Current Botany 2011, 2(7): 14-16
ISSN: 2220-4822
Available Online: http://currentbotany.org/

Effect of silver thiosulphate on *In Vitro* plant regeneration of *Solanum nigrum* (Linn.) – An important antiulcer medicinal plant

T.M. Sridhar[1], D. Preethi[1] and C.V. Naidu[2*]

[1]*Department of Biotechnology, Sri Venkateswara University, Tirupathi-517502, A.P., India*
[2]*Department of Biotechnology, Dravidian University, Kuppam-517425, A.P., India*

Abstract

Effect of Silver Thiosulphate (Ethylene inhibitor) on shoot regeneration using axillary bud explants of *Solanum nigrum* was studied. Ethylene inhibitor silver thiosulphate favoured the shoot morphogenesis. Highest frequency of regeneration (95%), maximum number of shoots (4.2) was achieved with 40 μM/L STS, added to MS medium. The optimum range of STS concentrations recorded was between 10-40 μM/L. At higher concentration adventitious root formation was observed and successful field establishment was also achieved. Ethylene inhibits the shoot morphogenesis and also affects the root formation. Ag^+ ions inhibit ethylene action in a wide variety of ethylene induced responses in plants by reducing the receptor capacity to bind ethylene. Thus, silver thiosulphate may be useful as a media supplement to develop efficient protocols for *in vitro* propagation of *Solanum nigrum* as it favours the shoot and root formation.

Keywords: *Solanum nigrum*, Silver thiosulphate, Axillary bud explants, Shoot initiation, Adventitious roots

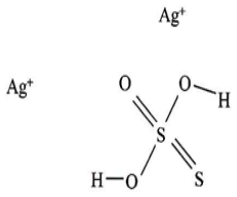
INTRODUCTION

Solanum nigrum L. (Black night shade) a member of the solanacae, has a wide range of medicinal values. The herb is antiseptic, antidysentric and antidiuretic used in the treatment of cardiac, skin disease, psoriasis, herpivirus and inflammation of kidney. The fruits and leaves have been traditionally used against various nerve disorders [1]. It has very important gastric ulcerogenic activities [2] Berries are bitter, pungent and are useful in heart diseases, piles, dysentery [3]. *Solanum nigrum* presently grown as a homestead plant, it is often cultivated in homestead gardens as pot plants. The plant has been considered ethonobotanically important due to its use in traditional and health care system for curing severe ulcers, gastritis and stomachache. Most prominent medicinal properties are the presence of alkaloids, solamargin and solasonine which yield solasodine as glycone has great demand in pharmaceutical industries. Solasodine has embryogenic, teratonic as well as antifungal and antiviral activities [4].

The role of ethylene in plant tissue culture is not clear, although it can change the organogenic capacity of explants *in vitro*. Ethylene is a gaseous plant hormone involved in many aspects of plant life cycle such as seed germination, root hair development, root nodulation, flower senescence, abscission, and fruit ripening [5]. *In vitro* studies have indicated that ethylene can affect callus growth, shoot regeneration and somatic embryogenesis *In vitro* [6]. Thus, by regulating the production or action of ethylene, the growth and development of some tissue cultures can be controlled to a certain extent [7].

However, there are no reports on the effect of ethylene inhibitors on regeneration from *Solanum nigrum*. Hence, in the present study, an attempt was made to study the effect of silver thiosulphate on plant regeneration from axillary bud explants of *Solanum nigrum*.

Structure of Silver thiosulphate (Ag_2+ H_2S_2 – $4O_3$)



MATERIALS AND METHODS

Collection of Plant material

Healthy axillary bud explants of *Solanum rigrum* (L.) were collected from two month old seed germinated field grown plants growing in biotechnology garden, S.V.University, Tirupati A.P. India.

