식물생명공학회지 제33권 제4호
J Plant Biotechnol
Vol. 33, No. 4, 249-255 (2006)

Effect of Ethylene Inhibitors on *In Vitro* Shoot Multiplication and their Impact on Ethylene Production in Cucumber (*Cucumis sativus* L.)

A. Vasudevan[1]* N. Selvaraj[2], A. Ganapathi[3], V. Ramesh Anbazhagan[4] and C.W. Choi[1]

[1]Department of Biology and Medicinal Science, Pai Chai University, Daejeon 302-735, Korea
[2]Department of Botany, Periyar E.V.R. College (Autonomous), Tiruchirappalli 620-023, Tamilnadu, India
[3]Department of Biotechnology, Bharathidasan University, Tiruchirappalli 620-024, Tamilnadu, India
[4]Division of Forest Resources, College of Forest Sciences, Kangwon National University, Chunchon 200-701, Korea

Abstract

Effects of ethylene inhibitors like silver nitrate ($AgNO_3$), cobalt chloride ($CoCl_2$) and salicylic acid (SA) on multiple shoot induction and their impact on ethylene production using embryonal cotyledon cultures of *Cucumis sativus* L. were examined. The optimum concentration of $AgNO_3$ (40 μM), $CoCl_2$ (20 μM) and SA (20 μM), separately, induced maximum number of shoots on Murashige and Skoog's (MS) medium supplemented optimally with 4.44 μM BA and 0.25 μM NAA. Among the three ethylene inhibitors tested, $AgNO_3$ produced maximum number of shoots when compared to $CoCl_2$ and SA. Ethylene production was monitored in all the treatments with $AgNO_3$/$CoCl_2$/SA and it was observed that the treatment with $AgNO_3$ alone showed increase in ethylene production when compared to $CoCl_2$ and SA. Even though ethylene concentration was the highest in $AgNO_3$ treated explants, maximum number of shoots was obtained.

Introduction

Cucumber (*Cucumis sativus* L.) is one of the major vegetables in the tropics, subtropics and milder areas of the temperate zones of both hemispheres. In recent years, cucumber tissue culture has been employed to produce cultivars with improved agronomic traits such as virus resistance (Chee and Slightom 1991) and fungal resistance (Raharjo et al. 1996, Tabei et al. 1998). However, plant regeneration in cucumber still encounters many problems such as abnormal embryo development, poor differentiation of callus into shoots, poor survival of regenerated plants in soil and undesirable changes in ploidy level of regenerated plants (Ziv and Gadasi 1986, Malepszy 1988, Gambley and Dodd 1990). Further, the frequency of regeneration in earlier studies was dependent on the source of explants, the cultivars, growth regulator combinations and physical conditions of culture.

In recent years, there has been accumulating evidences that growth and differentiation of plant cells and tissues *in vitro* can be affected considerably by ethylene. Ethylene is readily produced by plants and its production is associated with poor regeneration or recalcitrance of culture materials (Chi et al. 1990). Many reports have demonstrated the positive effect of $AgNO_3$ on plant tissue culture (Mohiuddin et al. 1997, Mhatre et al. 1998, Saly et al. 2002). Ethylene inhibitors such as silver nitrate ($AgNO_3$), cobalt chloride ($CoCl_2$) and salicylic acid (SA) have been shown to be effective for shoot regeneration by inhibiting the ethylene production in cucumber (Roustan et al. 1992, Mohiuddin et al. 1997, Mhatre et al. 1998), muskmelon (Yadav et al. 1996), or its function by blocking certain steps in the pathway of ethylene synthesis in *Brassica campestris* (Pua et al. 1996) and *Chicorium intybus* (Bais et al. 1999). Addition of these compounds *in vitro* may be helpful for overcoming the recalcitrance or in enhancing regeneration. The present study aimed to determine the effect of ethylene inhibitors on shoot regeneration efficiency from the embryonal explants of an important commercial cucumber cultivar Poinsett 76. In particular, we examined the interaction of ethylene and other plant growth regulator, SA. In addition, ethylene production from cultured explants and its impact on shoot induction frequency was investigated at the first time. Such studies are providing an update in regenerating large number of shoots from a single explant.

