Review

Role of polyamines and ethylene as modulators of plant senescence

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Under optimal conditions of growth, senescence, a terminal phase of development, sets in after a certain physiological age. It is a dynamic and closely regulated developmental process which involves an array of changes at both physiological and biochemical levels including gene expression. A large number of biotic and abiotic factors accelerate the process. Convincing evidence suggests the involvement of polyamines (PAs) and ethylene in this process. Although the biosynthetic pathways of both PAs and ethylene are interrelated, S-adenosylmethionine (SAM) being a common precursor, their physiological functions are distinct and at times antagonistic, particularly during leaf and flower senescence and also during fruit ripening. This provides an effective means for regulation of their biosynthesis and also to understand the mechanism by which the balance between the two can be established for manipulating the senescence process. The present article deals with current advances in the knowledge of the interrelationship between ethylene and PAs during senescence which may open up new vistas of investigation for the future.

1. Introduction

Senescence is an integral part of the normal developmental cycle of plants and can be viewed on a cell, tissue, organ or organization level. Senescence, a highly ordered and genetically regulated process, is largely an oxidative process involving a general degradation of the cellular structures and the mobilization of the products of degradation to other parts of the plants. The process is mainly characterized by a cessation of photosynthesis, disintegration of organelle structures, intensive loss of chlorophyll and proteins, a dramatic increase in lipid peroxidation, membrane leakage, breakdown of cell wall components, disruption of cell membranes leading to cellular decompartmentalization and loss of tissue structure (Paliyath and Droillard 1992; Buchanan-Wollaston 1997). A distinct difference, however, exists between the term aging and senescence. The former comprises all those degenerative changes and cellular wear-and-tear that occur in time without reference to death, the latter is considered as the final developmental phase which culminates in death (Nooden 1988).

Senescence, though a terminal developmental stage, can also be accelerated by an array of both abiotic and biotic factors, such as light, temperature, nutrients and pathogens (Smart 1994). It is a dynamic and closely regulated developmental process which involves highly coordinated changes in gene expression (Hensel *et al* 1993). For example, an increase in the synthesis of enzymes which participate in degradation of protein, chlorophyll and lipid

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Abbreviations used: PAs, polyamines; PAO, polyamine oxidase; PCA, perchloric acid; Put, putrescine; Spm, spermine; Spd, spermidine; ODC, ornithine decarboxylase; SAM, S-adenosylmethionine; SAMDC, S-adenosylmethionine decarboxylase; ACC, 1-aminocyclopropane-1-carboxylic acid; EFE, ethylene forming enzyme; AOA, aminooxyacetic acid; AVG, aminoethoxyvinyl glycine; MGBG, methylglyoxal-bis-(guanylhydrazone); ADC, arginine decarboxylase; DFMA, DL-difluoromethyl arginine; DFMO, DL-difluoromethyl ornithine.

might be expected to involve changes in gene expression and genes responsible for a senescence-specific protease have already been identified in *Arabidopsis* (Hensel *et al* 1993; Drake *et al* 1996; Han Frey *et al* 1996; Humbeck *et al* 1996; Buchanan-Wollaston 1997; Gan and Amasino 1997; Nam 1997).

A large number of experimental evidences attest to the promotive role of ethylene in the phenomenon of senescence. By contrast, polyamines (PAs) are reported to be effective anti-senescence agents and found to retard chlorophyll loss, membrane deterioration and increases in RNase and protease activities, all of which help to slow down the senescence process (Evans and Malmberg 1989). Thus these two groups act in opposite directions and often play a crucial role either in suppressing the onset of senescence or triggering and promoting the event. Although the results from several studies with different plant species provide convincing evidences that interaction between PAs and ethylene do modulate fruit ripening and senescence (Saftner and Baldi 1990; Katoh *et al* 1987) such well defined relationships are not generally seen (Evans and Malmberg 1989) in all organisms or tissues. Possibility of other parallel routes may not be discounted since biology after all is known to have several backups. The requirement for PAs and ethylene in primary metabolism is not in question; rather it is the nature and extent of their relationship to both the physiological and biosynthetic processes that remain unresolved. The present article examines various aspects of the involvement of PAs and ethylene in the regulation of senescence.

