EFFECT OF ETHYLENE SYNTHESIS/ACTION INHIBITORS ON LEAF SENESCENCE AND FLORAL ABSCISSION

By

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DEPARTMENT OF CROP PHYSIOLOGY
UNIVERSITY OF AGRICULTURAL SCIENCES
BANGALORE

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EFFECT OF ETHYLENE SYNTHESIS/ACTION INHIBITORS ON LEAF SENESCENCE AND FLORAL ABSCISSION

By

J. PADMALATHA

Thesis Submitted to the
University of Agricultural Sciences, Bangalore
in partial fulfilment of the requirements
for the award of the Degree of

Master of Science (Agriculture)

in

CROP PHYSIOLOGY

BANGALORE APRIL 1987

To My Beloved Parents

Sri. J. Muneppa

and

Smt. Shashirekha

DEPARTMENT OF CROP PHYSIOLOGY
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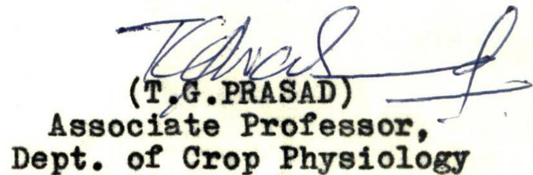
BANGALORE

CERTIFICATE

This is to certify that the thesis entitled "EFFECT OF ETHYLENE SYNTHESIS/ACTION INHIBITORS AND THEIR EFFECT ON LEAF SENESCENCE AND FLORAL ABSCISSION" submitted by Mrs.J. PADMALATHA in partial fulfilment of the requirements for the degree of MASTER OF SCIENCE (AGRICULTURE) in CROP PHYSIOLOGY to the University of Agricultural Sciences, Bangalore is a record of research work carried out by her during the period of her study in this University under my guidance and supervision and that no part of the thesis has been submitted for the award of any other degree, diploma, associateship, fellowship or other similar titles.



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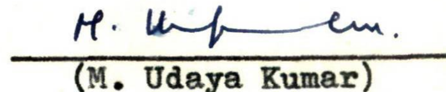
Members: 1. 
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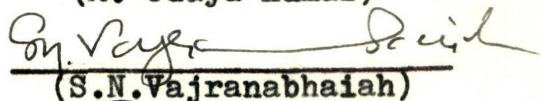
2. 
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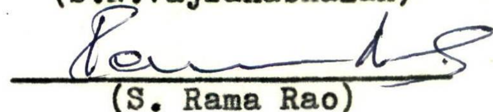
3. 
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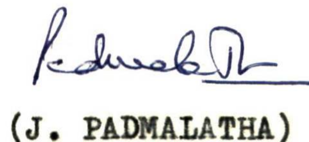

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INTRODUCTION

I. INTRODUCTION

Ethylene is a simple organic compound produced by any plant part. It is biologically active in trace amounts and is referred to as a gaseous plant hormone.

Ethylene is a powerful natural regulating substance of plant metabolism. It is found to influence many processes either by acting directly or by interacting with other hormones in trace amount . It was shown to regulate many plant growth and developmental processes such as germination of seeds and pollen, stem growth, root growth, expansion of leaves, flower induction, exudation of latex in rubber, sex expression, senescence, abscission of leaves and flowers and fruit ripening.

Ethylene production in plant tissue is regulated by many physiological and environmental factors. Various physical and chemical stressed like diseases, wounding and high radiation were shown to influence ethylene production. Induced rates of ethylene production is observed during the terminal life of the organ followed

by senescence and abscission. This assumes an active role for ethylene in senescence and abscission.

Leaf senescence induced by different abiotic stresses (moisture stress, temperature, low light) is a major constraint for productivity in many crop plants. Since a positive correlation exists between the leaf area duration and biomass production.

Abscission is the final stage of senescence. A high rate of flower shedding has been reported in many crop species especially in pulses. Limitation of nutrients, hormonal imbalance and environmental factors have been considered to be some of the important factors influencing premature floral abscission.

Controlling senescence and abscission could be of great economic value. Since both these processes are related to ethylene, controlling ethylene synthesis would be an approach to retard senescence and abscission.

Recently this field of study has gained a lot of interest. Ethylene biosynthetic pathway, and the important sites of regulation in the biosynthetic pathway have been well documented. Many organic and inorganic compounds have been found to regulate ethylene production and action.

A number of metal ions like silver, copper, nickle and cobalt, organic chemicals like benzoic acid, sodium benzoate, propyl esters of pyrogallol-n-propyl gallate and phenols have been shown to inhibit ethylene synthesis and/or action. Some of these chemicals act as free radicle scavengers.

Mode of action of these chemicals, sites of inhibition during the ethylene biosynthetic pathway and their role as competitive inhibitors at the site of action have been extensively studied. Chemical nature of these substances, optimum concentrations to be used for ethylene inhibition, duration of treatment also have been given a lot of importance, with this background, experiments have been conducted with the following objectives.

Objectives:

i) To assess the role of ethylene synthesis/ action inhibitors on leaf senescence,

ii) To determine the effect of some chemicals which retard senescence and floral absci-ssion in excised inflorescence,

iii) To assess the effect of a few effective concentrations of ethylene synthesis/ action inhibitors on pod set and producti-vity in field beans, under field conditions.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

Various physiological and biochemical changes preceding the process of senescence and abscission, role of endogenous plant hormones on senescence and abscission and the effect of exogenous application of various chemicals on senescence and abscission are briefly reviewed.

Senescence

Senescence refers to the deteriorative changes which are natural causes of death. Some of these deteriorative processes that result in senescence, if it occurs during the active growth stage affect plant growth and development resulting in reduction of plant productivity.

Metabolic changes in leaf senescence

Dwivedi *et al*. (1979) studied various biochemical changes in excised leaves of *Oryza sativa*. They reported that during senescence, under both turgid and water stressed conditions, there was reduction in chlorophyll,

total protein, starch, soluble sugars, total carbohydrates and non-reducing sugar content.

Bhivare *et al*. (1984) showed that leaf age had a marked effect on the concentration of organic and inorganic constituents.

Jana and Choudhuri (1982) reported that during senescence, chlorophyll content, RNA, DNA, protein activity of alkaline pyrophosphatase decreased while a few amino acids, activity of protease and RNase increased.

Chlorophyll degradation

One of the major prominent symptoms of senescence is yellowing. Loss of chlorophyll has been the main criterion for assessing senescence by many workers. Tetley and Thimann (1974) reported that detached first leaves from green or etiolated oat seedlings loose chlorophyll and carotenoids. Further Tanaki (1982) showed the degeneration of chlorophyll during senescence of maize leaf discs. Chlorophyll deficient types of soybean have fewer light complexes

and a higher ratio of PS II to PS I (Eskins _et al_., 1983).

Reduction in protein content

Decreased protein synthesis or increased protein degradation would account for the decline in protein content (Beevers, 1976).

Reduction in protein content and rise in total amino acids in senescing organs was observed by many workers (Hendry and Stobart, 1977, Storey and Beevers, 1977).

Proteolysis has been used as a measure of senescence in some cases (Osborne, 1968; Tetley and Thimann, 1974).

Garcia _et al_. (1983) indicated that protein synthesising capacity increases after 10-12 hours of dark incubation in isolated chloroplast of _Hordeum vulgare_.

Change in nucleic acids

Senescence is associated with marked changes in nucleic acid content. Reduction in RNA content during senescence was noted by many workers (Osborne, 1968).

Thimann (1980) stated that decrease in DNA was smaller than to that of RNA.

Decrease in total RNA content occurs due to increased activity of RNase enzyme (Osborne, 1968). Thimann (1980) opined that reduction in RNA can be due to decreases in the rates of synthesis or due to increase in the activity of RNase.

Scott *et al*. (1983) stated that there was preferential loss of plant DNA during the senescence induced due to nitrogen deficiency.

Plant growth regulators and leaf senescence

Growth regulators play a predominant role in leaf senescence. Osborne and Cheah (1982) reported that retardation or acceleration of senescence can be duplicated by the direct addition of appropriate growth

regulator to the leaf tissue, though the accelerations was evident only in leaves that are already past full maturity.

Osborne & Hallaway (1964) observed that auxins such as 2,4-D effectively retard senescence when supplied to leaves at particular stages of growth and development. Supraoptimal concentrations of IAA stimulated ethylene production opposing the senescence retarding effect of IAA (Aharoni et al., 1979).

Senescence retarding property of auxins was further supported by many researchers (Gepstein and Thimann, 1981).

Hsia et al. (1978) said that gibberellic acid retarded chlorophyll loss during senescence. Activity was due to sustained membrane integrity.