* Corresponding author: e-mail: vasudevan@pcu.ac.kr
Received Mar. 30, 2006 ; Accepted Oct. 30, 2006

Materials and Methods

Culture Method

Seeds of *Cucumis sativus* L. cv. Poinsett 76 (Indo-American hybrid seeds Ltd., Bangalore, India) were soaked in tap water for 15 min disinfested with 70% alcohol (v/v) for 1 min and 2.5% (v/v) commercial bleach 'Teepol' (5.25% sodium hypochlorite; Reckitt and Benckiser of India Ltd, Kolkatta, India) for 15 min followed by three rinses with sterile distilled water. Seeds were further disinfested by soaking in 0.1% mercuric chloride (w/v) for 8 min and germinated in darkness for 48 h in 25 X 150 mm test tubes (Borosil, India) containing sterile moist cotton. Embryonal cotyledon explants were isolated from one day-old germinating seeds under sterile conditions and were used as explants. To optimize the concentration of phytohormone for shoot regeneration, embryonal cotyledon explants were cultured on ten ml of shoot regeneration medium which was agar-solidified MS (Murashige and Skoog 1962) medium supplemented with different concentrations and combinations of 6-benzyladenine (BA) (0-8.88 μM), kinetin (Kn) (0-9.2 μM) and α-naphthalene acetic acid (NAA) (0.05-1.0 μM). The effect of $AgNO_3$, $CoCl_2$ and SA were investigated by culturing the explants on MS medium containing 4.44 μM BA and 0.25 μM NAA with different concentrations of $AgNO_3$ (10-50 μM), $CoCl_2$ (10-40 μM) and SA (10-40 μM) individually. Silver nitrate and SA were filter sterilized through 0.22 μm Millipore filter (Sigma, USA) and added to the medium after autoclaving. After 4 wk of culture, the adventitious shoots that formed on the explants were counted. Shoots developed from the explants were excised and transferred to rooting medium containing different concentrations of IBA (0.4, 2.4, 4.9 and 7.3 μM; Indole-3-butyric acid). The medium was adjusted to pH 5.8 before autoclaving at 121°C for 15 min. All cultures were incubated at 25°C under 16 h photoperiod of cool-white illumination (Philips, India) at 30 μmol m^{-2} s^{-1}.

Acclimatization

Plants with profuse rooting were thoroughly washed in tap water to remove the agar and later transplanted to plastic pots containing a mixture of autoclaved sand, soil and vermiculite (1:1:1, v/v/v). Potted plants were grown in a growth chamber at 85% relative humidity for 4 wk. Upon new leaf growth, the plants were kept in a shade house for 4 wk before transferring to the field.

Statistical Analysis

Regeneration frequency (number of explants with shoots/total number of explants cultured) and shoot number per explant were assessed for 20 replicates. A complete randomized design was used in all experiments and a one-way analysis of variance (ANOVA) and comparisons between the mean values of treatments were carried out using Duncan's Multiple Range Test (DMRT). Significance was determined at the 5% level (Gomez and Gomez 1976).

Ethylene Measurement

The embryonal cotyledon explant was cultured in a 9-ml glass tube (Borosil, India) (5 cm in length, 1 cm in diameter) containing 2.5 ml MS medium with or without different concentrations of $AgNO_3$ (10-50 μM), $CoCl_2$ (10-50 μM) and SA (10-50 μM). The glass tube was sealed with an airtight seal septum until ethylene measurement. Each treatment consisted of five to six replicates with each glass tube containing one explant. Cultures were allowed to stand hood and at the end of 3 h, one milliliter of gas was sampled from each glass tube and measured using gas-chromatograph (Theologis 1992). Ethylene produced by the cultured explant was examined at the interval of one week each during 4 wk culture period.