2. Polyamines

PAs form a class of aliphatic amines that are ubiquitous in living organisms and have been implicated in a wide range of biological processes, including plant growth, development and response to stress (Smith 1985a). Their role in regulation of plant growth and development has been recently reviewed (Galston and Kaur-Sawhney 1995; Kumar *et al* 1997). The major forms of PAs are putrescine (Put), spermine (Spm) and spermidine (Spd) and are found in every plant cell. Less common PAs, like 1,3-diaminopropane and homospermidine, which differ from the common PAs in the number of methylenic moieties between the amine groups, have been detected in a broad spectrum of biological systems including plants (Rodriguez-Garay *et al* 1989), algae (Hamana and Matsuzaki 1982), bacteria (Tait 1985) and animals (Hamana and Matsuzaki 1985). The association of the uncommon PAs with the capacity of same biological systems to grow or function under extreme conditions has provided opportunities for new investigations into their potential functions. Despite their ubiquitous presence in all cells, very little is known about their intra-cellular compartmentalization. At cellular pH values, these compounds behave as cations, and can interact with anionic macromolecules such as DNA, RNA, phospholipids and certain proteins (Tabor and Tabor 1984; Heby and Persson 1990). Polyamines can also be compared with buffers that resist changes in pH.

The common PAs are essential for normal growth of all organisms (Tabor and Tabor 1984) but their precise molecular functions *in vivo* are still not clear. PAs could be involved in various responses to hormonal signals either as hormonal second messengers or as growth regulators (Galston and Kaur-Sawhney 1987). According to the classical definition a plant hormone should have a significant physiological or developmental effect, and be active even at relatively low concentrations moving from site of production to site of action. The levels of PAs in plants are significantly higher than those of plant hormones. The concentration necessary for biological effects are often in the order of millimolar, rather than the micromolar levels. Most of the living cells including plant cells can carry out *de novo* synthesis of PAs but the physiological significance of PA transport from the application zone and/or site of synthesis to the target site or tissue in plants is still not clear. In a recent report, Kakkar *et al* (1998) have dealt at length on the various aspects of PA uptake and transport in plants. Galston (1983) has suggested that polyamines have distinct physiological and developmental effects on plants and therefore, should be regarded as members of a more loosely defined category of plant growth regulators, rather than as hormones *per se*.

The adsorption and exchange of PAs on pectic polysaccharides can contribute to pectic signal modulation in pathogenesis and in differentiation (D'Orazi and Bagni 1987; Messiaen *et al* 1997). PAs are known to bind to the negatively charged phospholipid components or other anionic sites on membranes, thus altering the stability characteristics of such membranes. PA binding is also known to affect membrane fluidity and thus PAs may modulate the activities of membrane-associated enzymes indirectly (Slocum *et al* 1984). Binding of PAs to proteins in petunia protoplast (Mizrahi *et al* 1989) also suggests the direct interaction between the PAs and the membranes. In addition, Roberts et al (1986) using microsomal membranes from *Phaseolus*, reported that exogenously applied PAs were associated with membrane lipids, thereby substantially reducing membrane fluidity.

In addition to their free forms, amine conjugates (polyamine and aromatic amine conjugates) covalently bound to hydroxycinnamic acids have also been shown to occur at high levels in plants (Martin-Tanguy 1985) and are thought to be correlated with developmental phenomena. They occur as both water soluble and insoluble forms. The increased accumulation of polyamine conjugates occurs in parallel to enhanced activity of ornithine decarboxylase (ODC) (Burtin *et al* 1991) which is responsible for PA synthesis. At the time that flowering is initiated, the conjugates move from leaves (their presence in leaves is no longer detectable) to the young floral tissue (Havelange *et al* 1996). They may act as storage forms of PAs, from which the free bases may be released during growth or these conjugates may be transported as and when required (Martin-Tanguy 1997). Although the presence of polyamine oxidases have been established (Smith 1985b), it is thought that synthesis and conjugation rather than catabolism are responsible for regulation of PAs. The hypothesis, that a temporal correlation between changes in levels of free and conjugated PAs and developmental phenomena, requires knowledge of the molecular genetics of PAs and their metabolic regulation.