Kumar and Khan (1983) reported that GA and benzyl adenine prevented the raise in RNase activity.

Benzyl adenine treatment of intact primary leaves of bean prevents the decline in the level of

chlorophyll, protein, RNA, DNA and elevated the activities of corresponding hydrolases (Naito et al., 1979).

Thimann and Satler (1979) reported that kinetin prevents senescence in excised oat leaves by keeping the stomata open. In tobacco and tomato flux of BA in xylem sap, was increased due to debudding treatment. This retarded chlorophyll loss. Kinetin retarded senescence and stimulated metabolism of sugars in maize leaf discs (Tanaki, 1982). Lesham (1984) observed that cytokinin induced reduction in lipoxygenase and superoxide dismutase in senescing foliage.

ABA accelerates senescence and ABA levels increases sharply during senescence (Dumbroff et al., 1977 Gepstein and Thimann, 1980, 1981).

ABA promotes senescence of Lupinus albus cotyledons by speeding up the translocation of nutrients from cotyledons to axis (Elkinsway, 1983).

Ethylene

It is a well established fact that ethylene

induces leaf senescence and abscission (Burg, 1968) Abels and Holm (1966) reported that ethylene stimulates senescence and thus abscission.

Ethylene supplied through leaves or roots induced endogenous ethylene production and also stimulated senescence within 75 hours (Hall, 1977). During the senescence of tobacco, pinto bean and sugarbeat leaves, ethylene production occurred (Aharohi and Lieberman, 1979). Similarly Morgan and Durham (1980) reported involvement of ethylene in the senescence of Melia leaves.

Barmore (1975) reported that ethylene treatment increased the chlorophyllase activity in the rind tissue of calmodulin by 195 per cent. This was further supported by the observations made by Shimokawa et al (1978). He reported that ethylene induced degreening in citrus resulted in increased chlorophyllase activity.

Gepstein and Thimann (1981) reported chlorophyll loss during senescence of oat leaves and observed that loss of chlorophyll was low in white light associated with low ethylene evolution, when the loss

of chlorophyll was rapid, ethylene evolution was also accelerated. Kao and Yang (1983) have shown that during senescence of excised rice leaves ethylene production enhances. Light, cyclohoximide and cold temperature which retarded chlorophyll degradation also inhibited ethylene production. Legge and Thompson (1983) studied ethylene biosynthesis and membrane changes during plant senescence in senescing carnation flowers and epicotyls from etiolated peas. They reported that the enzyme capable of converting ACC to ethylene was primarily associated with microsomal membrane fraction. A sequential pattern of lipid rigidification was observed for microsomal membranes which correlated temporarily with the pattern of C_2H_4 production.

Effect of growth regulators on ethylene synthesis and action

Higher concentrations of auxins applied particularly during ageing was found to stimulate ethylene production leading to early senescence or ripening (Aharoni *et al.*, 1979). It was found that auxin induced formative effects such as flowering was due to ethylene production (Clark and Kerns, 1942).

The region of plant parts having higher endogenous auxin content were also shown to produce higher rates of ethylene (Sakai *et al.*, 1970).

Enhanced ethylene production was observed with the application of synthetic auxins like, 2,4-D (Hansen, 1946) which promoted fruit ripening. Picloram, an auxin like herbicide (Hansen, 1946), 2,4-5-Trichlorophenoxy acetic acid (Chalutz *et al.*, 1969) were also found to stimulate ethylene production.

In the ethylene biosynthetic pathway, IAA stimulates ethylene production by inducing synthesis or activation of ACC synthase which catalyses the conversion of SAM to ACC (Yu *et al.*, 1979). ACC is the *in vivo* precursor of ethylene. The activity of ACC forming enzyme was higher in seedling sections of *Pisum sativum* (incubated with IAA than in sections incubated in water alone (Jones and Hanskende, 1979).

Yoshii and Imaseki (1982) investigated the pathway of ethylene biosynthesis in auxin treated mungbean hypocotyls. They said that the enzyme which converts ACC to ethylene is already present in the

tissue and that auxin induced production of the enzymatic system responsible for the conversion of methionine to ACC.

ACC synthase was inactivated by its substrate SAM during its catalytic action (Satoh and Esashi, 1986).

Higher concentrations of cytokinins were also found to stimulate ethylene synthesis. Leibermann _et al_. (1977) showed that Isopentenyl (IPA) adenosine + IAA and IPA + IAA + GA_3 reduced ethylene production in tissue slices from preclimateric, climateric as well as post-climateric apples. GA_3 had little effect on ethylene production at all stages of fruit growth.

Gepstein and Thimann (1981) reported that IAA inhibits ACC induced reversal of chlorophyll loss in leaves of oat seedlings. Kinetin, cobalt, silver and IAA were shown to inhibit the chlorophyll loss induced by ethylene in ACC applied tissue.

When ABA and BA were simultaneously applied to the tissue ABA inhibited IAA induced ethylene production

The degree of inhibition was solely determined by ABA concentration (Kondo, 1975).

Effect of inorganic ions on ethylene synthesis and action

Inorganic ions like cobalt and nickel were shown to inhibit ethylene induced effect in many plant tissues (Kang et al. (1967). Lau and Yang (1976) showed that cobalt strongly inhibited the in vivo conversion of ^{14}C methionine to ^{14}C ethylene exerting its effect by inhibiting ethylene formation.

Aharoni et al. (1979) stated that cobalt and silver ions bind to the receptor site where ethylene actually acts. This was further confirmed by Yu and Yang (1979). Cobalt seems to form complexes with sulfhydryl group of proteins (Thimann, 1956 and Yu and Yang, 1979).

However other potent sulfhydryl agents such as P-chloromercuribenzoate, N-ethylmaleimide and S, 5^1 -dithio-bis-2-nitrobenzoic acid were not as effective as cobalt or nickel (Yang and Hoffman, 1984).

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According to a recent review by Yang and Hoffman (1984) inhibitors of ethylene biosynthesis were classified into 2 types.

1. Vinyl glycine analogs - Compounds which inhibit pyridoxal phosphate linked enzymes irreversibly.

2. Hydroxyl amine analogs - Which react with pyridoxal phosphate co-enzymes to form stable oximes.

Rhizobitoxine inhibits methionine biosynthesis through inactivation of B-crystathionase (Owens et al., 1968; Giovanelli et al., 1971). Although AVG (Amino ethoxy vinyl glycine) was found to be an irreversible inhibitor of ethylene production in apple tissue, the inhibition of AVG on ACC synthesis in vitro was reversible as inhibited enzyme activity was fully recovered when AVG was removed from the incubation medium (Boller et al., 1979; Hoffman and Yang, 1982; Yoshii and Imaseki, 1982).

Yu et al. (1979) showed L-canaline and Amino oxyacetic acid (AOA) hydroxyl amine analogue markedly inhibit IAA induced ethylene production.

Romani *et al*. (1980) showed that AVG inhibits ethylene production and ripening in pear fruits.

Recently, Bufler (1984) showed that AVG effectively blocked the inhibition of autocatalytic ethylene production and ripening of apples, Addition of AVG to a reaction mixture containing mungbean hypocotyl tissue and 40 µM SAM prevented ACC synthase inactivation and increased the half life of the enzyme about 2 fold (Satoh and Esashi, 1986).

Action inhibitors

Silver ions and carbon dioxide were classified as ethylene action inhibitors. The inhibitory action of silver and CO_2 on ethylene were explained in two ways.

1. A dissociable ethylene receptor complex acting like a switch that could turn on a number of reactions when ethylene combines with its receptor.

2. An actual reaction with ethylene at receptor site which produces reactants from the ethylene molecule resulting in physiological actions.

Ag^+ with IAA, kinetin and CO_2 increased ethylene production 160 folds over control but they inhibited senescence (Aharoni _et al._, 1979).

Aharoni and Lieberman (1979) stated that tobacco leaf disc senescence was delayed by Ag+ and CO_2 which blocked ethylene binding to its receptor site, but increased ethylene production.

$AgNO_3$, AVG and CO_2 were shown to inhibit senescence of oat leaves in light and dark (Gepstein and Thimann, 1981).

Beyer (1976) reported that silver ion oppose the effect of ethylene presumably by blocking the ethylene action at its receptor sites so that the receptor site did not react with ethylene.

Among ethylene synthesis inhibitors, silver ion was most potent anti ethylene agent.

Veen and Kwakkenbos (1983) suggested that the binding of C_2H_4 to its receptor leads to an increase in the production of ACC and of C_2H_4 by blocking the

C_2H_4 action. Silver ion inhibits the autocatalytic increase in ethylene and the accompanying rise in ACC content.

