AgNO₃ - 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  
AgNO₃: silver nitrate  
AVG: aminoethoxyvinylglycine  
BA: 6-benzylaminopurine  
DFMA: α-DL-difluromethyl arginine  
DFMO: α-DL-difluromethyl 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 (AgNO₃) 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 AgNO₃, 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).

AgNO₃ 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 AgNO₃ 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 AgNO₃ action on plants is unclear. However, few existing evidences suggest its interference in ethylene perception mechanism (Beyer, 1976c). In recent years, AgNO₃ 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 Kend e, 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

![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.](image-url)
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In the 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
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 week antagonists such as CO₂ 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 AgNO₃ 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 AgNO₃ 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.

<table>
<thead>
<tr>
<th>Plant</th>
<th>Response</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Albizzia julibrissin</td>
<td>In vitro shoot formation</td>
<td>Sankhla et al. 1995</td>
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<tr>
<td>Andrographis paniculata</td>
<td>Somatic embryogenesis</td>
<td>Martin, 2004</td>
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<td>Apple</td>
<td>Higher efficiency of regeneration and transformation</td>
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<td>Arachis hypogea</td>
<td>Regeneration</td>
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<td>Arachis hypogea</td>
<td>Multiple shoot formation</td>
<td>Ozudogru et al. 2005</td>
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<tr>
<td>Albizzia procera</td>
<td>Plant regeneration</td>
<td>Kumar et al. 1998b</td>
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<td>Bactris gasipaes</td>
<td>Enhance embryogenic competence</td>
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<tr>
<td>Brassica campastris</td>
<td>Shoot regeneration</td>
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<td>Brassica juncea</td>
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<td>Capsicum annuum</td>
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<td>Cicer arietinum</td>
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<td>Cichorium intybus</td>
<td>Shoot length, shoot number, flowering</td>
<td>Bais et al. 2001a; Bais et al. 2001b; Bais et al. 2001c</td>
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<tr>
<td>Coffea arabica</td>
<td>Shoot growth</td>
<td>Ganesh and Sreenath, 1996</td>
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<td>Somatic embryogenesis</td>
<td>Giridhar et al. 2003</td>
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<td>Direct somatic embryogenesis</td>
<td>Giridhar et al. 2004</td>
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<tr>
<td>Coffea canephora</td>
<td>Shoot growth</td>
<td>Giridhar et al. 2003</td>
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<td>Direct somatic embryogenesis</td>
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<td>Fuentes et al. 2000</td>
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<td>Cucumis sativus</td>
<td>Shoot regeneration</td>
<td>Mohiuddin et al. 1997</td>
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<td>Sex expression</td>
<td>Atsmon and Tabbak, 1979</td>
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<td>Daucus carota</td>
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<td>Decalepis hamiltonii</td>
<td>In vitro root formation and shoot formation</td>
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<td>Egyptian maize</td>
<td>Enhanced the formation of embryogenic type II callus</td>
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<td>Eleusine coracana</td>
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<td>Glycine max</td>
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<td>Gossypium sp.</td>
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<td></td>
<td>Enhance multiple shoot production from hypocotyl segments</td>
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<td>Helianthus annuus</td>
<td>Regeneration and shoot organogenesis</td>
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<td>Hordeum vulgare</td>
<td>Somatic embryogenesis</td>
<td>Castillo et al. 1998</td>
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<td>Plant regeneration</td>
<td>Jha et al. 2007</td>
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<td>Ipomoea batatas</td>
<td>Shoot regeneration</td>
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<td>Manihot esculenta</td>
<td>Shoot organogenesis</td>
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<td>Morus Alba</td>
<td>Modification of sex expression</td>
<td>Thomas, 2004</td>
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<td>Nicotiana plumbaginifolia</td>
<td>Shoot regeneration</td>
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<td>Oryza sativa</td>
<td>Androgenesis</td>
<td>Lentini et al. 1995</td>
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<td>Paspalum scrobiculatum</td>
<td>Plant regeneration</td>
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<td>Passion fruit</td>
<td>Regeneration</td>
<td>Reis et al. 2003</td>
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<td>Penistium glaucum</td>
<td>Plant regeneration</td>
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<tr>
<td>Penistium glaucum</td>
<td>Regeneration from zygotic embryos</td>
<td>O’Kennedy et al. 2004</td>
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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 (Feier et al. 1984), the promotive effect of ethylene inhibitors, such as AgNO₃, 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 AgNO₃ and putrescine on shoot regeneration in Chinese radish. Miyazaki and Yang (1987) reported the influence of putrescine and AgNO₃ 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 AgNO₃ 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, AgNO₃, 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 Ca²⁺ 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 Ca²⁺ ions. It is reasonable to conclude that Ca²⁺ 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.
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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 Flamant, 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 AgNO₃. The addition of N6-benzyladenine with AgNO₃ greatly enhanced the rate of sprouting. At low concentration, AgNO₃ was found to cause delayed senescence resulting in improved growth of the proliferated shoots in Coffea canephora (Fuentes et al. 2000). AgNO₃ 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 AgNO₃ (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 AgNO₃.

The effects of the ethylene precursor, 1-aminoacyclopropone-1-carboxylic acid (ACC), and two inhibitors, silver thiosulfate and aminooxyvinlyglycine (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 AgNO₃ 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 AgNO₃ in the culture medium to restore the regeneration potential. AgNO₃ enhanced shoot regeneration frequency in silk tree (Albizzia 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 AgNO₃ 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 marigold (Misra and Datta, 2001), Cassava (Zang et al. 2001), Petunia (Gavlinertvatanata 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 AgNO₃ on in vitro root
formation of *Decalepis hamiltonii* were studied. Addition of 40 µM AgNO₃ 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 AgNO₃ 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 µM AgNO₃. AgNO₃ not only induced shoot multiplication but also influenced rooting of vanilla explants. The plantlets obtained on medium containing 40 µM AgNO₃ exhibited 100% survival. Silver nitrate also induced rooting and flowering in vitro on the rare, rheophytic woody medicinal plant, *Rotula aquatica* Lour. Dipping of the basal end of shoots in NAA (2.69 µM) and silver nitrate (11.7 µM) 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 Jaffé, 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 Jaffé, 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-methycyclopropane (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 AgNO₃, all the leaves had abscised on the 7th day in ethylene. Plants treated with increasing concentrations of AgNO₃ and placed in ethylene showed progressively less leaf abscission. Treatment with 25 mg/l of AgNO₃ 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 AgNO₃ 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 AgNO₃ 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 AgNO₃ 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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