Results and Discussion

Optimization of Culture Conditions for Shoot Regeneration

Among the different concentrations and combinations of BA, Kn and NAA tested, BA (4.44 μM) with NAA (0.25 μM) was found favorable for adventitious shoot induction and multiplication with a frequency of 62.6% for embryonal cotyledon explant (Table 1). In this medium, each explant regenerated 9.2 shoots. In the absence of plant growth regulators (control), no shoot regeneration was observed. Similarly BA and NAA induced multiple shoots from various explants of cucumber viz., cotyledon (Selvaraj 2002), shoot tip (Vasudevan et al. 2001, Selvaraj 2002), hypocotyl (Wehner and Locy 1981, Rajasekaran et al. 1983, Selvaraj 2002), and shoot tip of *Momordica dioica* (Shiragave and Chavan 2001). Hence, a combination of 4.44 μM BA and 0.25 μM NAA was chosen as a basal medium for investigating the effect of $AgNO_3$, $CoCl_2$ and SA on their role in enhancing shoot regeneration frequency and other growth parameters.

Effect of Ethylene Inhibitors on Shoot Regeneration

In the present study the addition of $AgNO_3$ (10-50 μM) was beneficial to shoot regeneration (Table 2). The high frequency of shoot regeneration for embryonal cotyledon (84.2%) with maximum number of shoots (37.4/explant) as well as increase in the length of shoots (7.6 cm) were achieved on medium containing 40 μM $AgNO_3$; shoot number was four times more than that obtained on medium without $AgNO_3$. $AgNO_3$ concentration below 40 μM also induced multiple shoots but at a lower extent (Table 2).

Table 1. Effect of BA, Kn and NAA on multiple shoot induction from cotyledon explants of cucumber cv. Poinsett 76.

Concentration (μM)		Percentage of explants with shoots	Mean number of shoots		Shoot length (cm)	No of nodes
			Initial culture	After 2nd transfer		
0		9.4±0.16qr	1.6±0.16j	2.4±0.16n	1.7±0.16lm	1.1±0.08lm
		(F=6291.25)***	(F=6.410)***	(F=15.83)***	(F=58.40)***	(F=21.87)***
BA						
0.44		12.6±0.11p	1.8±0.16hi	3.0±0.34l	2.2±0.11k	1.4±0.11kl
0.88		18.4±0.11mn	1.9±0.32h	3.6±0.11jk	2.4±0.23k	1.6±0.11k
1.76		23.6±0.11k	2.1±0.16gh	4.2±0.11i	3.6±0.11h	2.4±0.11i
2.22		32.2±0.11hi	2.5±0.40e	4.8±0.46g	3.8±0.11gh	2.6±0.11h
4.44		43.4±0.23e	2.8±0.16d	5.4±0.23ef	4.6±0.11e	3.4±0.11ef
8.88		15.8±0.20no	1.8±0.16hi	3.4±0.23k	2.6±0.11jk	1.8±0.11jk
		(F=1238.32)***	(F=31.645)***	(F=13.52)**	(F=31.66)***	(F=15.92)**
Kn						
0.92		9.2±0.23r	0.9±0.12l	2.0±0.34o	1.2±0.11n	0.8±0.08n
1.84		12.4±0.23q	1.6±0.11j	2.8±0.11lm	2.0±0.34kl	1.2±0.11ln
2.76		17.2±0.11n	1.8±0.11hi	3.2±0.11kl	2.6±0.23jk	2.0±0.34j
3.68		22.4±0.11l	2.0±0.11gh	3.8±0.46j	3.0±0.11ij	2.2±0.30ij
4.64		30.8±0.46i	2.4±0.11ef	4.6±0.23gh	3.8±0.11gh	2.8±0.11g
9.28		10.8±0.23q	1.2±0.11k	2.4±0.11n	1.8±0.11l	1.2±0.23l
		(F=5507.31)***	(F=16.954)***	(F=56.89)***	(F=187.12)***	(F=75.28)***
BA	NAA					
4.44	0.05	46.6±0.11d	2.8±0.11d	5.6±0.11e	4.4±0.11ef	3.6±0.11e
	0.10	58.4±0.23b	3.6±0.34b	7.4±0.23c	6.0±0.23c	5.0±0.34bc
	0.25	62.6±0.11a	4.8±0.46a	9.2±0.23a	7.2±0.11a	5.8±0.23a
	0.50	38.4±0.23fg	2.4±0.11ef	4.8±0.46g	3.6±0.11h	2.6±0.11gh
	0.75	32.8±0.11q	2.1±0.08g	4.0±0.23ij	2.8±0.11j	2.0±0.11j
		(F=1942.95)***	(F=91.393)***	(F=24.54)***	(F=40.80)***	(F=58.07)***
Kn	NAA					
4.64	0.05	27.4±0.23j	3.6±0.11b	5.2±0.30f	4.0±0.40g	2.8±0.11g
	0.10	38.6±0.40f	3.4±0.11c	6.4±0.23d	5.2±0.11d	4.0±0.23d
	0.25	49.8±0.34c	3.5±0.08bc	7.8±0.50b	6.4±0.23b	5.2±0.11b
	0.50	22.6±0.11kl	2.1±0.08g	4.2±0.41i	3.2±0.30i	2.4±0.23i
	0.75	19.4±0.23m	1.6±0.11j	3.4±0.23k	2.2±0.11k	1.4±0.23kl