Aharoni (1985) studied the effect of silver ions and ethylene on auxin metabolism and auxin induced ethylene production in tobacco leaf discs and he stated that the most pronounced effect of Ag^+ in increasing ethylene production as well as the strongest antagonistic effect of exogenous ethylene were found between 24 and 48 hrs of incubation, ethylene exerted its auto-inhibitory effect by a feed back control on the IAA induced ethylene biosynthesis.

Burg and Burg (1965 and 1967) proposed that CO_2 is a competitive inhibitor of ethylene. They pointed out that CO_2 has close structural analog which substitute for ethylene (Burg and Burg, 1965). When CO_2 was added to the system of pea stem sections in different concentrations of ethylene, CO_2 has been found to block or retard ethylene action.

The fact that CO_2 was able to overcome or block ethylene action has been taken advantage of in several

cases. Many workers demonstrated that ethylene played an intermediate role in the action of various chemicals or treatments.

CO_2 treatment inhibited ethylene production and respiration of apples (Chaves and Tomes, 1984), Bufler (1984) showed that CO_2 delayed induction of ACC synthase activity and ripening of preclimacteric apples.

Proft _et al_. (1985) noticed that during the _in vitro_ culture of Magnolia, high CO_2 and low ethylene concentrations correspond with low chlorophyll content and reduced growth.

Effect of calcium

Rao and Swamy (1977) suggested that $CaCl_2$ treatment and petiole removal could markedly delay senescence in detached betel leaves by retarding chlorophyll and protein degradation.

Lieberman and Wang (1982) observed that high concentrations of calcium and magnesium eliminate lipid peroxidase protein leakage and also conversion of ACC to ethylene.

Green (1983) stated that KCl and $CaCl_2$ applied to cucumber cotyledons singly increased ethylene evolution. Whereas they strongly inhibited ethylene evolution when mixed together.

Evensen (1984) showed that calcium at a concentration of 50 mM increased ethylene production by 2 to 3 times in potato discs. Endogenous levels of 1-aminocyclopropane-1-carboxylic acid increased in parallel with ethylene production and ACC levels are 3-5 times higher in calcium treated discs than in control. This indicated the primary effect of calcium on a step of ethylene biosynthesis preceds ACC production. Since a consistent increase in ACC dependent ethylene production was observed in the presence of calcium, they opined that membrane stabilization by calcium could also be involved in its effects on ethylene production.

Lesham <u>et</u> <u>al</u>. (1984) suggested that in senescence induced pea plants internal calcium ions promoted senescence by activating calmodulin which in turn mediated the action of phospholipase A_2 on membranes which also had increased capabilities to convert ACC to C_2H_4.

Recently Burns and Evensen (1986) reported that application of 5 mM $CaCl_2$ resulted in increased ethylene production in pericarp discs obtained from ripening tomato.

Effect of phenolic compounds free radical scavengers and other chemicals

A number of other compounds were also shown to inhibit ethylene production/action like protein synthesis inhibitors (Apelbaum and Yang, 1981) short chain organic acids, oxidative phosphorylation inhibitors like 2,4-Dinitrophenol (DNP).

DNP was found to inhibit auxin dependent ethylene production in mungbean hypocotyl tissue (Yu et al., 1980). They have also reported that at lower concentrations DNP inhibits conversion of ACC to C_2H_4. At higher concentrations, inhibitory effect was attributed to inhibition of S. Adenosyl methionine (SAM) synthesis.

Shortchain organic acids were found to reduce the endogenous ACC levels (Apelbaum et al., 1981).

Leibermann and Kunishi (1975) reported that inhibitors of protein synthesis like actinomycin-D, cordycepin, L-amanitin and cycloheximide inhibit auxin induced ethylene synthesis.

Fuchs and Lieberman (1968) reported that inhibitors like cycloheximide and N-ethylmaleimide caused considerable inhibition of kinetin induced ethylene production.

Fuhrer et al. (1982) reported that polyamines inhibit senescence by inhibiting ethylene production and activity of enzyme ACC synthase.

Polyamines spermine and spermidine, diamines, putrescine and cadaverin were found highly effective in preventing chlorophyll loss in excised leaves of radish (Altman, 1982). Chen et al. (1982) have reported that spermine, spermidine and Co^{2+} inhibited ethylene production.

Floral abscission

Flower and immature pod drop is a major factor limiting seed yield in many crops. In field beans only

84 to 90 per cent of the flower buds opened into flowers and 39 to 63 per cent of the flowers produced young pods (Kombal, 1969). High percentage of floral abscission to the extent of 80 to 90 per cent was recorded in cowpea (Summer field et al., 1974) and in field bean (Soper, 1952; Rawlands, 1961). High percentage of floral absci-ssion in different crops has been reported by several other workers. Kaul et al. (1976) showed high flower abscission in cowpea, greengram and blackgram and Soundrapandian et al., 1975 in black gram. Huff and Dybing (1980) reported high percentage of abscission of flowers in soybean.

A number of edaphic, environmental and plant factors influence abscission. In Capscicum annum, number of flowers produced decreased with reducing light intensity (Park and Jeong, 1977). Bean flower buds have been found to abscise in large numbers under long day conditions (Bentley et al., 1975; Periera et al., 1971).

High temperatures were shown to induce floral abscission in tomato (Levy et al., 1978) soybeans (Mann and Jawarski, 1970), and in hot pepper (Song et al., 1978).

Mineral elements which are associated with abscission of plant parts are nitrogen, phosphorus, potassium, sulphur, calcium, magnesium, zinc and iron. The deficiency of these minerals in soil were reported to increase the abscission of flowers and pods (Kozlowki, 1973). Rubie et al. (1979) showed that nitrogen application increased the total dry matter accumulated and number of pods produced by plant.

Calcium plays an important role in abscission through its presence in calcium pectate, the cementing substance between the cells (Leopold, 1964). Cell wall of the abscission layer had lower affinity for calcium with progressive development of abscission zone due to degradation of cell wall (Poovaiah and Rasmussen 1973a). They have also shown that, calcium chloride treatment resulted in delayed abscission.

The other important elements, the deficiency of which accelerates abscission are zinc (Takaki and Kushizaki, 1970) and Boron (Linskens, 1964).

Many workers proved the importance of current photosynthesis during reproductive stage for increased pod fruit set and development.

Duarte and Adams (1972) suggested that leaf number was highly correlated with pod number.

After pollination and fertilization of the ovary, further growth and development or suspension of growth resulting in abscission of flower seems to lie much in the synthetic activity in the ovary. Several biochemical and physiological changes occur during abscission.

Studies conducted to find the sequence of processes prior to abscission showed that there is synthesis and secretion of enzymes which initiate cellulose and hemicellulose degradation in the abscission zones associated with increased enzyme activities which are involved in the formation of abscission layer cells.

Increased activity of the enzymes polygalactrr-uronase and cellulase (Greenberg, et al., 1975; Perger, 1979) lead to the abscission of citrus and Phaseolus vulgaris explants. Cellulase and pectinase enzymes accelerated the degradation of absaission layer cells during leaf shedding (Otakhanov and Imamalier, 1982).

Poovaiah and Rasmussen (1973b) showed increased peroxidase activity is closely related with the development of abscission layer. Henry *et al*. (1974) observed high activity of peroxidase in abscission layer before abscission is induced. In field beans, reduction in source capacity by defoliation enhanced flower drop (Chinnaswamy, 1979). Similar results were reported by many researchers (Stewart *et al*., 1978; Enyi, 1975; Lockwood *et al*., 1977).

Competition between vegetative and reproductive organs for current photosynthates leads to incomplete development of many sinks leading to their abscission (Egli and Legett, 1973; Tanaka and Fuzita, 1975). Farrington and Pate (1981) observed that in *Lupinus angustifolius*, interaction of vegetative and reproductive growth at flowering lead to floral abscission. In groundnut also, varieties which showed higher partitioning of assimilates to the reproductive growth showed higher pod yield (Williams *et al*., 1975; Duncan *et al*., 1978).

Role of hormones

Changes in the level of endogenous growth

regulators in the abscissing organs are often correlated with abscission of leaves and flowers.

Auxins are found to have a major role in controlling senescence and abscission. Levels of extractable and diffisable auxins are known to decrease with age of the leaves (Wetmore and Jacobs, 1953). Addicot and Lynch (1951) suggested that higher auxin concentration in the proximal side promotes abscission. On the other hand higher auxin concentration in distal side of the abscission zone delays abscission. Rubinstein and Leopold (1963) showed that auxin effects were inhibitory to abscission at initial stages,whereas at later stages, the same concentrations of auxins promoted abscission. Morgan and Durham (1972) suggested that the natural role of auxin in suppression of abscission should have two components (i) an adequate amount of auxin needs to be synthesised in the leaf blade and (ii) a continuous flow of auxin from disal to promimal side of abscission zone.

