

## AgNO<sub>3</sub> - a potential regulator of ethylene activity and plant growth modulator

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**Abbreviations:** ACC: 1-Amino-cyclopropane-1-carboxylic acid  
ADC: arginine decarboxylase  
AgNO<sub>3</sub>: silver nitrate  
AVG: aminoethoxyvinylglycine  
BA: 6-benzylaminopurine  
DFMA: α-DL-difluoromethyl arginine  
DFMO: α-DL-difluoromethyl ornithine  
IAA: indole acetic acid  
NAA: α-naphthalene acetic acid  
ODC: ornithine decarboxylase  
PA: polyamine  
Put: putrescine  
SAM: S-adenosyl-L- methionine  
STS: silver thiosulphate  
Spd: spermidine  
Spm: spermine

The aim of this review is to critically analyze the role of silver nitrate (AgNO<sub>3</sub>) in modulating plant growth and development. In recent years, basic studies on ethylene regulation opened new vistas for applied research in the area of micro-propagation, somatic embryogenesis, *in vitro* flowering, growth promotion, fruit ripening, and sex expression. Silver nitrate has proved to be a very potent inhibitor of ethylene action and is widely used in plant tissue culture. Few properties of silver nitrate such as easy availability, solubility in water, specificity and stability make it very useful for various applications in exploiting plant growth regulation and morphogenesis *in vivo* and *in vitro*. Silver ion mediated responses seem to be involved in polyamines, ethylene- and calcium- mediated pathways, and play a crucial role in regulating physiological process including morphogenesis. The molecular basis for regulation of

morphogenesis under the influence of silver nitrate is completely lacking. This review compiles published reports of silver nitrate-mediated *in vitro* and *in vivo* studies and focuses on fundamental and applied aspects of plant growth modulation under the influence of silver nitrate.

In recent years, advances in plant genetic engineering have opened new avenues for crop improvement and various plants with novel agronomic traits have been produced. The success of plant genetic engineering relies on several factors which include an efficient tissue culture system, for regeneration of plants from cultured cells and tissues (Pua et al. 1996). Shoot generation and rooting are important in the realization of the potential of the cell and tissue culture techniques for plant improvement (Purnhauser et al. 1987). Silver ions in the form of nitrate, such as AgNO<sub>3</sub>, play a

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major role in influencing somatic embryogenesis, shoot formation and efficient root formation which are the prerequisites for successful genetic transformation (Bais et al. 1999; Bais et al. 2000a; Bais et al. 2000b; Bais et al. 2001a; Bais et al. 2001b; Bais et al. 2001c). Silver ions are also employed in the form of silver thiosulphate in several tissue culture studies (Eapen and George, 1997).

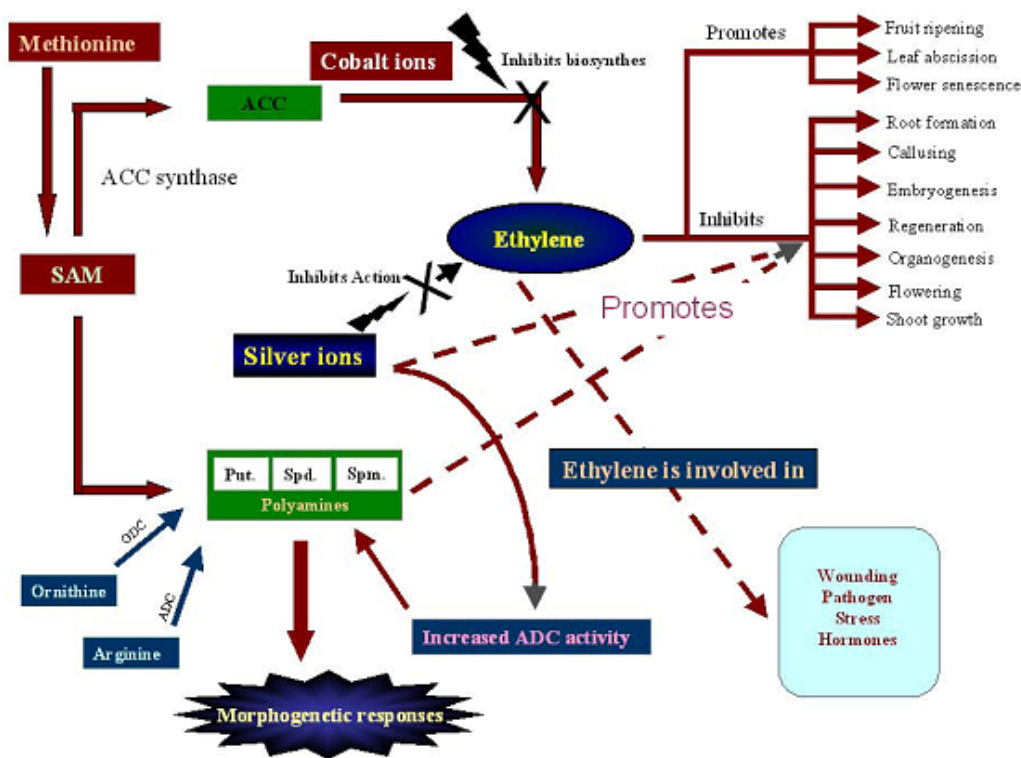
Ethylene is recognized as a ubiquitous plant hormone (Lieberman, 1979; Yang, 1985), which influences growth and development of plants (Abeles, 1973; Yang and Hoffman, 1984; Mattoo and Suttle, 1991). *In vitro* studies have indicated that ethylene can affect callus growth, shoot regeneration and somatic embryogenesis *in vitro* (Purnhauser et al. 1987; Songstad et al. 1988; Roustan et al. 1989; Roustan et al. 1990; Biddington, 1992; Pua and Chi, 1993). Thus, by regulating the production or action of ethylene, the growth and development of some tissue cultures can be controlled to a certain extent (Beyer, 1976c; Davies, 1987; Purnhauser et al. 1987; Songstad et al. 1988; Chi and Pua, 1989; Bais et al. 2000a; Giridhar et al. 2003).