Surface sterilization

Explants were washed thoroughly under running tap water to remove the traces of dust etc. followed by treatment with 10% teepol/tween-20 for 5 minutes. Then the explants were sterilized in 70% ethanol for a minute, and finally with 0.01% $Hgcl_2$ for 1-2 minutes and washed 3-4 times with sterile double distilled water.

Culture medium

Young axillary bud explants (1-2 cms) were inoculated on MS medium [8] containing 3% sucrose and gelled with 0.8% agar supplemented with various concentration of *Silver thiosulphate* (STS) in the range of 10-120 μm/L. The pH of the medium was adjusted to

Received: July 14, 2011; Revised September 11, 2011; Accepted September 11, 2011.

*Corresponding Author

C.V. Naidu
Department of Biotechnology, Dravidian University, Kuppam-517425, A.P., India

Tel: +91 877 2260386; Fax: +91-8570278209
Email: challagundlav@yahoo.co.in (C.V. Naidu)*
thulasimsreedhar@gmail.com (T.M. Sridhar)

5.8 before gelling with agar and autoclaved for 20 minutes at 121^{0}C for 15 lbs pressure.

Sub culturing

The cultures were maintained by regular subculture at 4 week intervals on fresh MS medium.

Culture conditions

The growth room conditions maintained for *in vitro* cultures were 26 ± 2^{0}C and 60-70% relative humidity, light intensity was 3000 lux with a photoperiod of 18 hrs day light and 6 hrs dark. Each experiment was conducted at least thrice with 20 replicates per treatment.

RESULTS AND DISCUSSION

Effect of Silver thiosulphate on shoot regeneration from axillary buds (Table-1 and Figure-1)

Experiments were performed to scrutinize the effect of STS on shoot bud regeneration. Initiation of shoot bud was observed after one week of inoculation. Highest frequency of regeneration (95%) and maximum shoot number (4.2 ± 0.17) was achieved with 40 μM/L STS, supplemented with MS medium. But maximum shoot length (6.5 ± 0.50) was observed in 80 μM/L STS, lower concentrations of STS favoured high frequency and maximum number of shoot induction. The optimum range of STS concentrations is recorded between 10-40 μM/L. Ethylene inhibits the shoot morphogenesis and also affects the root formation. Ag^+ ions inhibit ethylene action in a wide variety of ethylene induced responses in plants by reducing the receptor capacity to bind ethylene [9, 10]. In STS supplemented MS basal medium increased regeneration frequencies were reported in *Stevia rubaudiana* [11]. The effects of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), and two inhibitors, Silver thiosulfate and aminoethoxyvinylglycine (AVG), were tested in yellow passion fruit (*Passiflora edulis*) axillary buds cultured in vitro [12]. The organogenesis was assessed by the number of buds per explant, mean leaf area per explant, and shoot length. ACC-supplemented medium significantly inhibited all evaluated responses. When ethylene action and biosynthesis were inhibited, a significant enhancement of buds and leaf area was observed. The results suggest beneficial effects of silver ions in the form of silver nitrate on *in vitro* development of axillary buds.

Table 1: Influence of silver thiosulphate (STS) added to MS medium on regeneration of plantlets from axillary bud explants of *Solanum nigrum*

STS (μm/L)	Regeneration frequency (%)	Number of shoots / explant	Length of shoot (cm)	Callus
10	70	1.5 ± 0.50	4.0 ± 0.28	C+
20	72	2.0 ± 0.28	4.5 ± 0.17	C+
30	78	2.8 ± 0.18	5.0 ± 0.28	-
40	95	4.2 ± 0.17	3.6 ± 0.53	-
50	65	1.8 ± 0.18	5.8 ± 0.17	-
80	40	1.4 ± 0.34	6.5 ± 0.50	-
100	24	1.0 ± 0.28	3.0 ± 0.18	-
120	15	1.0 ± 0.18	5.2 ± 0.17	-