Although the precise modes of action of PAs are yet to be understood (Walden *et al* 1997), extensive studies support their role in modulation of a variety of physiological processes ranging from cell differentiation to stress responses. The involvement of PAs in floral induction has been documented by spraying the compounds directly on the plants. In *Iris hollandica* buds, Spd has been suggested to be a marker for floral induction. In Douglas fir, *Pseudotsuga menziesii*, putrescine was the dominant PA in vegetative buds, while Spd predominated in floral buds. Even though the PAs have been implicated in numerous physiological processes (Kakkar and Rai 1993; Kumar *et al* 1997), at present however, it is not possible to relate any specific free or conjugated polyamine with these processes. In the absence of productive genetic analysis, many of the steps that contribute to floral morphology aberrations are unclear.

3. Ethylene

Ethylene, a multipurpose signaling molecule in plants and considered as a plant hormone, orchestrates many aspects of plant growth and development such as fruit and flower senescence and defense against pathogens (Yang and Hoffman 1984; Abeles *et al* 1992). The production of ethylene is also observed due to flooding, mechanical wounding and pathogen infection, leaf and floral senescence and abscission, during fruit ripening and in response to chemical inducers (Theologis 1992). Wounding is regarded as one of the primary events in the induction of the senescence syndrome in detached leaves and leaf discs, while ethylene is implicated as a regulator of the rate of the process (Philosoph-Hadas *et al* 1991). In the current text main emphasis is given on the involvement of ethylene with leaf and flower senescence and fruit ripening.

Ethylene is generated from methionine by a well defined pathway (Yang and Hoffman 1984) involving the conversion of S-adenosylmethionine (SAM) to cyclic amino acid, 1-amino cyclo propane-1-carboxylic acid (ACC), a reaction catalyzed by ACC synthase and subsequent oxidation of this intermediate to ethylene by ACC oxidase. Numerous studies have suggested a role for ethylene in a diverse array of processes including the triple response of germinating seedlings, leaf and flower senescence, fruit ripening and wound responses (Abeles *et al* 1992; Zarembinsiki and Theologis 1994).

The role of ethylene in leaf senescence is well documented. Senescence of juvenile leaf lamina of *Hedera helix* was found to increase many folds by exposure to ethylene at 10 fl/litre (Horton and Bourguoin 1992). Aharoni (1989) suggested that abscissic acid (ABA) and ethephon, enhanced chlorophyll loss and antagonized the senescence retarding effects of GA3 and kinetine in lettuce leaf discs. However, effectiveness of IAA and kinetine was noted at supra-optimal concentrations in retarding leaf senescence. In the ethylene forming enzyme (EFE)-antisense tomato plants, leaf senescence is delayed and wound-ethylene synthesis is also reduced. Inhibiting ethylene synthesis caused a reduction in carotenoid production in fruit, which was associated with reduced expression of phytoene synthase required for the production of *b*-carotene and lycopene (Gray *et al* 1993).

Analysis of the *in vitro* translated proteins revealed that the abundance of most of the leaf mRNAs significantly diminished during the progression of senescence, whereas some translatable mRNAs increased during senescence (Buchanan-Wollaston 1994; Smart *et al* 1995). Cytokinin is a known senescence retardant and senescence is also delayed in transgenic plants producing cytokinin by expression of a bacterial gene encoding isopentenyl transferase (IPT); the enzyme catalyzing the first step of cytokinin biosynthesis (Smart *et al* 1991; Gan and Amasino 1995). In contrast to cytokinins, ethylene treatment often promotes senescence. This suggests a molecular strategy to intervene in leaf senescence by blocking ethylene production or perception in transgenic plants. Transgenic tomato plants expressing antisense genes that inhibit ACC oxidase showed significantly reduced production of ethylene and retarded senescence of leaves (John *et al* 1995). Leaf senescence is also delayed in *Arabidopsis* plants with the dominant *etr*1 mutation, which renders plants insensitive to ethylene (Grbic and Bleecker 1995). Thus it is expected that transgenic plants over-expressing *etr*1 and some other mutant genes involved in ethylene perception may result in a delayed senescence phenotype.