Roberts and Osborne (1981) stated that auxin flux from abscising organ decrease in turn increasing ethylene production. George (1982) noted that ability

of petals to respond to IAA appears to be a function of physiological age. IAA induces senescence in <u>Dianthus caryophyllus</u> petals by the duration and amount of ethylene production. Nago and Sakai (1985) reported that reduction in fruit removing force was inhibited by 100 µM NAA and 100 µM 2,4-D.

Gibberellins can have moderately promotive effect on abscission (Jacobs, 1966). The stimulatory effect of gibberellin is said to be due to enhancement in ethylene formation (Abeles and Rubinstein, 1964) Addicot, 1970; Marynick, 1977 and Gorrod and Harris (1978) reported delay in senescence with the application of GA (GA_3), at 200 mg/litre to isolated petals of carnation Cv. Whitesim.

Cytokinins retard senescence and thus have an inhibitory effect on absission (Osborne and Moss, 1963). In streptocarpus leaves, a decrease in endogenous cytokinin levels have been correlated with abscission (Vanstaden, 1973). However, Abeles <u>et al</u>. (1967) had shown that cytokinin can stimulate ethylene biosynthesis which in turn can promote abscission. Pre-treatment of leaves with cytokinin enhanced the

delaying effect of IAA on abscission of debladed petioles in coleus (Sastry and Ramarao, 1967). Further, pre-treatment of leaves with cytokinin prevented alanine induced abscission (Viswanath _et al_, 1970).

Abscisic acid (ABA)

It is very well established that abscisic acid promotes abscission (Craker and Abeles, 1969; Jackson and Osborne, 1972, Addicot, 1982). Varma (1976) reported that in cotton, compared to abscising bolls, retained bolls contained less ABA.ABA treatment to intact or explant bolls promoted abscission.

Porter (1977) observed a sharp increase in ABA content in flowers of _Lupinus luteus_ a day before abscission. External application of ABA to leaves increased abscission of flowers.

Apte and Laloraya (1982) stated that ABA induces abscission of petioles of _Phaseolus vulgaris_ explants. Guinn (1982) reported that abscission of young cotton bolls increased when they were placed in dim light due to increased levels of ABA.

Ethylene

Members of each of the five groups of plant hormones have been implicated in the regulation of senescence and floral abscission. However, ethylene seems to have been dealt with most extensively, because of its potential hazard as a pollutant. A wide range of flowers are affected by ethylene to various degrees, Various symptoms associated are fading and in rolling of the corolla of Ipomea (Kende and Baumgartner, 1974). Fading and wilting of sepal tips in orchid (Akamine, 1963) and abscission of flowers and petals.

Exposure of mature carnation flowers to ethylene resulted in a dual response i.e., in rolling of petals and increased synthesis of ethylene. In young carnation buds, exposure to ethylene did not stimulate ethylene production but a promotion of petal expansion was observed (Camprubi and Nichols, 1978, 1979; Nowak and Rudnicki, 1979).

Similarly exposure of mature open Ipomea flowers to ethylene resulted in a dual response. However, younger buds responded only with the inrolling

of the corolla without a concomitant increase in ethylene synthesis (Kende and Hanson, 1976, 1977).

The time course of ethylene production follows a typical profile composed of three distinct phases (1) low steady rate (2) an accelerated rise to maximum emanation (3) a last phase in which production is declining. Various events associated with senescence can be studied with reference to these phases (Maxie *et al.*, 1973).

Exposure of mature carnation flower tissue to ethylene generally hastens the onset of senescence and the development of typical aging symptoms (Mayak and Kofranek, 1976). Mor and Reid (1981) found that petals detached from mature flowers senesced and showed the climacteric rise in ethylene simultaneously with parent flowers. However, petals detached from young flowers on the day of flower opening senesced much later and produced less ethylene than did detached petals of older flowers, Senescence includes processes leading to cell disorganization.

Kende and Baumgartner (1974) have proposed a

model based on changes in compartmentation to account for autocatalytic ethylene synthesis. By increasing the permeability of the tonoplast, ethylene could enhance the flow of substrates from the vacuole to the cytoplasm where an ethylene - generating system is located. Hanson and Kende (1975) and Mayak et al. (1977), indicated that one of the first changes to take place in response to ethylene was an enhanced efflux across the tonoplast. Halevy and Mayak (1979), Matile (1978) have also demonstrated cell membrane disintegration, especially the tonoplast leading to uncontrolled mixing of vacuole contents with the cytoplasm. However, Suttle and Kende (1978) concluded that a rise in ethylene production was observed in Tradescantia petals during the initial period of fading without corresponding increase in pigment leakage, a natural indicator of changes in permeability.

Suttle and Kende (1980) stated that there is a lag of abcut 2 hours between ethylene application and increased permeability in Tradescantia petals. The increase in permeability is accompanied by a massive loss of phospholipids (Borochov et al., 1978). John et al. (1982) concluded that the climacteric range in

ethylene production during senescence of carnation flowers facilitated physical changes in membrane lipids that presumably lead to loss of membrane lipids.

Vander Westhuisen and de Swardt (1978) observed a rise in the level of methionine at about the physiological stage associated with commendement of the rise in ethylene production.

Nichols (1977) proposed that in carnation after pollination, the style, through its endogenous ethylene production is thought to trigger petal wilting. Glissen (1977) using petunia flowers, reported that the penetrating pollen tubes activate the stylar tissue which then leads to the wilting of the flower.

Stead and Moore (1979) demonstrated that a stimulus was transmitted from the pollinated stigma through the style and the ovary stimulating petal abscission.

Bufler _et al_. (1980) observed that the rise in ethylene production was associated with 30 fold increase

in ACC in the petals of cut carnations. Roberts et al. (1984) reported that exposure of excised pedicels with the flower attached to ethylene results in abscission within 12 hrs in tomato. Mor et al. (1985) studied the cause of the uneven production of ethylene by upper and basal portions of detached petals of carnation. They suggested that ACC synthase activity in the basal portion of the petals was 38 to 400 times more than that in the upper portion.

Application of 1-Amino cyclopropane-1-carboxylic acid (ACC) to rib segments of excised ipomea flowers resulted in the formation of ethylene in greater quantities than produced under natural conditions (Jorg et al., 1980). Whitehead et al. (1984) stated that during senescence of carnation petals, ethylene production increased more than 1000 fold but the ACC concentration in petals increased only 3 fold. The concentrations of conjugated ACC was generally lower than that of free ACC.

Cook et al. (1985) observed a characteristic rise in ACC, in the initial stages but the greatest increase in ACC are associated with the decline in

ethylene production during the later stages of lower senescence of cut carnations. They suggested that ACC synthase in the basal portion of the petals was 38 to 400 times than that in the upper portions.

Regulation of abscission

Sastry and Rama Rao (1967) reported that IAA delays abscission of debladed petioles of Coleus blumei. Poovaiah and Rasmussen (1973) observed that application of IAA 10^{-5}M and cycloheximide inhibited abscission of petiolar explants of beans.

Cook et al. (1985) suggested that cytokinins delay the onset of senescence and reduce ethylene sensitivity and production in cut carnation flowers by affecting the biosynthesis and action of ethylene in the tissue. Benzyl adenine prevents the rise in endogenous ACC levels and reduces the capacity of the tissue to convert ACC to ethylene.

Ethylene synthesis inhibitors

Suttle and Kende (1978) reported that AVG completely inhibits ethylene production in petals of

Tradescantia and also inhibits partially the loss of membrane permeability.

Konze *et al*. (1980) observed that ACC dependent ethylene production in protoplasts obtained from flower tissue of *Ipomea tricolor* was inhibited by n-propyl gallate, silver nitrate, cobalt chloride and potassium cyanide.

Rhizobitoxine and some of its analogues inhibited the synthesis of ethylene from methionine. They also reduced ethylene production and extended longivity of carnation (Baker *et al*., 1977). Yu *et al*. (1979) and Amrheim and Wenkler (1979) reported that amino oxy-acetic acid (AOA) is an inhibitor of ACC synthase.

Addition of AOA to the vase solution of carnations greatly extended longivity and suppressed the climacteric rise in respiration and ACC and ethylene production (Brown and Mayak, 1980; Fujino *et al*., 1981).