Observations: after 4 weeks, values are mean ± SE of 20 independent determinants

Figure-1

Legends for figure-1
A) Shoot initiation from axillary bud explants on MS+ STS (Silver Thiosulphate) (10 μm/L), A) bar 1 cm = 0.9 cm
B-C) multiple shoot formation on MS+STS (40 μm/L)
B) bar 1 cm =0.5 cm; C) bar 1 cm = 1.0 cm
D-E) elongation of multiple shoots showing root formation on MS+STS (80 μm/L); D) bar 1 cm = 1.0 cm; E) bar 1 cm = 1.5 cm

In vitro rooting

At higher concentration (80 μm/L of STS) adventitious root formation was observed and successful field establishment was also achieved. These findings were in agreement with earlier reports in *Decalepis hamiltonii*, where silver nitrate favoured the shoot morphogenesis and root formation [13, 14]. The effect of silver nitrate on *in vitro* rooting was also elucidated in *Vanilla planifolia* [15]. Where, silver nitrate not only induced shoot multiplication but also influenced rooting of Vanilla explants. The plantlets obtained on medium containing 40 μm/L $AgNO_3$ exhibited 100% survival.

To understand the role of silver ions in regulating morphogenesis, it is important to know the aspects of ethylene biosynthesis. In brief, the biosynthesis of ethylene starts with conversioin of the amino acid methionine to S-adenosyl-L- methionine (SAM, also called Adomet) by the enzyme Met Adenosyltransferase. SAM is subsequently converted to 1-aminocyclopropane-1-crboxylic-acid (ACC) by the enzyme ACC synthase (ACS). The activity of ACS is the rate-limiting step in ethylene synthesis. The final step requires oxygen and involves the action of the enzyme ACC-oxidase (ACO), formerly known as the ethylene forming enzyme (EFE) [16]. Ag^+ ions from ethylene inhibitors such as silver thiosulphate and aminoethoxyvinylglycine (AVG) inhibit ethylene action in a wide variety of ethylene-induced responses in plants. The ethylene inhibiting effect of Ag^+ is believed to be due to an interference with ethylene binding [17] whereas, AVG blocks the activity of aminocyclopropane carboxylic acid (ACC, an ethylene precursor) synthetase, which plays a key role in regulating ethylene production [18]. This positive effect of Ag^+ ions and AVG in shoot organogenesis suggest that ethylene produced by cultured explants inhibits shoot organogenesis of those explants [19]. The beneficial effects of the

ethylene inhibitors on organogenesis have been widely reported [20, 13]. The early aging of the plants is inhibited by silver. Ag^+ present in silver thiosulphate blocks the production of ethylene and there by promotes good growth of plant. Earlier the effect of silver thiosulphate on shoot organogenesis was studied in both cultivars, Caniho and Helena [21].

CONCLUSION

The influence of exogenous supplemented silver ions in the form of silver thiosulphate in plant tissue culture media significantly regulates ethylene activity in most of the plant systems. This was clearly evident from the results obtained in direct shoot organogenesis from axillary bud explants of *Solanum nigrum*. However, the basic molecular mechanism of interaction between silver ions and the ethylene receptors yet to be fully understood. Hence, further research in this line is highly necessary to elucidate the actual role of silver ions on shoot morphogenesis and how they influence the regulation of ethylene action in *in vitro* cultures.

ACKNOWLEDGEMENT

The authors are grateful to the UGC (New Delhi, India) for granting major research project and giving financial assistance in the form of fellowship.

REFERENCES

[1] Perez, G.R.M., Perez, L.A., Garcia, D.L.M. and Sosa, M.H. 1998. Neuropharmacological activity of *Solanum nigrum* (L.) fruit. J. Ethanopharmacology. 62:43-48.

[2] Akhtar, M.S and Munir, M. 1989. Evaluation of gastric ulcerogenic effects of *Solanum nigrum, Brassica olevacae* and *Ocimum basillicum* in rats. J.Ethanopharmacology. 27:163-176.

[3] Kritikar, K.R. and Basu, B.S. 1987. Indian Medicinal Plants. 3:1784-1781.