Numerous workers have shown a relationship between ethylene and senescence induction, and attempts have been made to depict ethylene as the initiator of the chain of events associated with flower senescence (Borochov and Woodson 1989). In ethylene sensitive flowers like carnation, orchids, peak ethylene production precedes senescence. Application of exogenous ethylene enhances the process and inhibition of ethylene synthesis or action slows it down (Reid and Wu 1992). In such flowers, ethylene is regarded as a signal, mediating a sequence of events that eventually lead to the death of the organ. However, in certain ethylene insensitive flowers like Iris, flower senescence was shown to proceed without any ethylene induction (Celikel and van Doorn 1995). The decline in content of membrane components such as phospholipids is a key event in flower senescence which alters membrane integrity and this loss can be modulated by ethylene (Borochov *et al* 1997).

Fruits are categorized into two groups depending on their exhibition of a respiratory peak and ethylene production during ripening (Lelievre *et al* 1997). With the advancements in molecular biology, a considerable progress has been made during the past decade in understanding the possible relationship between ethylene and fruit ripening. Inhibition of ethylene biosynthesis by amino-oxyacetic acid (AOA), amino-ethoxyvinylglycine (AVG), free radical scavengers or action of silver ions, when either sprayed or infiltrated at non-toxic concentrations into the fruit prior to harvest, delay but do not prevent fruit ripening (Hobson *et al* 1984). Removal of ethylene by hypobaric storage also results in delaying ripening and senescence of fruits and flowers (Dilley 1977). These studies suggest a direct role for ethylene in fruit ripening.

Evidence for the regulation of ethylene biosynthetic pathway during fruit ripening originally came from studies describing ACC accumulation and increase in ACC oxidase and ACC synthase activity in a variety of climacteric fruits (Abeles *et al* 1992). Transgenic tomato plants expressing antisense genes that inhibit either of the two ethylene biosynthesis enzymes showed significantly reduced production of ethylene and retarded senescence of fruits (Hamilton *et al* 1990; Oeller *et al* 1991). These data provided evidence for a role for ethylene as a ripening hormone. Similar observations were also recorded in case of melon and apple (Ayub *et al* 1996; Bolitho *et al* 1997). The inhibition of ethylene responses in plants has also been reviewed recently by Sissler and Serek (1997).

4. Polyamine, ethylene and senescence

PAs and ethylene use a common precursor, SAM, for their biosynthesis (figure 1). But these two molecules show opposite effects in relation to senescence. Based on the available information, the interaction between polyamines and ethylene can be schematically represented as shown in figure 2. It is the balance between two opposite roles that is crucial for one or the other developmental states in the plant. The interaction between ethylene and PAs during *Hibiscus* flower senescence were observed by Chae *et al* (1995). The activities of ACC synthase and ACC oxidase were fairly high during the pre-climacteric stage in which ethylene production was very low. Regulation of expression of wound-inducible ACC synthase was studied by Li *et al* (1992) in tomato. It was found that PAs inhibited the accumulation of the wound-inducible ACC synthase transcript. They suggested that both, salicylic acid and PAs may specifically regulate ethylene biosynthesis at the level of ACC synthase transcript accumulation. No relation between endogenous PAs levels and ethylene were observed during post bloom period in *Pyrus communis* flowers (Crisosto *et al* 1992). In contrast to the usual sharp decline in PAs observed during fruit ripening process, some varieties of long keeping tomato showed a time-dependent increase in Put (Saftner and Baldi 1990). In cherimoya fruit, a relationship between the rise in Put content and the ripening process was observed (Escribano and Merodio 1994). Further, the rise in Put levels in this fruit was correlated with greatly enhanced arginine decarboxylase (ADC) activity and it was suggested (Escribano *et al* 1996) that the ripening related changes in Put levels are regulated via ADC mediated synthesis and that no conjugation is involved in the process. The endogenous content of Put declined during the ripening of normal tomato fruit but remained constant in ripening inhibited fruits. It is thought that anti-senescence activity of Put can be attributed to its ability to stabilize and protect membranes (Hong and Lee 1996). Other PAs like Spm and Spd seem to be more active in retarding senescence. Spm was more effective in preventing senescence-related events than similar treatments with other known senescence retardants (Apelbaum *et al* 1981; Kaur-Sawhney and Galston 1991). The decline of endogenous Spm or Spd in senescing leaf tissue could result from activation of the catabolic pathway via PA oxidase activity which could be blocked by PA-oxidase inhibitor guazatine (Smith 1985b).