Values represents the treatment means of 20 replicates
Values with the same letter within the column are not significantly different according to Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ level
***, Highly significant ($p<0.001$); **, Highly significant ($p<0.01$)

$AgNO_3$ treatment at 50 μM inhibited shoot multiplication in shoot number (9.6/explant) and length of shoots (3.4 cm). The efficiency of $AgNO_3$ on multiple shoot induction from cotyledon and hypocotyl explants was reported in melon Roustan et al. (1992) and in cucumber Mohiuddin et al. (1997). However, they were able to obtain only lower number of shoots per explant (about 25 shoots). But in the present experiment, we could get more number of shoots (37.4 shoots/explant) at the end of second subculture. As Ag^+ ions can prevent a wide variety of ethylene-induced plant responses, including growth inhibition and senescence, the effect was 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. In a similar way, $AgNO_3$ enhanced shoot regeneration in *Nicotiana plumbaginifolia* and *Triticum aestivum* (Purnhauser et al.1987), *Zea mays* (Songstad et al. 1991), *Brassica campestris* ssp. Oleifera (Burnett et al. 1994), ssp. Pereinensis (Chi et al. 1990) and *Raphanus sativus* (Pua et al. 1996).

Treatment with $CoCl_2$ was also effective for improving shoot regeneration frequency (Table 2). In the present study, a high frequency of shoot regeneration was achieved by $CoCl_2$ (30 μM) with embryonal cotyledon explant (68.6%). The number of shoots was 27.2/explant with a mean shoot length of 5.6 cm. Roustan et al. (1992), Mhatre et al. (1998) reported that $CoCl_2$ induced multiple shoots but a lower frequency of 11 shoots/explant in melon and cucumber, respectively. In some cases, $CoCl_2$ was equally effective as $AgNO_3$ in eliciting morphogenesis (Chraibi et al. 1991).

Among the different concentrations of SA tested, 30 μM concentration induced maximum shoot proliferation (Table 2) but less than $AgNO_3$ and $CoCl_2$ treatments with 20.6 shoots/explant. Our study was in conformity with Mhatre et al. (1998) who reported that acetyl SA induced multiple shoots at 20 μM concentration in cucumber (about 9 shoots/explant). Experiments with apple suspension culture SA showed

Table 2. Effect of ethylene inhibitors in multiple shoot induction on MS medium containing EA (4.44 μM) and NAA (1.59 μM).