Several polyamines e.g., putrescine, cadavenine spermidine and spermine which were reported to retard the senescence of leaf protoplasts also inhibited

ethylene production in senescing petals of Tradescentia (Suttle, 1980).

Amino ethoxylene vinyl glycine and methoxy vinyl glycine (MVG) rhizobitoxine analogues shown to be specific inhibitors of ethylene synthesis from methionine by blocking the conversion of S-adenosylmethionine to 1-aminocyclo propane-1-carboxylic acid (Lieberman, 1979).

Silver ion

Veen (1979) reported that silver ions have antiethylene action. Movement of silver ions are faster in the form of silver thiosulphate (STS) aninoic complex. Anti-ethylene action of silver is preserved in this complex as shown by a significant improvement of the longivity of carnation flower. Bufler et al. (1980) observed that pre-treatment of flowers with silver thiosulphate retarded flower senescence and prevented the increase in ACC. An increase in ACC in the remaining flower parts, which appeared to precede its increase in the petals was only partially prevented by the STS pre-treatment.

Reid et al. (1980) stated that STS doubled vase

life of carnation flowers. The effect could be achieved by treating stems with solutions containing as little as 1.0 mM with a pulse as short as 10 minutes. Silver uptake estimations indicated that a minimum of 0.5 μ mol Ag was required per stem for maximum vase life and more than that was toxic.

Meeteren et al. (1982) suggested that light controlled flower bud abscission was mediated by ethylene.in Lilium. As a consequence of the influence of light on ethylene action darkness also caused an increase in ethylene evolution and floral bud abscission. This could be prevented by injecting flower buds with 0.2 mM STS at the beginning of dark period.

A combined STS and sucrose treatment improved flower quality by promoting bud opening of spikes and delayed floret senescence and abscission in both fresh and stored flowers in sweet peas (Mor et al., 1984).

Cook et al. (1985) in their work on carnations compared the effect of antisenescence agents such as AVG, silver ions and cobalt ions with BA on ethylene

sensitivity and production. Benzyladenine prevents the rise in endogenous ACC levels and reduces the capacity of the tissue to convert ACC to ethylene.

Veen (1985) suggested that STS has an antagonistic effect on ACC stimulated growth of pistils in carnation buds.

Carbon dioxide (at 4 per cent) is a competitive inhibitor of ethylene and it completely prevented the development of inrolling of carnation petal tissue associated with naturally occurring changes in ethylene production (Mayak and Dilley, 1976). Kende and Hanson (1976) reported that CO_2 delayed but did not prevent the inrolling of the corolla in Ipomea flowers.

In Tradescantia petals, CO_2 delayed but did not prevent the rise in ethylene evolution and pigment leakage (Suttle and Kende, 1978).

Cobalt is an inhibitor of ethylene biosynthesis and has been shown to extend vase life of cut flowers. (Lau et al., 1976; Fujino and Reid, 1983; Venkatarayappa et al., 1980).

Calcium salts at 10^{-3} to 10^{-1}M applied at the state-I inhibited abscission of bean petioles probably by retarding the senescence of pulvinar tissue (Poovaiah and Leopold, 1973a). (Poovaiah and Rasmussen 1973b) reported that $CaCl_2$ treated bean leaves had higher calcium content localized in abscission zone and prior to abscission, there was a corresponding decrease in calcium content in the abscission zone.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

Experiments were conducted to study the effect of ethylene synthesis/action inhibitors on the senescence of leaf discs of soybean, abscission of debladed coleus petioles and floral abscission in field beans. Plant material used in these experiments and the methods adopted to study senescence and abscission processes are explained in brief in this chapter.

3.1 Effect of ethylene synthesis/action inhibitors on senescence:

Plant material - Soybean leaves:

Soybean variety KSHB-2 was used inthis study. Healthy, bold seeds were selected and sown on raised beds with a spacing of 15 cm between plants and 30 cm between rows. Cultural practices and plant protection measures were taken according to the recommendations in the package of practices for the cultivation of soybean variety KSHB-2 (Anon., 1982). Leaves were used from the plants aged around 30 - 35 days. Third or fourth trifoliate leaf from the top was used in the experiments.

Chemicals tested:- Ethylene synthesis inhibitor cobalt chloride, free radical scavenger benzoic acid,

Ethylene action inhibitor silver thiosulphate (STS) were used in the experiments. Effect of calcium, aluminium alone or in combination with other ethylene synthesis/action inhibitors were also studied.

3.1 Experiments

Experiments were conducted to study the effect of different chemicals on chlorophyll degradation in senescing soybean leaf discs under normal (non-induced) conditions.

Second or third fully expanded leaves from top of soybean plants were excised and were washed with distilled water. Leaf punches measuring 0.75 cm diameter were taken by using a leaf punch. Ten leaf discs were used per each replication. Leaf discs were placed in petridishes (9 cm diameter) containing 6 ml of test solution with three replications and incubated under dark conditions at 35°C. After 72 hrs chlorophyll content retained in leaf discs was measured.

3.1.1 Effect of silver thiosulphate on chlorophyll degradation:

This experiment was conducted to study the effect of STS on leaf senescence. Different concentrations of STS were used to know the optimum concentration required for retarding chlorophyll degradation.

Leaves from soybean plants were excised and were washed with distilled water and surface dried by gently blotting between folds of filter paper. Ten such leaf discs were placed in 9 cm petridishes containing 6 ml of test solution with three replications and incubated under dark conditions at 35°C. After 72 hrs, chlorophyll content retained in leaf discs was measured. Concentrations of STS used in this experiment are given in results chapter.

3.1.2 Cobalt:

Leaf discs of soybean were incubated in different concentrations of cobalt chloride solutions for 72 hrs. The effect of cobalt on chlorophyll degradation was studied.

3.1.3 Aluminium:

Leaf discs of soybean plant were incubated in different concentrations of aluminium chloride solutions. Amount of chlorophyll retained in the leaf discs was measured at the end of 72 hrs of incubation period.

3.1.4 Effect of cobalt, STS and their combinations with aluminium on senescence of soybean leaf discs:

Trifoliate leaves of soybean were excised from plants aged 30 days. Leaves were washed with distilled water and surface dried. Cut ends of petioles were kept immersed in different test solutions. For increased uptake of test solutions into conical flasks containing test solutions and leaves were kept under light (1000 lux.) for 6 hours. Leaf discs taken from these leaves were incubated in respective test solutions with which they were pretreated at 35°C in dark. Three replications were maintained for each treatment. At the end of 72 hrs of incubation period, chlorophyll retained in the leaf discs was measured.

3.1.5 Effect of benzoic acid, cobalt and STS on ABA induced chlorophyll degradation:

Experiments were conducted to study the effect of different chemicals on leaf senescence induced by ABA. Soybean leaf discs were pre-incubated in different concentrations of abscisic acid solution (ABA-0, ABA 10^{-5}M and ABA 10^{-4}M) for 24 hrs at 35°C. Later, leaf discs were transferred to petridishes containing water, benzoic acid (100 uM), cobalt chloride (100 uM)

and STS (100 μM). After 48 hrs of dark incubation at 35°C, chlorophyll content and per cent leakage of solutes were measured.

3.1.6 Calcium.

Calcium solutions of different concentrations were prepared using calcium chloride salt. Leaf discs were incubated in petridishes containing different concentrations of calcium for 72 hrs. At the end of the incubation period, chlorophyll content retained in the leaf discs was measured.

3.1.7 Effect of calcium on senescence under normal conditions and in the presence of EGTA:

Experiments were conducted to see the involvement of calcium in the senescence process. EGTA was used to sequester calcium ions. Cut ends of petioles of trifoliate leaves were kept immersed in different treatment solutions of calcium and EGTA for 6 hrs under light. Leaf punches from these leaves were incubated in dark in the respective treatment solutions in petridishes with which they were pre-treated. At the end of incubation period, chlorophyll retained in leaf discs and per cent leakage of solutes were measured.

3.1.8 Effect of calcium pre-treatment on chlorophyll degradation under normal conditions and in the presence of EGTA:

Soybean leaves were enriched with different concentrations of calcium by keeping the cut ends of leaf petioles in conical flasks containing treatment solutions for 6 hrs. Leaf punches taken from leaves of each treatment were incubated in the same treatment solutions with which they were pre-treated. After 24 hours, leaf discs were transferred to different petri-dishes containing water, EGTA, calcium and calcium + EGTA. After 48 hrs of dark incubation at 35°C, chlorophyll content and per cent leakage of solutes from the leaf discs were measured.