As discussed earlier the ethylene production is associated with the biosynthesis of ACC (Nichols *et al* 1983; Nichols and Frost 1985). Most of the observations indicate that various PAs can delay senescence in a number of plant tissues by inhibiting ACC synthesis (Lee *et al* 1997; Hong and Lee 1996). The ethylene biosynthesis could also be modulated by the *in vivo* biosynthesis of PAs since ethylene and PA biosynthetic pathways share SAM as a common intermediate and could compete for the available SAM during senescence (Katoh *et al* 1987). Although PA could act by suppressing ethylene synthesis and stabilizing cellular membranes however, this is not universal. In carnation, treatment with PA does not always increase the flower longevity and may even result in an accelerated senescence (Downs and Lovell 1986). It has also been observed that treatment with PA could result in the stimulation of ethylene synthesis in some tissues

(Pennazio and Rogerro 1990). An increase in the endogenous level of free Put was noted during senescence of carnation flowers (Roberts *et al* 1984). No differences in free PAs distribution were reported by Serrano *et al* (1991) in climacteric and non-climacteric carnation flowers at the pre climacteric stage. However, the Spd content was noted about 3 times of Put, but as senescence progressed, this situation reversed in climacteric flowers, while it remained same in non-climacteric flowers.

Lee *et al* (1997) found that Spm delayed the senescence of cut carnation flowers and reduced ethylene production and ACC content and the activities and transcript amounts of ACC oxidase and ACC synthase in petals. Jones and Woodson (1999) studied the expression pattern of ACC synthase genes in carnation and found that the genes were differentially regulated in a tissue-specific manner. It was suggested that there may be additional members of carnation ACC synthase gene family responsible for ACC synthase expression in vegetative tissue. Comparing ethylene production with the changes of endogenous PA levels from control and methylglyoxal-bis-(guanylhydrazone) MGBG of Spm-treated petals during the entire incubation period, it was suggested that the endogenous PAs possibly suppress ethylene production. Treatment of cut carnation with aminotriazole retarded senescence and increased flower longevity and it also inhibited the climacteric peak of ethylene production but the treatment had no effect on the levels of PAs (Serrano *et al* 1999). In petunia flowers (Botha and Whitehead 1992), initial decline in PAs during the pre-climacteric stages was not accompanied by a concomitant increase in ethylene production. The reason for this can be found in the fact that the synthesis of ACC by the enzyme ACC synthase is a rate limiting step and not the availability of SAM in the pathway of ethylene biosynthesis during the pre-climacteric phase (Yu *et al* 1979; Nichols *et al* 1983). The inhibition of Put and Spd synthesis by D-arginine and MGBG respectively did not result in stimulation of ethylene synthesis during the pre-climacteric phase of petunia flowers. This provides further support for the claim that the availability of SAM is not involved in the control of ethylene synthesis during this period (Botha and Whitehead 1992).