AgNO<sub>3</sub> has been known to inhibit ethylene action (Beyer, 1976a) and cobaltous ions are known to inhibit ethylene synthesis (Lau and Yang, 1976) (Figure 1). Silver ion is capable of specifically blocking the action of exogenously applied ethylene in classical responses such as abscission, senescence and growth retardation (Beyer, 1976c). These

observations led to its application in tissue culture. Addition of AgNO<sub>3</sub> to the culture media greatly improved the regeneration of both dicot and monocot plant tissue cultures (Beyer, 1976c; Duncan et al. 1985; Davies, 1987; Purnhauser et al. 1987; Songstad et al. 1988; Chi and Pua, 1989; Veen and Over Beek, 1989; Bais et al. 2000a; Giridhar et al. 2003). The exact mechanism of AgNO<sub>3</sub> action on plants is unclear. However, few existing evidences suggest its interference in ethylene perception mechanism (Beyer, 1976c). In recent years, AgNO<sub>3</sub> has been employed in tissue culture studies for inhibiting ethylene action because of its water solubility and lack of phytotoxicity at effective concentrations (Beyer, 1976a).

### Ethylene

Ethylene is a gaseous plant hormone involved in many aspects of plant life cycle (Figure 1) such as seed germination, root hair development, root nodulation, flower senescence, abscission, and fruit ripening (Johnson and Ecker, 1998; Bleecker and Kende, 2000). Its biosynthesis (Wang et al. 2002) is tightly regulated by internal signals and environmental stimuli from biotic and abiotic stresses, such as pathogen attack, wounding, hypoxia, ozone, chilling, or freezing (Wang et al. 2002). The role of ethylene in morphogenesis has been well documented in an earlier review (Kumar et al. 1998a). Mutants have also been identified that display a constitutive triple response in the



**Figure 1. Ethylene biosynthesis and its influences on various physiological processes.** Ethylene is involved in many aspects of the plant life cycle, including plant morphogenesis, defense, flowering, sex expression, embryogenesis, seed germination, root hair development, root nodulation, flower senescence, abscission, fruit ripening etc. and a number of evidences proved the usefulness of silver nitrate in the inhibition of ethylene-induced adverse effects.

absence of ethylene (Kieber et al. 1993). This can be divided into subgroups based on whether or not the constitutive triple response can be suppressed by inhibitors of ethylene perception and biosynthesis, such as silver ions and aminoethoxyvinyl glycine (AVG). Mutants that are unaffected by these inhibitors are termed constitutive triple-response (*ctr*) mutants, whereas mutants whose phenotype reverts to normal morphology are termed ethylene-overproducer (*eto*) mutants, which are defective in the regulation of hormone biosynthesis. To date, data is lacking on the molecular basis for silver ion interaction with the mutants, which are insensitive to ethylene.