Compound (μM)	Percentage of explants with shoots	Number of shoots		Mean shoot length (cm)	No of nodes
		Initial culture	After 2nd transfer		
BA + NAA	62.6d	4.8gh	9.2j	7.2b	5.8b
$AgNO_3$					
10	48.6±0.12g	7.2±0.12d	13.4±0.17g	4.6±0.12g	3.2±0.16fg
20	62.4±0.17de	8.4±0.17cd	16.2±0.11ef	5.4±0.18e	4.6±0.18d
30	71.6±0.11b	10.6±0.12b	21.6±0.24c	6.2±0.12c	5.0±0.21c
40	84.2±0.16a	13.8±0.24a	37.4±0.17a	7.6±0.14a	6.2±0.24a
50	34.6±0.30k	6.0±0.17f	9.6±0.12i	3.4±0.12ij	2.6±0.21gh
$CoCl_2$					
10	30.8±0.11l	2.8±0.14k	6.6±0.12l	2.2±0.12m	1.4±0.12ij
20	36.4±0.11j	3.6±0.11ij	8.2±0.14k	2.8±0.14k	1.6±0.12hi
30	68.6±0.34c	8.8±0.24c	27.2±0.24b	5.6±0.21de	4.4±0.24de
40	52.4±0.17f	7.2±0.12de	16.4±0.17e	4.8±0.21fg	3.6±0.18f
50	44.6±0.22i	4.8±0.14g	11.4±0.14h	3.4±0.18i	2.0±0.14h
Salicylic acid					
10	21.8±0.18n	1.8±0.17m	3.0±0.12no	1.6±0.12h	1.0±0.08j
20	26.4±0.20m	2.4±0.21kl	3.8±0.14n	2.6±0.16kl	1.6±0.12i
30	62.4±0.34e	5.8±0.14fg	20.6±0.17cd	5.8±0.24d	4.8±0.20cd
40	47.6±0.28gh	4.4±0.24h	9.6±0.12ij	5.0±0.18f	4.2±0.18e
50	34.8±0.24jk	3.6±0.17i	5.4±0.12m	3.8±0.16gh	3.0±0.21g

Values represents the treatment means of 20 replicates
Values with the same letter within the column are not significantly different according to Duncan's Multiple range Test (DMRT) at $p \leq 0.05$ level
***, Highly significant ($p<0.001$); **, Highly significant ($p<0.01$)

reduced ethylene production over a short period of time but less effective over longer periods (Leslie and Romani 1988). Increased concentration of 10 to 30 μM, enhances the shoot growth and development, suggesting that there is a correlation between a decrease in ethylene level and an increase in SA level.

Ethylene Production from Cultured Explants

The addition of $AgNO_3$ to shoot regeneration medium enhanced the level of ethylene produced by the cultured explants (Figure 1). Increasing $AgNO_3$ concentrations coincided with an increase in ethylene production in embryonal culture. In the untreated cucumber explants the maximal ethylene level was 2.9 μl/l after 3 wk in the culture. Then the capacity of explant to produce ethylene declined gradually after a wk (1.4 μl/l). In the presence of $AgNO_3$ at 40 μM the ethylene production was dramatically increased after 3 wk over the control (Figure 1). On the 28th day, ethylene production by $AgNO_3$ treated or non treated tissue had decreased. Although ethylene production is stimulated in response to $AgNO_3$, the mechanism is unknown. Ethylene over production as a result of Ag^{2+} ion treatment has been reported in tomato fruits (Penarrubia et al. 1987). Theologis (1992) reported that ethylene production by the stimulation of $AgNO_3$ can be explained by receptor interference by Ag^{2+} ions that triggers cells to overproduce ethylene.

When embryonal cotyledon explants were cultured with $CoCl_2$, ethylene production was strongly inhibited. Its effectiveness as an inhibitor of ethylene production was concentration-dependent (Figure 2). Co^{2+} inhibits ethylene production by blocking the conversion of 1-aminocyclo-propane 1-carboxylic acid to ethylene (Yang and Hoffman 1984). In the presence of this inhibitor, shoot regeneration increased with the level of inhibition of ethylene production.

In the present investigation, the amount of ethylene production in SA treated explants was strongly inhibited. In the presence of SA at 30 μM, the ethylene level was strongly inhibited after 3 wk (Figure 3), supporting the previous assumption that SA inhibits ethylene production by blocking the action of ethylene forming enzyme (EFE) (Leslie and Romani 1986). In the present study, like $CoCl_2$ treatment, SA induced multiple shoots by inhibiting ethylene production. SA may have an inhibitory effect on ethylene biosynthesis on signaling (Jirage et al. 2001), on the other hand, ethylene can also inhibit SA accumulation.