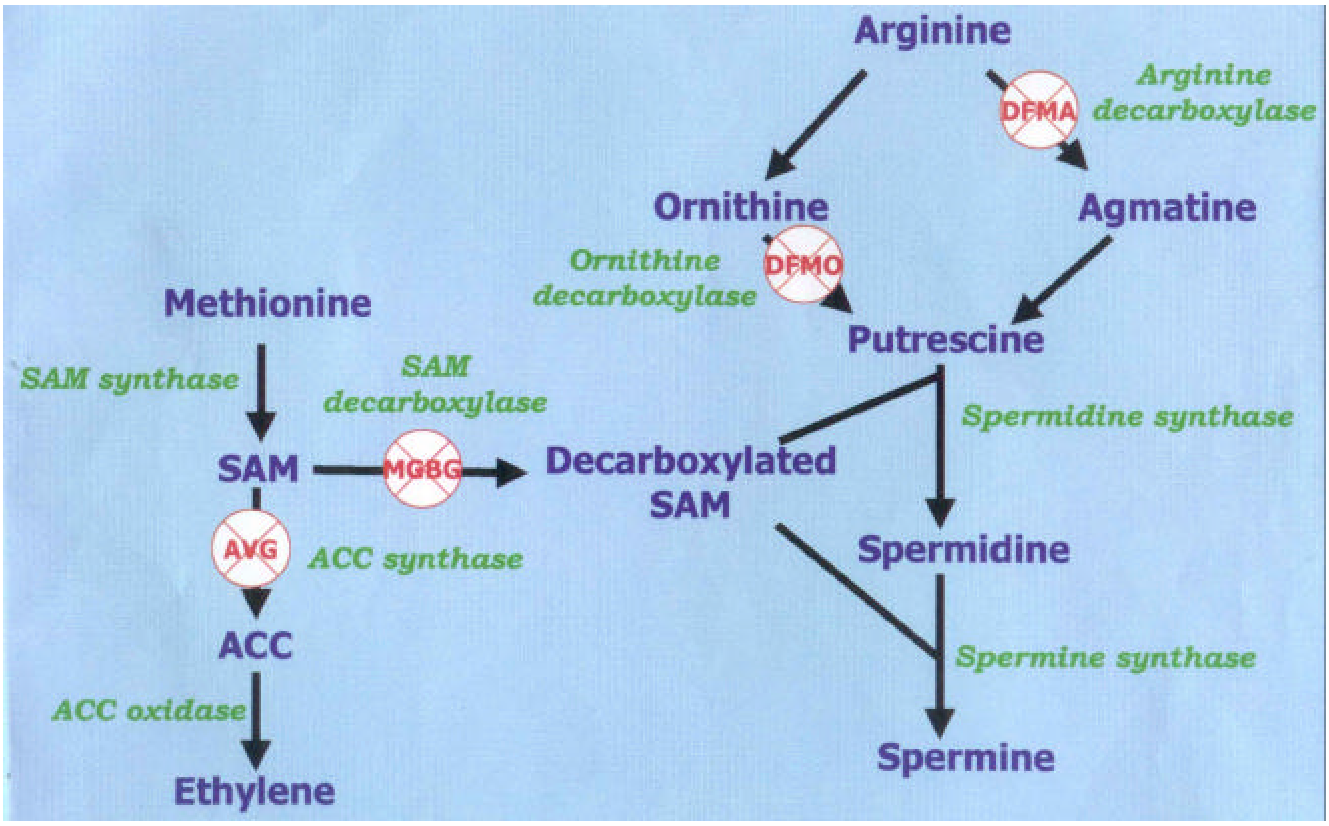

Figure 1. Partial metabolic pathway for the biosynthesis of ethylene and the common polyamines is depicted. SAM is the key common intermediate in the pathway. The names of the important enzymes are also shown in italics. The inhibitors are indicated by large encircled crosses. AVG, Aminoethoxyvinyl glycine; DFMA, DL-difluoromethyl arginine; DFMO, DL-difluoromethyl ornithine; MGBG, methylglyoxal-bis-(guanylhydrazone).

In recent years, genes for several key biosynthetic enzymes of PA have been cloned from different plant species (Perez-Amador *et al* 1995; Michael *et al* 1996). These include ODC and SAMDC. Additionally, plant SAM synthetase, which catalyzes the conversion of ATP and L-methionine into SAM has been cloned and characterized (Esparto *et al* 1994). Genes encoding ADC have been cloned from several species (Watson and Malmberg 1996) and the sequences were found to be highly conserved. In a comparative study of salt tolerant and salt sensitive rice cultivars, Chattopadhyaya *et al* (1997) using Northern blot analysis showed differential accumulation of ADC mRNA during salinity stress, correlating with the differences in ADC enzyme activities. A more detailed analysis of the conserved residues in ADC and ODC sequences has already been conducted (Michael *et al* 1996) along with an analysis of the only plant ADC gene (from *Datura*) to have been characterized. Several of SAMDC genes that have been characterized from plants, were cloned by differential screening of cDNA libraries from rapidly dividing and expanding cell types (MadArif *et al* 1994: Chang *et al* 1996). The plant SAMDC genes provide an exciting opportunity to test the importance of introns. Factors that control the expression of the polyamine biosynthesis genes are the subject of many studies in mammalian systems.