## Ethylene biosynthesis

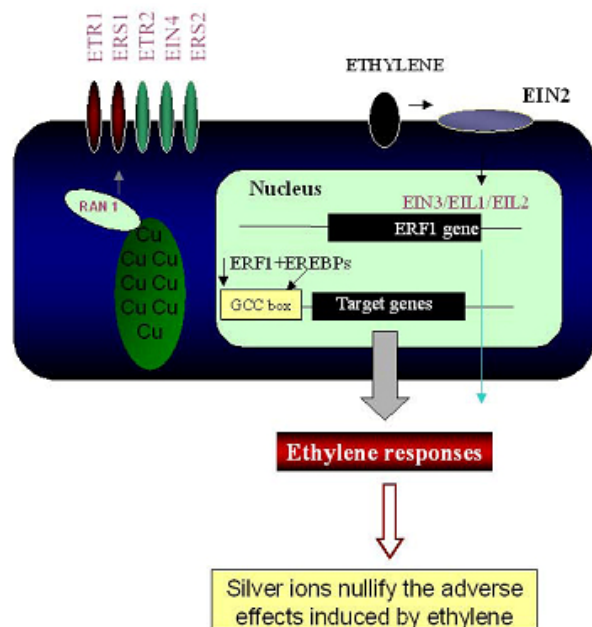
To understand the role of silver ions in regulating morphogenesis, it is important to know the aspects of ethylene biosynthesis (Figure 1). The biochemistry of ethylene biosynthesis has been a subject of intensive study in plant hormone physiology (reviewed by Wang et al. 2002). In brief, the biosynthesis of ethylene starts with conversion 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-carboxylic-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) (Wang et al. 2002).

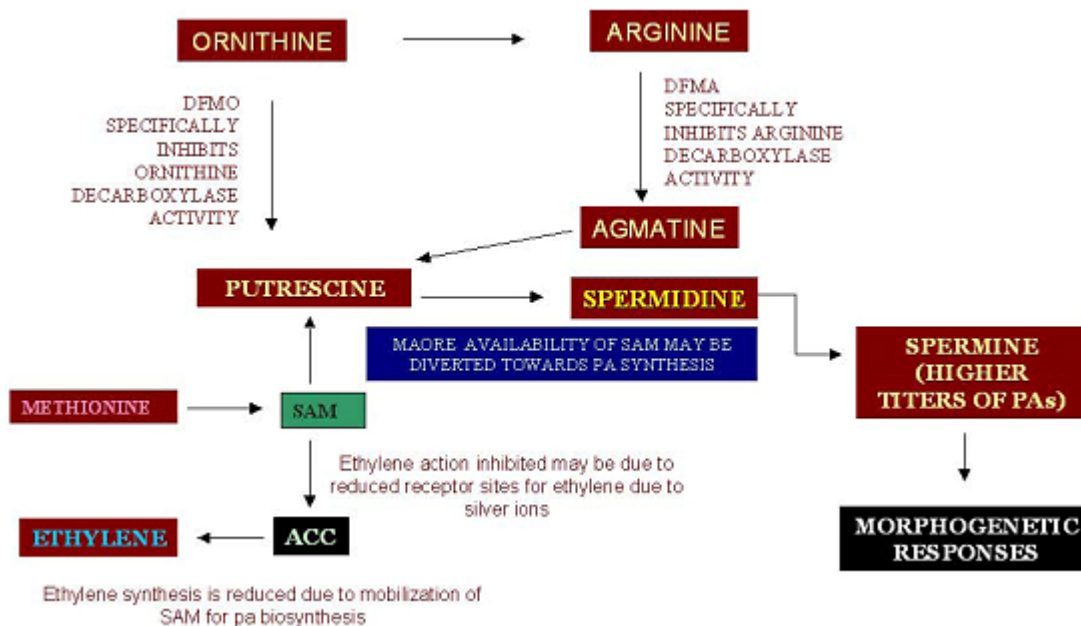
A major breakthrough in the ethylene synthesis pathway was the establishment of *S*-adenosylmethionine (*S*-AdoMet) and ACC as the precursors of ethylene (reviewed in Yang and Hoffman, 1984; Kende, 1993). On the basis of this knowledge, the enzymes that catalyze these reactions were characterized and purified. The first successes in molecular cloning of the ACC (Sato and Theologis, 1989) and ACO (Hamilton et al. 1991; Spanu et al. 1991) genes led to the demonstration of these enzymes belonging to a multi-gene family and are regulated by a complex network of developmental and environmental signals responding to both internal and external stimuli (reviewed by Johnson and Ecker, 1998). In addition to being an essential building block of protein synthesis, nearly 80% of cellular methionine is converted to *S*-AdoMet by *S*-AdoMet synthetase (SAM synthetase) at the expense of ATP utilization (Ravanel et al. 1998). *S*-AdoMet is the major methyl donor in plants and is used as a substrate for many biochemical pathways, including polyamines and ethylene biosynthesis (Ravanel et al. 1998).