We conclude that ethylene plays an important role in the growth of cell cultures and it is also possible to improve the frequency of shoot regeneration in cucumber by supplementing the regeneration medium with ethylene inhibitors. Inhibitors of

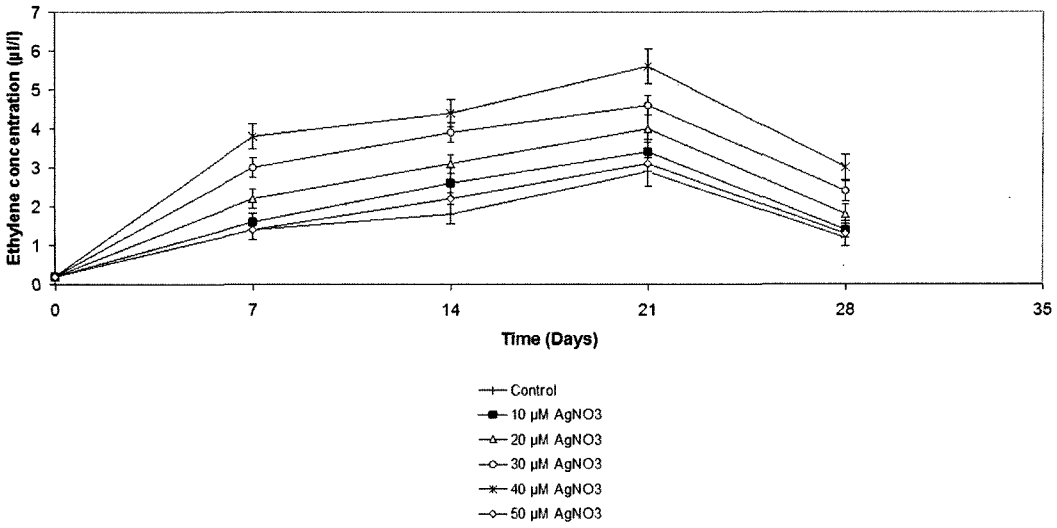

Figure 1. Effect of various concentrations of $AgNO_3$ (10-50 µM) on ethylene production in cucumber cv. Poinsett 76.

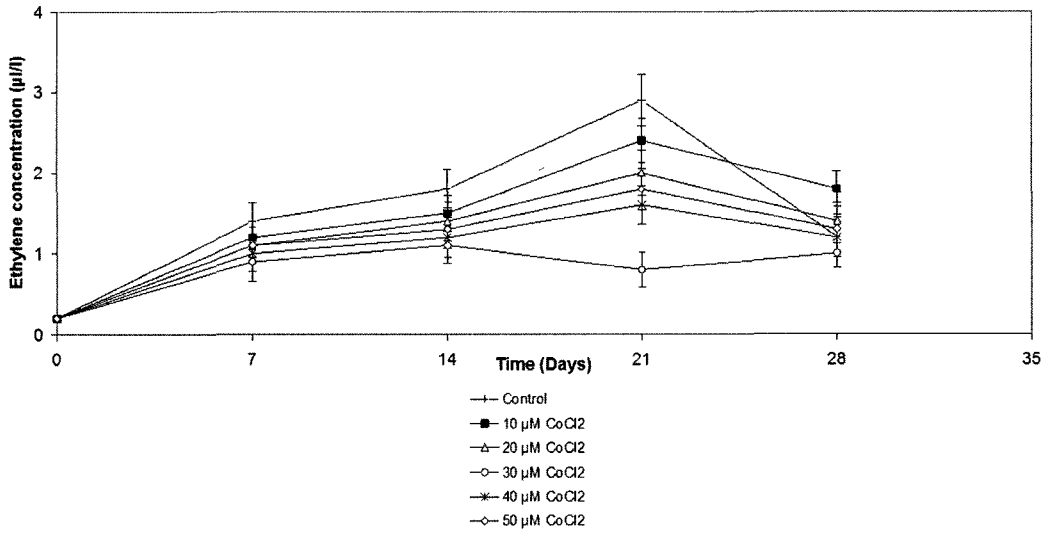

Figure 2. Effect of various concentrations of $CoCl_2$ (10-50 µM) on ethylene production in cucumber cv. Poinsett 76.