Ca 1 mM
a) H_2O
b) EGTA 1 mM
c) Ca 1 mM
d) Ca 1 mM + EGTA 1 mM

Ca 2 mM
a) H_2O
b) EGTA 1 mM
c) Ca 2 mM
d) Ca 2 mM + EGTA 1 mM

Ca 5 mM

a) H_2O

b) EGTA 1 mM

c) Ca 5 mM

d) Ca 5 mM + EGTA 1 mM

3.1.9 Effect of calcium and aluminium on senescence of soybean leaf discs:

Excised leaves were enriched with treatment solutions by keeping the cut ends of petioles in the treatment solutions. Leaf discs taken from these leaves were incubated in the same treatment solutions. At the end of 72 hrs of incubation period, chlorophyll content retained in leaf discs were measured.

Floral abscission.

Experiments were conducted by using leaf petiolar explants from coleus plants to study the role of ethylene synthesis/action inhibitors on abscission.

3.2.1 Plant material:

Coleus blumei - Benth plants were multiplied from a single clonal stock. When the plants were at one and half to two months old, leaf petiolar explants were taken for the studies. Leaves were debladed keeping the length of petioles at 2.5 cm. Three centimeter section of internode with the petioles were used in the experiments.

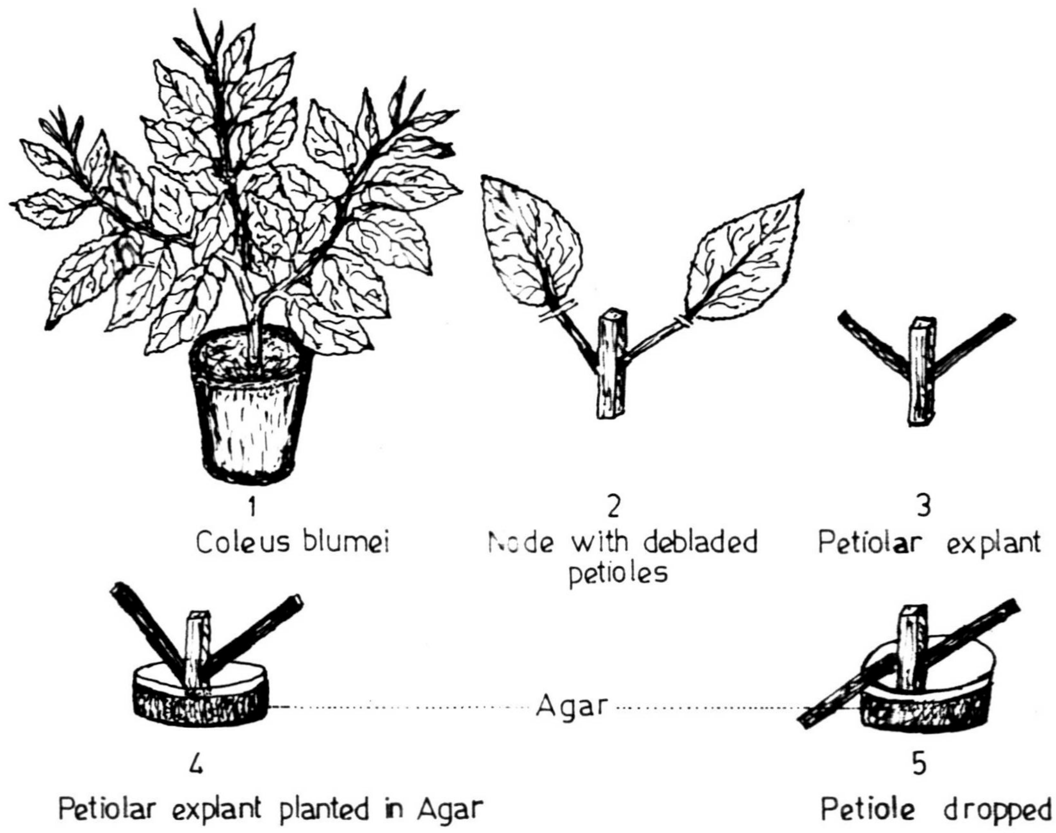


FIG. PREPARATION OF PETICLAR EXPLANTS FROM COLEUS PLANTS FOR STUDIES ON ABSCISSION

Explants were soaked in 10 ml of each treatment solution taken in petriplates at room temperature. Later, explants were planted on agar block (1.5%) in 9 cm petriplates. Eight explants were planted in each petriplate. Two replications were maintained for each treatment. Petriplates were placed in a humid chamber (100% relative humidity).

To study the effect of different chemicals under ethylene inductive conditions, ethylene was released in the humid chamber from ethrel solution to give 100 ppm in the chamber of capacity of 7.5 litres. At regular time intervals, number of petioles abscised were recorded.

3.2.1 Effect of silver thiosulphate on abscission of petiolar explants:

Petiolar explants were soaked in different concentrations of STS solution for 16 hrs. At the end of 16 hrs, they were surface dried by blotting the moisture and planted over agar medium. Number of petioles abscised were recorded at different time intervals

3.2.2 **Standardization of pretreatment time required to delay abscission of coleus petiolar explants by STS:**

This experiment was conducted to know the minimum duration of silver thiosulphate (50 µM). Pretreatment time required to inhibit abscission of petioles effectively.

Explants were soaked with STS, 50 µM for 0 hrs, 8 hrs, 16 hrs and 24 hrs. After the pre-treatment, explants were planted on agar medium and placed in humid chamber. Observations were recorded at regular intervals.

3.2.3 **Effect of STS and cobalt on abscission of coleus petiolar explants:**

With an objective to study the comparative effectiveness of ethylene synthesis inhibitor - cobalt and ethylene action inhibitor - STS. Explants were soaked in 50 uM concentration solution of $CoCl_2$ and STS for 16 hrs and then planted on agar medium. Number of petioles abscised at different time intervals were counted.

3.2.4 **Effect of ethylene synthesis, action inhibitors and free radical scavengers on abscission of coleus petiolar explants:**

This experiment was conducted to see the effect of different ethylene synthesis/action inhibitors and free

radical scavengers on abscission of petioles.

Coleus explants were soaked in different treatment solutions for 16 hrs, at room temperature. After pre-treatment, explants were surface dried and planted over agar medium. One set of explants treated with different chemicals, were maintained in ethylene free atmosphere. In another set, the explants were maintained in ethylene medium. Ethylene concentration of 100 ppm was released by using ethrel solution in humid chamber.

3.2.5 Floral abscission:

Experiments were conducted to study the effect of ethylene synthesis/action inhibitors on abscission of flowers and buds in field bean.

Plant material.

Field bean, variety Hebbal Avare-3 plants were selected for the experiment. Plants were raised under field conditions adopting a spacing of 30 cm between rows and 15 cms between plants in a row. Cultural practices and fertilizer dosages were followed according to the recommendations in package of practices for the cultivar Hebbal Avare-3 (Anon., 1982).

First formed inflorescences having approximately equal number of flowers and buds were excised from field bean plants and used in the experiments.

Method of treatment.

Field bean inflorescences were brought to the laboratory and the inflorescences were sprayed with 5 ml of treatment solution individually with a hand sprayer. After spraying, cut ends of inflorescences were kept immersed in water or ethrel (50 ppm) solutions taken in conical flasks (50 ml). Four replications were maintained for each treatment. Number of pods, flowers and buds abscissed were recorded at regular time intervals. Different treatment solutions used in these experiments were:

1. Water
2. STS 50 µM
3. STS 100 µM
4. STS 200 µM
5. Co 50 µM
6. Co 100 µM
7. Co 200 µM

Field experiments.

Effect of foliar application of ethylene synthesis inhibitor-cobalt and action inhibitor-silver on floral abscission was studied under field conditions.

Experiments were conducted at the Gandhi Krishi Vignana Kendra (GKVK), University of Agricultural Sciences, Bangalore, during kharif season (July to November 1985) and Rabi season (November to February 1986). Fertilizer dosage and other cultural practices were followed according to the recommendations in package of practices for the cultivar Hebbal Avare-3 (Annon., 1982).

3.2.6 Experiment-I

Field bean variety Hebbal Avare-3 was selected for the studies. The experiment was laid out in a randomised block design. Plants were raised with a spacing of 45 cms between rows and 30 cms between plants within a row. When the flowers started opening in the basal nodes of inflorescence, different concentrations of aqueous solutions of cobalt and STS were sprayed on the inflorescence using a hand sprayer.

Thirty representative plants were selected from each treatment and the following observations were taken after the plants attained maturity.

1. Leaf dry weight
2. Pod dry weight
3. Stem dry weight
4. Number of pods/plant

Treatment solutions used.