## Ethylene signal perception

Ethylene is perceived by a family of five membrane-localized receptors that are homologous to bacterial histidine kinases involved in sensing environmental changes (Figure 2). Ethylene binding occurs at the N-terminal transmembrane domain of the receptors, and a copper co-factor is required for the binding. The system typically consists of a histidine kinase as the sensor that autophosphorylates an internal histidine residue in response to environmental signals, and a response regulator that activates the downstream components upon receiving a phosphate from the histidine residue of the sensor on its aspartate residue (Wurgler-Murphy and Saito, 1997). Five ethylene receptors exist in *Arabidopsis*: ETR1, ETR2, ERS1, ERS2, and EIN4 (Chang et al. 1993; Hua et al. 1995; Hua and Meyerowitz, 1998; Sakai et al. 1998). Further characterization of ethylene binding to ETR1 has revealed that it occurs at the hydrophobic pocket located at the N-terminus of the receptors and requires a transition metal, copper, as a co-factor (Figure 2) (Schaller and Bleecker, 1995; Rodriguez et al. 1999; Wang et al. 2002). Further findings indicated that RAN1 is involved in the delivery of copper to the ethylene receptor and that this copper-delivery pathway is required to create functional ethylene receptors in plants (Figure 2) (Wang et al. 2002). Cu ions are also known to form complexes with ethylene (Coates et al. 1968). But the studies of Beyer (1976c) revealed that the effect of silver ions could be explained on the basis that silver ions substitute for Cu ions, thereby interfering with ethylene action. This may be due to the similarity in size, the same oxidation state, and the ability of both Cu ion and Ag ion to form complexes with ethylene (Coates et al. 1968). The possibility of the anti-ethylene property of silver was later well explored in various plant



**Figure 2. Involvement of copper ions in ethylene-mediated response and ethylene signal transduction.** Copper ion is hypothesized to be transported into the cell and brought to the RAN1 protein. The RAN1 protein then delivers copper ion to the ETR1 apoprotein, which then becomes functional and is possibly transported to the plasma membrane. In the absence of ethylene, ETR1 functions to activate CTR1, which regulates the ethylene response pathway (Woeste and Kieber, 2000).



**Figure 3. Interplay of polyamine and ethylene biosynthetic pathway.** Enhanced polyamine biosynthesis and ethylene action inhibition due to the binding of silver ions. (Bais et al. 2000b).

systems. At present there are no concrete evidences to show the involvement of silver ions with signaling networks which leads to down regulation of physiological responses governed by ethylene. Therefore, focus on the elucidation of molecular basis for diverse developmental process in plants such as abscission, flowering, fruit ripening, morphogenesis and sex expression, that are known to be regulated by silver ions, would be interesting.

### Possible mechanisms of action of silver nitrate on ethylene action inhibition

Silver ions are capable of generating ethylene insensitivity in plants (Zhao et al. 2002). Ethylene-insensitive mutations (Hall et al. 1999) and silver ions are thought to perturb the ethylene binding sites (Rodriguez et al. 1999). The ethylene receptor, ETR1, contains one ethylene-binding site per homodimer and binding is mediated by a single copper ion (Cu) present in the ethylene-binding site. The replacement of the copper co-factor by silver also serves to lock the receptor into a conformation such that it continuously represses ethylene responses (Zhao et al. 2002).

There are different views and experimental evidences on this subject. According to one view, the ethylene action in plants is inhibited by weak antagonists such as CO<sub>2</sub> and strong antagonists like silver compounds. This is possibly due to oxidation of ethylene by a metal-ion enzyme system (Abeles, 1973). In *Arabidopsis*, insensitivity to ethylene is conferred by dominant mutation in receptors (Bleecker et al. 1988). Another hypothesis is that AgNO<sub>3</sub> inhibits ethylene action by means of silver ions by reducing the receptor capacity to bind ethylene (Yang, 1985), which

would result in higher titers of ethylene in the tissues, thus inhibiting the earlier steps of its own pathway. Miyazaki and Yang (1987) reported the influence of putrescine and AgNO<sub>3</sub> on the competitive utilization of SAM. Bais et al. (2000b) also postulated that the utilization of SAM by putrescine for its conversion to spermidine would possibly result in a lower availability of SAM for ethylene biosynthesis (Figure 3). The introduction of ethylene antagonists into the culture media affects the level of ACC, thereby affecting ethylene levels (Gong et al. 2005).

### Polyamines

Other important substances responsible for regulation of morphogenesis are polyamines. The polyamines (PAs) are organic compounds having two or more primary amino groups. Polyamines have been implicated in several important cellular processes like cell division, morphogenesis, protein synthesis, DNA replication, and plant response to abiotic stress (Tabor and Tabor, 1984; Smith, 1985; Smith, 1993; Van Den Broeck et al. 1994; Walden et al. 1997; Kumar and Rajam, 2004). They bind to DNA, and are essential for cell viability (Flink and Pettijohn, 1975). Polyamines are also known to be involved in DNA helix stabilization, stabilization of loops in RNA molecules, membrane permeability, DNA replication, cell division, gene expression, regulation of enzyme activities, membrane stabilization, morphogenesis, fruit ripening etc. (Bais and Ravishankar, 2002; Kumar and Rajam, 2004). It has been postulated that polyamines and related compounds are a type of growth regulator or secondary hormonal messenger (Galston, 1983; Davies, 1987). PAs are found in plant cells at significantly higher levels than plant

Table 1. Composition of medium in transformation experiment.