[4] Kim, Y.C., Che-Quing Ming, Gunatilaka, A.A. and Kingston, D.G. 1996. Bio active steroidal alkaloids from *Solanum umbelliferum.* Journal of Natural Products. 59(3):283-285.

[5] Bleecker, A.B. and Kende, H. 2000. Ethylene: A gaseous signal molecule in plants. Annual Review of Cell and Developmental Biology. 16: 1-18.

[6] Pua, E.C. and Chi, G.L. 1993. *De novo* shoot morphogenesis and plant growth of mustard (*Brassica juncea*) in vitro in relation to ethylene. Physiologia Plantaruim. 88(3): 467-474.

[7] Giridhar, P., Indu, E.P., Vinod, K., Chandrasekhar, A. and Ravishankar, G.A. 2003. Effect of silver nitrate on *in vitro* shoot growth of Coffee. Tropical Science. 43(3): 144-146.

[8] Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with Tobacco tissue culture. Physiol. Plant., 15:473-497.

[9] Beyer, E.M. 1979. Effect of silver ion, carbon dioxide and oxygen on ethylene action and metabolism. Plant Physiol., 63: 169-173.

[10] Yang, S.F. and Hoffman, N.E. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiol., 35: 155-189.

[11] Preethi, D. 2008. Studies on plant regeneration, phytochemicals and molecular marker analysis of *Stevia rubaudiana.* Bert-Ph.D Thesis (p. 80) submitted to S.V. University, Tirupati, A.P., India.

[12] Reis, L.B., Paiva Neto, V.B., Toledo Picoli, E.A., Costa, M.G.C., Rego, M.M., Carvalho, C.R., Finger, F.L. and Otnoi, W.C. 2003. Axillary bud development of passion fruit as affected by ethylene precursor and inhibitors. In Vitro Cellular Developmental Biology-Plant. 39(6): 618-622.

[13] Bais, H.P., Sudha, G., Suresh, B. and Ravishankar, G.A. 2000. $AgNO_3$ influences *in vitro* root formation in *Decalepis hamiltonii*. Current Science. 79:894-898.

[14] Reddy, B.O., Giridhar, P. and Ravishankar, G.A. 2001. *In vitro* rooting of *Decalepis hamiltonii* an endangered shrub by auxins and root-promoting agents. Current Science. 81(11): 1479-1481.

[15] Giridhar, P., Obul Reddy, B. and Ravishankr, G.A. 2001. Silver nitrate influences *in vitro* shoot multiplication and root formation in *Vanilla planifolia*. Current Science. 81(9): 1166-1170.

[16] Wang, K.L.C., Hai, L. and Ecker, J.R. 2002. Ethylene biosynthesis and signaling networks. Plant Cell., S131-S151 Supplement.

[17] Beyer, E.M. 1979. Effect of silver ion, carbon dioxide and oxygen on ethylene action and metabolism. Plant Physiology. 63: 169-173.

[18] Yang, S.F. and Hoffman, N.E. 1984. Ethylene biosynthesis and its regulation in higher plants. Annu. Rev. Plant Physiology. 35: 155-189.

[19] Chi, G.L., Barfield, D.G., Sim. G.E. and Pua, E.C. 1990. Effect of $AgNO_3$ and aminoethoxyvinylglycine on *in vitro* shoot and root organogenesis from seedling explants of recalcitrant Brassica genotypes. Plant Cell Reports. 9:195-198.

[20] Chraibi, K.M., Latche, A., Roustan, J.P. and Fallot, J. 1991. Stimulation of shoot regeneration from cotyledons of *Helianthus annuus* by the ethylene inhibitors, silver and cobalt. Plant Cell Reports. 10: 204-207.

[21] Burgos, L. and Alburquerque, N. 2003. Ethylene inhibitors and low kanamycin concentrations improve adventitious regeneration from apricot leaves. Plant Cell Reports. 21: 1167-1174.