1. Water
2. STS 50 µM
3. STS 100 µM
4. STS 200 µM
5. Co 50 µM
6. Co 100 µM
7. Co 200 µM

3.2.7 Experiment II

Field bean variety Hebbal Avare was grown during November 1985 - February 1986 at GKVK Farm. The experiment was laid out in a randomised block design, each plot measuring 1.8 x 3.0 m. Four replications were maintained for each treatment. Plants were raised with a spacing of 45 x 30 cm. Fertilizer application, cultural practices and plant protection measures were taken as recommended in the package of practices (Anon., 1982). When the flowers started opening at the basal nodes of the inflore-

scence, the following treatment solutions were sprayed on the inflorescence. Second spray was given after 10 days of first treatment.

Treatments.

1. Control
2. STS 25 µM
3. STS 50 µM
4. Co 50 µM
5. Co 100 µM
6. STS 25 µM + Co 50 uM
7. STS 25 µM + Co 100 uM
8. STS 50 µM + Ca 50 uM
9. STS 50 µM + Co 100 uM

Ten representative plants from each replication of each treatment were taken for observations. The following observations were taken after the pods attained maturity.

1. Number of pods
2. Pod dry weight
3. Leaf dry weight
4. Stem dry weight

Dry weight.

Dry weights of leaf, stem and pods were recorded after oven drying the samples - at 80°C for 24 hrs. Stem dry matter included all branches of stem and inflorescence.

Number of pods per plant.

The total number of mature pods produced by the plant were counted at harvest.

Estimation of chlorophyll content in leaf discs.

Chlorophyll content was estimated following the method explained here. Immediately after taking leaf discs from trifoliate leaves of soybean, fresh weight of 10 discs was estimated from all the treatments and replications. After incubating the leaf discs in the respective test solutions, chlorophyll content was estimated. To know the initial chlorophyll content before incubation in test solutions, total chlorophyll content was estimated in 3 sets of 10 leaf discs each.

Known weight of leaf discs were mascerated with a pinch of calcium carbonate into a thin paste using a pestle and mortar. To this, 10 ml of 80 per cent acetone was added and thoroughly mixed. The mixture was filtered under suction. Required volume of acetone was added to the residue and filtered until all the chlorophyll is extracted. The filtrate was collected.

The volume of the filtrate was made upto 30 ml and this extract was used for estimating chlorophyll 'a',

'b' and total chlorophyll. The optical density of the extract was measured at wave lengths of 645, 652 and 663 nM in a Doublebeam Spectrophotometer. Chlorophyll 'a', 'b' and total chlorophyll contents were estimated by using the following formulae.

$$\frac{\text{mg. chlorophyll 'a'}}{\text{g tissue}} = 12.7(D663) - 2.69(D645) \times \frac{V}{1000 \times W}$$

$$\frac{\text{mg. chlorophyll 'b'}}{\text{g tissue}} = 22.9(D\ 645) - 4.68(D\ 663) \times \frac{V}{1000 \times W}$$

Total chlorophyll = mg of chl.'a'/g tissue + mg of chl.'b'/g tissue

Where:

D - Optical density

V - Final volume of 80 per cent acetone

W - Fresh weight in grams

Estimation of loss in membrane integrity.

Leaf discs from three replications of each treatment incubated in three different petriplates were selected to know the loss in membrane integrity. The extent of membrane damage or leakage was quantified by measuring the amount of substances that leaked out from the leaf discs. Solutes which are having absorption peak at 273 nm were estimated by following the method of Leopold (1981).

Leaf discs taken out from the incubation medium were briefly washed with distilled water and surface dried on a filter paper. Leaf discs were floated on 10 ml of distilled water in a beaker (25 ml). The beakers were occasionally shaken, so that all the solutes will leak uniformly into the water. Leaf discs were left in water for three hours. The amount of substances leaked out into the medium was measured by taking absorbance at 273 nm. The contents of the beaker were then kept in a boiling water bath for 15 minutes for the complete destruction of the tissue and complete leakage of substances in water. After necessary dilutions, the amount of substances leaked was measured by measuring OD at 273 nm. The per cent of substances leaked out into the medium was calculated using the formula.

$$\text{\% leakage of substance or solutes} = \frac{\text{OD of leachate from leaf discs}}{\text{OD of leachate from the same leaf discs kept in boiling water}} \times 100$$

Method followed to prepare silver thiosulphate

Silver thiosulphate was prepared by mixing equal volumes of 1 mM Silver nitrate with 8 mM Sodium thiosulphate. This was used as stock solution are dilutions were made as and when required.

EXPERIMENTAL RESULTS

IV. EXPERIMENTAL RESULTS

Several laboratory experiments were conducted to study the role of ethylene synthesis and ethylene action inhibitors on leaf senescence.

In addition, some of the chemicals were used to study their effect on abscission of petioles by using leaf petiolar explants. Effectiveness of these substances on abscission of flowers and flower buds in excised fieldbean inflorescence was also assessed.

Some chemicals which were effective in reducing the floral abscission in excised inflorescence were used under field conditions to study their effectiveness in inhibiting floral abscission in fieldbean.

The results obtained in these experiments are presented in this chapter.

4.1 Senescence

Different chemicals which inhibit synthesis of ethylene synthesis and or action on senescence was studied under laboratory conditions. The amount of

chlorophyll retained in the leaf discs after incubation in different solutions was used as a measure of senescence.

4.1.1 Effect of ethylene action inhibitor - silver thiosulphate on senescence of soybean leaf discs

Ten leaf discs were incubated in water or on different concentrations of STS. At the end of 72 hrs of dark incubation period, senescence was measured by estimating chlorophyll 'a', 'b' and total chlorophyll content in leaf discs. Data are presented in Table 1.

Leaf discs of soybean leaves incubated in water showed a reduction of total chlorophyll content from 5.1 mg to 2.85 mg/gram fresh weight. Incubating leaf discs with STS solution of 50 µM or higher concentrations significantly retarded the chlorophyll degradation. The amount of chlorophyll retained in the leaf discs were 3.29, 3.98, 3.69 mg/g fresh weight in STS 50, 100 and 200 µM respectively. STS 10 µM was not effective in retarding senescence. Highest chlorophyll content was observed in leaf discs incubated in STS 100 µM. Further increase in the concentrations of STS in the medium was not effective in retaining

Table 1. Chlorophyll content of soybean leaf discs in the presence of silver thiosulphate in the incubation medium

Treatments (Concentration in uM)	Chl. 'a'	Chl. 'b'	Total Chl.
	(mg/g fresh weight of leaf discs)		
H_2O	1.888	0.97	2.85(44)*
STS 10	1.64	0.67	2.32(55)
STS 50	2.25	1.03	3.29(36)
STS 100	2.78	1.20	3.98(22)
STS 200	2.30	1.38	3.69(28)
CD	0.3051	0.1847	0.2432

* Per cent reduction in total chlorophyll in relation to initial chlorophyll values.

Initial chlorophyll content - 5.1 mg/g fresh weight.

higher amounts of chlorophyll when compared to STS 100 µM.

Chlorophyll 'a' content were significantly more in STS 50, 100 and 200 µM compared to water treatment. Higher chlorophyll 'b' content was also observed in these treatments.

Silver thiosulphate in the incubation medium inhibited chlorophyll degradation in excised soybean leaf discs. A minimum concentration of 100 µM is necessary to inhibit the chlorophyll degradation significantly.

4.1.2 Effect of cobalt on senescence of soybean leaf discs

Leaf discs from 30 days old soybean seedlings were incubated in water and different concentrations of cobalt chloride. After 72 hrs of incubation, under dark at 35°C, chlorophyll content in leaf discs was estimated. Data are presented in Table 2.

Cobalt significantly retarded senescence with increase in concentrations of cobalt from 10 to 50, 100 and 200 µM, total chlorophyll content retained in

Table 2. Chlorophyll content of senescing soybean leaf discs in the presence of cobalt in the incubation medium

Treatments (concentration in µM)	Chl. 'a'	Chl. 'b'	Total chlorophyll
	(mg/g fresh weight of leaf discs)		
H_2O	1.888	0.971	2.859(44)*
Co 10 µM	1.749	1.105	2.854(44)
Co 50 µM	1.881	1.053	2.934(42)
Co 100 µM	2.273	0.861	3.134(38)
Co 200 µM	2.418	1.465	3.883(24)
CD at 5%	0.3957	0.2273	0.3974

* Per cent reduction in total chlorophyll in relation to initial chlorophyll values.

Initial chlorophyll content - 5.1 mg/g fresh weight.

leaf discs increased accordingly total chlorophyll values were 2.854, 2.934, 3.134 and 3.883 mg/g fresh weight respectively. Highest chlorophyll content was observed in leaf discs incubated in Co 200 µM concentration. Cobalt at lower concentrations of 10 and 50 µM were not effective in retarding chlorophyll degradation during senescence.