Plant	Response	Reference
<i>Albizia julibrissin</i>	<i>In vitro</i> shoot formation	Sankhla et al. 1995
<i>Andrographis paniculata</i>	Somatic embryogenesis	Martin, 2004
Apple	Higher efficiency of regeneration and transformation	Seong et al. 2005
<i>Arachis hypogea</i>	Regeneration	Pestana, 1999
<i>Arachis hypogea</i>	Multiple shoot formation	Ozudogru et al. 2005
<i>Albizia procera</i>	Plant regeneration	Kumar et al. 1998b
<i>Bactris gasipaes</i>	Enhance embryogenic competence	Steinmacher et al. 2007
<i>Brassica campastris</i>	Shoot regeneration	Palmer, 1992
<i>Brassica juncea</i>	Microspore embryogenesis	Prem et al. 2005
<i>Capsicum annuum</i>	Shoot development and plant regeneration	Hyde and Phillips, 1996
<i>Cicer arietinum</i>	Somatic embryo induction	Patil et al. 1999
<i>Cichorium intybus</i>	Shoot length, shoot number, flowering	Bais et al. 2001a; Bais et al. 2001b; Bais et al. 2001c
<i>Coffea arabica</i>	Shoot growth Somatic embryogenesis Direct somatic embryogenesis	Ganesh and Sreenath, 1996 Giridhar et al. 2003 Giridhar et al. 2004
<i>Coffea canephora</i>	Shoot growth Somatic embryogenesis Direct somatic embryogenesis	Giridhar et al. 2003 Giridhar et al. 2004; Kumar et al. 2007 Fuentes et al. 2000
<i>Cucumis sativus</i>	Shoot regeneration Sex expression	Mohiuddin et al. 1997 Atsmon and Tabbak, 1979
<i>Daucus carota</i>	Somatic embryogenesis	Nissen et al. 1994
<i>Decalepis hamiltonii</i>	<i>In vitro</i> root formation and shoot formation	Bais et al. 2000a; Bais et al. 2000b; Reddy et al. 2001
Egyptian maize	Enhanced the formation of embryogenic type II callus	El-Itriby et al. 2003
<i>Eleusine coracana</i>	Plant regeneration	Kothari-Chajer et al. 2008
<i>Glycine max</i>	Shoot formation on hypocotyls	Wang and Xu, 2008
<i>Gossypium</i> sp.	Leaf abscission Enhance multiple shoot production from hypocotyl segments	Beyer, 1976a Divya et al. 2008
<i>Helianthus annuus</i>	Regeneration and shoot organogenesis	Chraibi et al. 1991
<i>Hordium vulgare</i>	Somatic embryogenesis Plant regeneration	Castillo et al. 1998 Jha et al. 2007
<i>Ipomoea batatas</i>	Shoot regeneration	Gong et al. 2005
<i>Manihot esculenta</i>	Shoot organogenesis	Zang et al. 2001
<i>Morus Alba</i>	Modification of sex expression	Thomas, 2004
<i>Nicotiana plumbaginifolia</i>	Shoot regeneration	Purnahauser et al. 1987
<i>Oryza sativa</i>	Androgenesis	Lentini et al. 1995
<i>Paspalum scrobiculatum</i>	Plant regeneration	Kothari-Chajer et al. 2008
Passion fruit	Regeneration	Reis et al. 2003
<i>Penisittum glaucum</i>	Plant regeneration	Oldach et al. 2001
<i>Penisittum glaucum</i>	Regeneration from zygotic embryos	O'Kennedy et al. 2004

<i>Pennisetum americanum</i>	Plant regeneration	Plus et al. 1993
<i>Phaseolus vulgaris</i>	Shoot development	Cruz de Carvalho et al. 2000
<i>Phoenix dactylifera</i>	Somatic embryogenesis	Al-Khayri and Al-Bahrany, 2001; Al-Khayri and Al-Bahrany, 2004
<i>Picea glauca</i>	Somatic embryogenesis	Kong and Yeung, 1994
<i>Picea glauca</i>	Maturation of somatic embryos	El Meskaoui et al. 2000
<i>Pisum sativum</i>	Shoot growth	Beyer, 1975
<i>Punica granatum</i>	Adventitious shoot regeneration.	Naik and Chand, 2003
<i>Quassia amara</i>	somatic embryogenesis	Martin and Madassery, 2005
<i>Raphanus sativus</i>	Shoot regeneration	Pua et al. 1996
<i>Rubus</i> sp	Inhibits callous formation during shoot multiplication	Tsao and Reed, 2002
<i>Sorghum bicolor</i>	Plant regeneration	Oldach et al. 2001
<i>Stenotaphrum secundatum</i>	Embryogenic callus shoot regeneration.	Fei et al. 2000
<i>Tagetes erecta</i>	Plant growth flowering and seed viability	
<i>Triticum aestivum</i>	Plant regeneration from callus Improved embryogenic callus frequency	Yu et al. 2008 Wu et al. 2006
<i>Vanilla planifolia</i>	Shoot growth and <i>in vitro</i> root formation	Giridhar et al. 2001
<i>Vicia faba</i>	Promoted root formation	Khalafalla and Hattori, 2000
<i>Vigna unguiculata</i>	<i>In vitro</i> regeneration	Brar et al. 1999
<i>Zea mays</i>	Regenerable callus Embryogenic calli from embryo scutellum	Songstad et al. 1988 Valdez-Ortiz, 2007

