong W (1999). Evaluation of a closed system, diffuse and humidity-induced convective through flow ventilation on the growth and physiology of cauliflower *in vitro*.Plant Cell Tissue Organ Cult. 59:113-123
Zobayed SMA, Armstrong J, Armstrong W (2001). Micropropagation of potato: Evaluation of closed, diffusive and forced ventilation on growth and tuberization. Ann. Bot.87(1):53-59.