5. Conclusions

PAs and ethylene appear to be involved in a number of physiological processes of plants. The biochemical mechanisms which have been suggested to explain the biosynthetic relationships between the two pathways are: (i) competitive demand for a limited pool of common precursor (SAM), and (ii) feedback inhibition of enzyme action system in one pathway by the product(s) of the competing pathway. However, the extent of linkage between PA and ethylene pathways remains a moot point, and the significance of these mechanisms during the course of normal plant growth and development has not

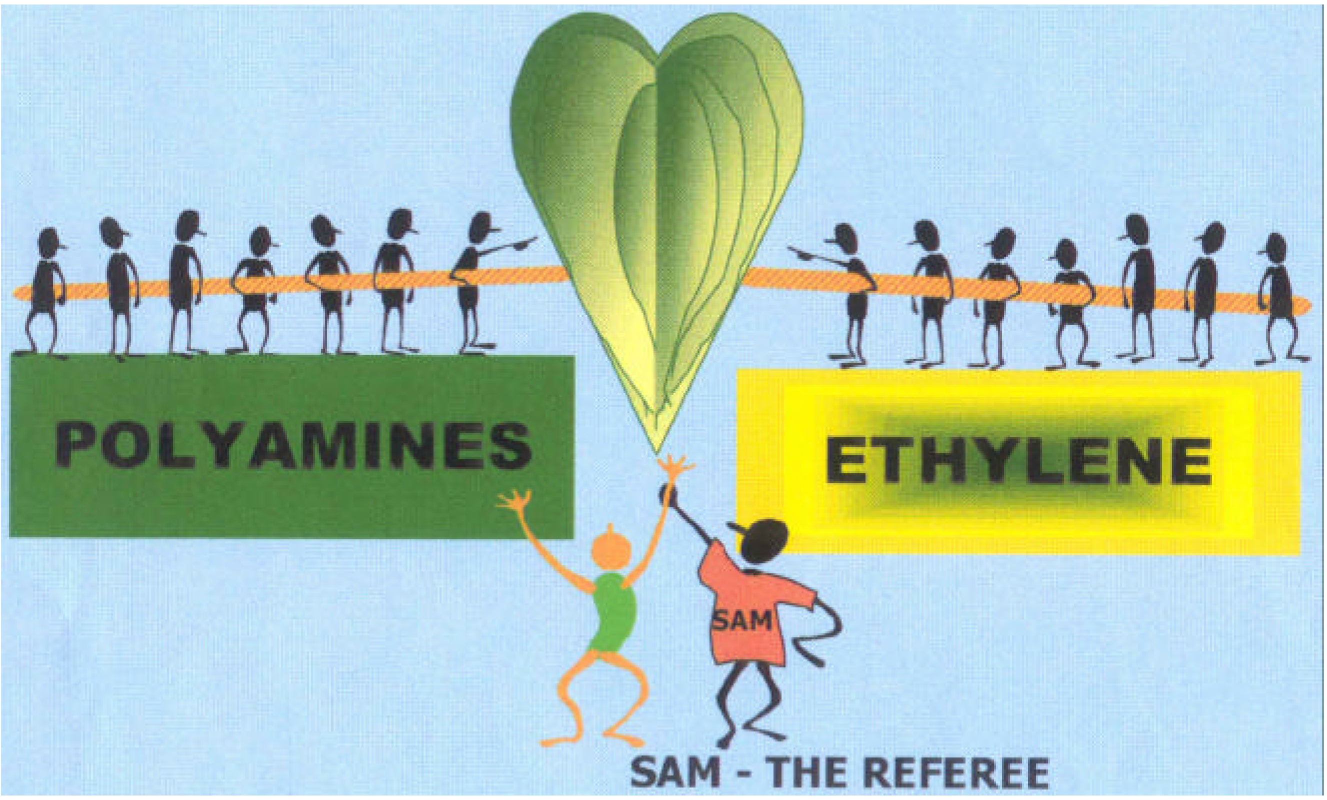

Figure 2. Schematic depiction of the “tug of war” between ethylene and polyamines has been given here. The site of action of the two modulators has been depicted as a leaf. The polyamine group is indicated by several small figurines holding the rope while in case of ethylene, the similar figurines represent various ethylene response elements. SAM has been depicted as the referee deciding the fate of the tug of war.

been demonstrated. Moreover, the requirement of PAs and ethylene in primary plant metabolism is not in question, rather it is the degree of their relationships at both the physiological and biosynthetic levels that remain unresolved.

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