hormones. There is evidence that PAs are taken up by cell suspension cultures (Evans and Malmberg, 1989). Interestingly, it seems that there is a strong link between ethylene, polyamines, and calcium-mediated signaling. This triangle is expected to be a potential target for silver ions. This is because both ethylene and polyamines are metabolically related (Figure 3) and utilize the same precursor, SAM, for their synthesis (Evans and Malmberg, 1989; Bais and Ravishankar, 2002). It has also been suggested that polyamines and ethylene may regulate each other's synthesis. For instance, ethylene has been shown to inhibit arginine decarboxylase and *S*-adenosyl methionine decarboxylase activities in pea seedlings (Apelbaum et al. 1985). These enzymes are necessary for polyamine synthesis (Smith, 1985). It has been proved beyond doubt that polyamines play crucial roles in plant growth and development as well as basic biological process (reviewed by Kumar and Rajam, 2004). Since polyamines have been reported to promote embryogenesis (Feirer et al. 1984), the promotive effect of ethylene inhibitors, such as AgNO<sub>3</sub>, on regeneration was thought to be due to enhanced polyamine synthesis rather than reduced ethylene production. Pua et al. (1996) clearly described the synergistic effect of AgNO<sub>3</sub> and putrescine on shoot regeneration in Chinese radish. Miyazaki and Yang (1987) reported the influence of putrescine and AgNO<sub>3</sub> on the competitive utilization of SAM. Bais et al. (2000b) postulated that, utilization of SAM by putrescine for its conversion to spermidine would

possibly result in a lower availability of SAM for ethylene biosynthesis (Figure 3). On the other hand, Pua and Chi (1993) also reported the same stimulatory effect of AgNO<sub>3</sub> feeding on ethylene production and its contribution to increased titers of polyamines in mustard. Polyamines also regulate the growth and secondary metabolism (Bais et al. 1999; Bais et al. 2001b; Bais and Ravishankar, 2002). Reports on somatic embryogenesis in carrot (Roustan et al. 1990; Nissen, 1994) indicate that the potent ethylene action inhibitor, AgNO<sub>3</sub>, causes the increase of ADC activity, which in turn increases the levels of endogenous polyamines in carrot embryogenic cultures.

### Involvement of calcium in polyamine-mediated response

Polyamines are associated with Ca<sup>2+</sup> ions in signaling events (Majewska-Sawka et al. 1998). They supported the hypothesis of transportation of spermidine/spermine within protoplasts through a carrier-mediated mechanism (Antognoni et al. 1994, Majewska-Sawka et al. 1997). Majewska-Sawka et al. (1998) found that spermidine/spermine may result in change in distribution of Ca<sup>2+</sup> ions. It is reasonable to conclude that Ca<sup>2+</sup> ions may be involved in the mechanism of polyamine action in plant cells (Bush, 1995). This aspect is very relevant here because, apart from ethylene regulation, silver nitrate is known to regulate the polyamine pool in plant systems.

### Application of silver nitrate in plant tissue culture

So far we have discussed the possible mechanisms of regulation of morphogenesis by silver nitrate. Interestingly, a large number of reports are accumulating on the utility of silver nitrate in tissue culture and other applications, with significant contributions towards the development of plant biotechnology and transgenic research. The following section deals with a brief compilation of published research pertaining to the effect of silver nitrate in plant morphogenesis (Table 1).