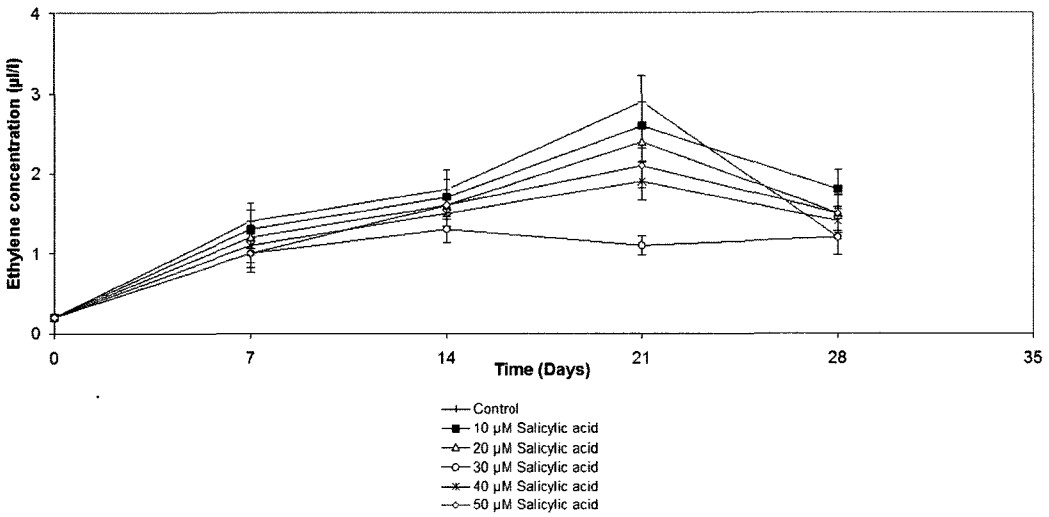

Figure 3. Effect of various concentrations of Salicylic acid (10-50 µM) on ethylene production in cucumber cv. Poinsett 76.

ethylene biosynthesis on receptor binding that confers enhancement of shoot induction provide efficient way for shoot propagation. As a result, we obtained more number of shoots from embryonal explants of cucumber. Further the ethylene production from embryonal cotyledon explants was analyzed for the first time in cucumber.

References

Bais HP, Sudha D, Ravishanker GA (1999) Putrescine influences growth and production of coumarins in hairy root cultures of witloof chicory (*Chicorium intybus* L. cv. Lucknow Local). J Plant Growth Reg 18: 159-165

Beyer EM, Page YM, Yang SF (1984) Ethylene. In: Wilkins MB (ed.) Advanced plant physiology, Pitman, London, pp 111-115

Burnett L, Arnoldo M, Yarrow S, Huang B (1994) Enhancement of shoot regeneration from cotyledon explants of *Brassica rapa* ssp. *oleifera* through pretreatment with auxin and cytokinin and use of ethylene inhibitors. Plant Cell Tiss Organ Cult 37: 253-256

Chee PP and Slightom JL (1991) Transfer and expression of cucumber mosaic virus coat protein in the genome of *Cucumis sativus*. J Amer Soc Hortic Sci 116: 1098-1102

Chi GL, Barfield DG, Sim GE, Pua EC (1990) Effect of AgNO3 and aminoethoxyglycine on *in vitro* shoot and root organogenesis from seedling explants of recalcitrant *Brassica* genotypes. Plant Cell Rep 9: 195-198

Chraibi BKM, Latche A, Roustan JP, Fallot J (1991) Stimulation of $AgNO_3$ and aminoethoxyglycine on in vitro shoot and root organogenesis from seedling explants of recalcitrant *Brassica* genotypes. Plant Cell Rep 9: 195-198

Gambley RL, Dodd WA (1990) An *in vitro* technique for production of *de novo* of multiple shoots in cotyledon explants of cucumber (*Cucumis sativus* L.). Plant Cell Tiss Org Cult 20: 177-183

Gomez KA, Gomez KA (1976) Statistical procedures for agricultural research with emphasis of rice. Rice. Res. Inst., Los Banos, Philliphines

Jirage D, Zhou N, Cooper B, Clarke JD, Dong X, Glazerbrook J (2001) Constitutive salicylic acid- dependent signaling in cpr1 and cpr6 mutants requires PAD4. Plant J 26: 395-407

Leslie CA, Romani RG (1988) Inhibition of ethylene biosynthesis by salicylic acid. Plant Physiol 88: 833-837

Malepszy S (1988) Cucumber (*Cucumis sativus* L.). In: Bajaj, YPS (ed.) Biotechnology in Agriculture and Forestry, Vol 6, pp. 276-293

Mhatre M, Mirza M, Rao PS (1998) Stimulatory effect of ethylene inhibitors and orthocoumaric acid in tissue cultures of cucumber, *Cucumis sativus* L. Ind J Exp Biol 36: 104-107

Mohinuddin AKM, Chowdhury MKU, Zaliha S, Abdullah ZC, Napis S (1997) Influence of silver nitrate (ethylene inhibitor) on cucumber *in vitro* shoot regeneration. Plant Cell Tiss Org Cult 51: 75-78

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant 15: 473-497

Penarrubia L, Aguilar M, Margossian L, Fischer RL (1992) An antisense gene stimulates ethylene hormone production during tomato fruit ripening. Plant Cell 4: 681-687

Pua EC, Sim GE, Chi GL, Kong LF (1996) Synergistic effect of ethylene inhibitors and putrescine on shoot regeneration from hypocotyl explants of Chinese radish (*Raphanus sativus* L. var. *longipinnatus* Bailey) *in vitro*. Plant Cell Rep 15: 685-690

Purnhauser LP, Medgyesy M, Czakó PJ, Dix K, Márton L (1987) Stimulation of shoot regeneration in *Triticum aestivum* and *Nicotiana plumbaginifolia* via. tissue cultures using the ethylene inhibitor $AgNO_3$. Plant Cell Rep 6: 1-4

Raharjo SHT, Hernandez MO, Zhang YY, Punza ZK (1996) Transformation of pickling cucumber with chitinase-encoding genes using *Agrobacterium tumefaciens*. Plant Cell Rep 15: 591-596

Rajasekaran K, Mullins MG, Nair T (1983) Flower formation *in vitro* by hypocotyl explants of cucumber (*Cucumis sativus* L.). Ann Bot 52: 417-420

Roustan JP, Latche A, Fallot J (1992) Enhancement of shoot regeneration from cotyledon of *Cucumis melo* by silver nitrate, an inhibitor of ethylene action. J Plant Physiol 140: 485-488

Saly S, Joseph C, Corbineau F, Lelu MA, Côme D (2002) Induction of secondary somatic embryogenesis in hybrid larch (*Larix* x *leptoeuropaea*) as related to ethylene. Plant Growth Regul 37: 287-294

Selvaraj N (2002) *In vitro* culture and genetic transformation of cucumber (*Cucumis sativus* L.). Thesis submitted to Bharathidasan University, Tiruchirappalli - 24, Tamilnadu, India

Shiragave R, Chavan NS (2001) *In vitro* propagation of *Momordica dioica* (Roxb) Willd. Asian J Microbiol Biotechnol Env Sci 3: 173-175

Songstad DD, Armstrong CL, Peterson WL (1991) Silver nitrate increases type II callus production from immature embryos of maize inbred B 73 and its derivatives. Plant Cell Rep 9: 699-702

Tabei Y, Kitade S, Nishizawa Y, Kikuchi N, Kayano T, Hibi T, Akutsu K (1998) Transgenic cucumber plants harboring a rice chitinase gene exhibit enhanced resistance to gray mold (*Botrytis cinerea*). Plant Cell Rep 17: 159-164

Theologis A (1992) One rotten apple spoils the whole bushel: the role of ethylene in fruit ripening. Cell 70: 181-184

Vasudevan A, Selvaraj N, Sureshkumar P, Ganapathi A (2001) Multiple shoot induction from shoot tip explant of cucumber (*Cucumis sativus* L.). Cucurbit Genet

Coop Rep 24: 8-12
Wehner TC, Locy RD (1981) Tissue culture propagation of field-grown cucumber selections. Cucurbit Genet Coop Rep 4: 20-22
Yadav RC, Saleh MT, Grumet R (1996) High frequency shoot regeneration from leaf explants of muskmelon. Plant Cell Tiss Org Cult 45: 207-214
Yang SF, Hoffman NF (1984) Ethylene biosynthesis and its regulation in higher plants. Annu Rev Plant Physiol 35: 155-189
Ziv M, Gadasi G (1986) Enhanced embryogenesis and plant regeneration from cucumber (*Cucumis sativus* L.) callus by activated charcoal in solid/liquid double layer cultures. Plant Sci 47: 115-122