### Somatic embryogenesis

Theoretically, each living plant cell is capable of forming somatic embryos. Somatic embryos are formed from vegetative plant cells. Applications of this process include: clonal propagation of genetically uniform plant material, elimination of viruses, provision of source tissue for genetic transformation, generation of whole plants from single cells called protoplasts, and development of synthetic seed technology. Plant growth regulators in the tissue culture medium can be manipulated to induce callus formation and subsequently changed to induce embryos from the callus or directly from intact tissues. The ratio of different plant growth regulators required to induce callus or embryo formation varies with the type of plant. The use of silver nitrate improved somatic embryogenesis in several plant species such as buffalograss (Fei et al. 2000), *Coffea* sp. (Fuentes et al. 2000; Giridhar et al. 2004), carrot (Nissen, 1994), white spruce (Kong and Yeung, 1994), *Triticum durum* (Fernandez et al. 1999), and *Zea mays* (Vain Hort and Flament, 1989; Vain Hort et al. 1989; Songstad et al. 1991).

### Multiple shoot induction and shoot regeneration

Silver nitrate is known to promote multiple shoot formation in different plants. *In vitro* shoot formation was improved by incorporating silver nitrate in the culture medium. Ganesh and Sreenath (1996) reported *in vitro* sprouting of apical buds of *Coffea* under the influence of AgNO<sub>3</sub>. The addition of N<sup>6</sup>-benzyladenine with AgNO<sub>3</sub> greatly enhanced the rate of sprouting. At low concentration, AgNO<sub>3</sub> was found to cause delayed senescence resulting in improved growth of the proliferated shoots in *Coffea canephora* (Fuentes et al. 2000). AgNO<sub>3</sub> enhanced *in vitro* shoot growth of *C. arabica* and *C. canephora* (Giridhar et al. 2003). Shoot regeneration of Chinese radish Cv Red coat was improved when cultured in media supplemented with 2030 μM AgNO<sub>3</sub> (Pua et al. 1996). *Brassica* sp. are poorly responsive to tissue culture manipulations (Narasimhulu and Chopra, 1988). *B. campestris* produces high levels of ethylene causing abnormal growth and development of the plant in tissue culture conditions (Lentini et al. 1988), and also inhibits *de novo* shoot regeneration *in vitro* (Chi et al. 1990; Chi et al. 1991; Palmer, 1992; Pua and Chi, 1993). The cotyledons and hypocotyls of 7 cultivars belonging to *B. campestris* spp. chinensis, spp. pekinensis and spp.

parachinensis exhibited improved shoot regeneration on culture media supplemented with growth regulators and AgNO<sub>3</sub>.

The effects of the ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), and two inhibitors, silver thiosulfate and aminoethoxyvinylglycine (AVG), were tested in yellow passionfruit (*Passiflora edulis*) axillary buds cultured *in vitro* (Reis et al. 2003). 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 nitrate on *in vitro* development of axillary buds.

Inhibition of ethylene action by AgNO<sub>3</sub> stimulated regeneration of shoots from cotyledon explants of *Helianthus annuus* (Chraibi et al. 1991). In many plants, the regeneration potential of cultured cells and tissues decreases with increased cycles of subcultures (Ogura and Shimada, 1978; Vasil, 1987). This phenomenon is evident in *Pennisetum americanum* (Pearl millet) and Plus et al. (1993) effectively addressed this issue by incorporating AgNO<sub>3</sub> in the culture medium to restore the regeneration potential. AgNO<sub>3</sub> enhanced shoot regeneration frequency in silk tree (*Albizia julibrissin*) and *Nicotiana plumbaginifolia*.

The work done in our laboratory has shown that exogenous feeding of putrescine and silver nitrate influenced morphogenesis in chicory (*Chichorium intybus*) shoot cultures (Bais et al. 2000b). Putrescine and AgNO<sub>3</sub> induced shoot multiplication and *in vitro* flowering. The chicory plants, which flower biennially, could be forced to flower experimentally for studies on *in vitro* pollination and seed development (Bais et al. 2000b).

Silver nitrate was found to be beneficial in the regeneration and clonal propagation of several economically important plants (Table 1) such as peanut (Pestana et al. 1999), cowpea (Brar et al. 1999), *Brassica* sp. (Eapen and George, 1997; Pua et al. 1999), *Capsicum* sp. (Hyde and Philips, 1996; Kumar et al. 2003a), watermelon (Lim and Song, 1993), *Coffea canephora* (Kumar et al. 2003b), Cucumber (Mohiuddin et al. 1997), Pomegranate (Naik and Chand, 2003), White marrygold (Misra and Datta, 2001), Cassava (Zang et al. 2001), Petunia (Gavinlertvatana et al. 1980), etc.

### *In vitro* rooting

*Decalepis hamiltonii* Wight, Arn (swallow root), belonging to Asclepiadaceae, is a monogeneric climbing shrub, native of the Deccan peninsula and forest areas of Western Ghats in India. It is used as a culinary spice due to its aromatic roots. *In vitro* root formation is a major issue in the tissue culture of this plant. Effects of AgNO<sub>3</sub> on *in vitro* root

formation of *Decalepis hamiltonii* were studied. Addition of 40  $\mu\text{M}$   $\text{AgNO}_3$  resulted in root initiation and elongation (Bais et al. 2000a; Reddy et al. 2001).

Vanilla is an important spice crop of commercial value. The effect of  $\text{AgNO}_3$  on rooting and shooting was elucidated in *Vanilla planifolia* (Giridhar et al. 2001). Maximum number of shoots and highest shoot length was obtained on medium containing 20  $\mu\text{M}$   $\text{AgNO}_3$ .  $\text{AgNO}_3$  not only induced shoot multiplication but also influenced rooting of vanilla explants. The plantlets obtained on medium containing 40  $\mu\text{M}$   $\text{AgNO}_3$  exhibited 100% survival. Silver nitrate also induced rooting and flowering *in vitro* on the rare, rhoeophytic woody medicinal plant, *Rotula aquatica* Lour. Dipping of the basal end of shoots in NAA (2.69  $\mu\text{M}$ ) and silver nitrate (11.7  $\mu\text{M}$ ) solution improved rooting efficiency (Sunandakumari et al. 2004).

### Modification of sex expression

The inhibition of ethylene action by silver nitrate was employed to suppress the development of female flowers and induce male flowers (Beyer, 1976c; Atsmon and Tabbak, 1979; Takahashi and Jaffe, 1984). Mulberry (*Morus alba* L.) is a dioecious plant and the male and female flowers are seen in separate plants. Bisexual flowers never occur under natural conditions (Thomas, 2004). By treating the nodal cuttings with silver nitrate, bisexual flowers could be induced in female plants. The histological analysis of these bisexual flowers showed both ovule and anther in the same flower (Thomas, 2004). Bisexual flowers were also induced in cucumber by silver nitrate treatment (Stankovic and Prodanovic, 2002). Ethylene and auxin promote the formation of female flowers whereas gibberellins promote the formation of male flowers (Mohan Ram and Jaiswal, 1970; Saito and Takahashi, 1986). The enhancement of femaleness by auxin possibly occurs through the induction of ethylene biosynthesis (Takahashi and Jaffe, 1984; Trebitsh et al. 1987). Ethylene evolution is highly correlated with sex expression in plants and dioecious plants produce more ethylene than monoecious ones (Rudich et al. 1972; Trebitsh et al. 1987). In view of all these evidences, silver nitrate may possibly be a potent candidate compound to regulate the sex expression in plants.

### Fruit ripening

Ethylene plays a crucial role in initiating and accelerating the ripening-related process. Treatment of tomato with silver ions has been shown to inhibit ethylene action and fruit ripening (Hobson et al. 1984). Furthermore, if silver ions were applied at stages of ripeness well after the breaker stage, ripening can be arrested (Tucker and Brady, 1987). The growth regulator 1-methylcyclopropane (1-MCP), like silver ions, is an extremely effective antagonist for plants or harvested plant products (Serek et al. 1995a; Serek et al. 1995b; Serek et al. 1995c; Sisler et al. 1996).

### Leaf abscission

Ethylene that stimulated leaf abscission in cotton is blocked by the silver ion (Beyer, 1976c). Without  $\text{AgNO}_3$ , all the leaves had abscised on the 7<sup>th</sup> day in ethylene. Plants treated with increasing concentrations of  $\text{AgNO}_3$  and placed in ethylene showed progressively less leaf abscission. Treatment with 25 mg/l of  $\text{AgNO}_3$  reduced the time required to reach 100% leaf abscission by 2 days. Silver nitrate treatment also reduced the growth retarding effects of ethylene. Other similar experiments with mature cotton plants have demonstrated a similar ability of  $\text{AgNO}_3$  to prevent young fruit and flower abscission (Beyer, 1976c).

### CONCLUDING REMARKS

In this review, an attempt has been made to discuss the role of ethylene, polyamines, and silver ions as potent regulators of morphogenesis in plants. The interplay of polyamines, ethylene, and calcium signaling is also discussed. The influence of exogenously applied silver ions in the form of  $\text{AgNO}_3$  in plant tissue culture media significantly regulates the ethylene activity in most of the plant systems. We have clearly brought out the major physiological effects of  $\text{AgNO}_3$  in plant systems *viz* direct or indirect organogenesis, somatic embryogenesis, *in vitro* rooting of micro shoots, induction of flowering, early flowering, sex expression, and control of leaf abscission. However, there is a gap in information on the molecular mechanisms of interaction between silver ions and the ethylene receptors. Further research on the regulation of morphogenesis through the use of metal ions like silver would throw light on an array of functions of these relatively simple molecules that play a marvelous role in influencing growth, development, and adaptation of plants to the environment. This opens new dimensions in understanding plant morphogenesis. Hence, it is necessary to elucidate the physiological mechanisms at the gene regulation level to find out the actual role of silver ions in signaling and to see how they influence regulation of ethylene action in plants.

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