Chlorophyll 'a' content retained in the leaves showed an increasing trend from 1.749 to 2.418 mg/g fresh weight with increase in concentrations of cobalt from 10 µM to 200 µM. Chlorophyll 'a' content in water treated leaf discs was 1.888 mg/g fresh weight. Chlorophyll 'b' content also followed the same trend. Values increased from 1.105 to 1.465 mg/g fresh weight with increasing concentrations of cobalt from 10 µM to 200 µM.

Cobalt at a concentration of 200 µM, significantly retarded senescence of soybean leaf discs.

4.1.3 <u>Effect of aluminium on senescence of soybean leaf discs</u>

Trifoliate leaves of soybean from plants aged

35 days old were selected for the experiment. Ten leaf discs were placed over filter paper in petriplates containing 6 ml of water or different concentrations of aluminium chloride. After 72 hours of incubation, chlorophyll retained in the leaf discs was estimated (Table 3).

Leaf discs of soybean leaves placed over water showed a significant reduction in total chlorophyll content from 4.4 mg/g fresh weight to 1.139 mg/g fresh weight. Leaf discs placed over solutions of aluminium retarded leaf senescence showing significantly higher values of chlorophyll 'a', 'b' and total chlorophyll. Highest values of total chlorophyll and chlorophyll 'a' were observed at Al. 900 µM concentration. Total chlorophyll content and chlorophyll 'a' content values were 3.281 mg/g fresh weight and 2.391 mg/g fresh weight.

This treatment was followed by Al.800 µM and Al1 µM treatments. In which total chlorophyll content 3.266 mg/g fresh weight and 3.273 mg/g fresh weight were observed respectively.

Table 3. Chlorophyll and total chlorophyll content of senescing soybean leaf discs incubated with different concentrations of aluminium

	Treatment (Concentration in uM)	Chl.'a'	Chl.'b'	Total chlorophyll
		(mg/g fresh weight of leaf discs)		
1.	H_2O	0.739	0.400	1.139(74)*
2.	Al 100 uM	1.212	0.709	1.921(56)
3.	Al 200 uM	1.337	0.474	1.811(59)
4.	Al 300 uM	1.416	0.812	2.228(49)
5.	Al 400 uM	1.518	0.694	2.212(49)
6.	Al 500 uM	1.630	0.678	2.308(48)
7.	Al 600 uM	1.836	0.609	2.445(44)
8.	Al 700 uM	1.695	0.867	2.562(42)
9.	Al 800 uM	2.140	1.126	3.266(25)
10.	Al 900 uM	2.391	0.990	3.281(25)
11.	Al 1000 uM	1.982	1.291	3.273(26)
12.	Al 2mM	1.524	0.918	2.442(45)
13.	Al 3 mM	1.449	0.835	1.284(71)
	CD at 5%	0.2281	0.1307	0.2585

* Per cent reduction in total chlorophyll in relation to initial chlorophyll values.

Initial chlorophyll content - 4.42 mg/g fresh weight.

An increasing trend in chlorophyll content was observed with increase in concentrations of aluminium from Al.200 µM to Al.900 µM. Total chlorophyll values increased from 1.881 mg/g fresh weight to 3.281 mg/g fresh weight. A further increase in aluminium concentration reduced the amount of chlorophyll retained in the leaves.

The same increasing trend was observed in chlorophyll 'a' and 'b' content. Chlorophyll 'a' values increased from 1.212 mg/g fresh weight to 1.982 mg/g fresh weight from Al. 100 µM to Al. 1000 µM (Al 1 mM) showing a peak at Al 900 µM and Al 800 µM which showed 2.391 and 2.140 mg/g fresh weight respectively. Chlorophyll 'b' values also increased in the same manner.

Aluminium in the incubation medium in the concentration range of 100 to 1000 µM inhibited chlorophyll degradation in the leaf discs of excised soybean leaf discs.

4.1.4 Effect of cobalt, STS and their combinations with aluminium on senescence of soybean leaf discs

Trifoliate leaves of soybean were excised from

plants aged 30 days. Cut ends of the petioles were kept immersed in different treatment solutions containing different concentrations of cobalt, STS and aluminium. To enhance rate of translocation of the solutions into leaves, these were placed under high light intensities for 6 hours. Leaf discs taken from these leaves were incubated with the respective treatment solutions for 48 hrs at 35°C under dark. Chlorophyll content was estimated at the end of incubation period (Table 4).

At the end of incubation period, total chlorophyll value dropped to 1.274 mg/g fresh weight in case of leaf discs treated with water from the original chlorophyll content of 5 mg/g fresh weight. Incubating the leaf discs with ethylene synthesis/action inhibitors significantly reduced senescence. Total chlorophyll content retained was 1.697, 2.367, 2.583 and 2.610 mg/g fresh weight in leaf discs incubated with Co 100 µM, Co 200 µM STS 100 µM and STS 200 µM respectively.

Aluminium at concentrations 500 µM and 1000 µM (1 mM) was found effective in retarding senescence.

Table 4. Chlorophyll content in senescing soybean leaf discs in the presence of cobalt, STS and aluminium in the incubation medium

Treatments (Concentration in µM)	Chl.'a'	Chl.'b'	Total Chl.	Per cent reduction
	(mg/g fresh wt.of leaf discs)			
Control	0.614	0.660	1.274	75*
Co 100	0.959	0.738	1.697	66
Co 200	1.776	0.591	2.367	53
STS 100	1.910	0.673	2.583	48
STS 200	1.998	0.612	2.610	48
Al. 500	2.568	1.259	2.327	43
Al. 500 + Co 100	2.245	1.225	3.470	31
Al 500 + Co 200	2.353	0.982	3.335	33
Al 500 + STS 100	0.998	1.460	2.458	51
Al 500 + STS 200	1.633	0.734	2.367	53
Al 1000	2.430	1.136	3.566	29
Al 1000 + Co 100	2.503	1.185	3.688	26
Al 1000 + Co 200	2.660	1.285	3.945	21
Al 1000 +STS 100	1.363	1.458	2.821	44
Al 1000 +STS 200	1.137	0.862	1.991	62
C.D.	0.1919	0.2136	0.3306	

* Per cent reduction in total chlorophyll in relation to initial chlorophyll values.

Initial chlorophyll content - 5 mg/g fresh weight.

Total chlorophyll retained in leaf tissue were 2.327 mg/g fresh weight and 3.566 mg/g fresh weight in leaf discs pre-treated with Al. 500 µM and Al. 1000 uM, respectively.

Highest total chlorophyll values, 3.945 followed by 3.688 mg/g fresh weight were observed in leaf discs treated with aluminium 1000 µM + cobalt 200 µM and aluminium 1000 uM + cobalt 100 uM respectively. Total chlorophyll values of leaf discs treated with Al 500 µM + Co 100 µM and Al 500 µM + Co 200 µM were 3.470 and 3.335 mg/g fresh weight.

Aluminium in combination with silver thiosulphate not effective in retarding chlorophyll degradation when compared to aluminium in combination with cobalt.

Chlorophyll 'a' content increased when the leaf discs were incubated with ethylene synthesis inhibitors Co 100 µM and Co 200 µM and ethylene action inhibitor STS at 100 and 200 µM. Highest chlorophyll 'a' content of 2.660 mg/g fresh weight was observed in leaf discs treated with Al 1000 µM in combination with Co 200 µM.

This was followed by 2.568 mg/g fresh weight in leaf discs treated with Al 500 uM and 2.503 mg/g fresh weight in leaf discs treated with Al 1000 uM in combination with Co-100 uM.

4.1.5 Effect of benzoic acid, cobalt and silver thiosulphate on ABA induced chlorophyll degradation in soybean leaves

Effect of different ethylene synthesis and action inhibitors on abscisic acid induced degradation of chlorophyll was studied. Leaf discs from soybean leaves were floated on water, or on 10^{-4} and 10^{-5} M concentrations of ABA (abscisic acid). After 8 hours, leaf discs were transferred to water, benzoic acid, cobalt and STS solutions and incubated for 72 hrs at 35°C under dark (Table 5).

Total chlorophyll content retained at the end of incubation was 2.406 mg/g fresh weight in leaf discs treated with water and transferred to water. Pretreatment with ABA 10^{-5} M and ABA 10^{-4} M resulted in marked reduction in the amount of chlorophyll retained in the leaves.

The amount of chlorophyll retained in the leaf discs pretreated with 10^{-5} and 10^{-4} M ABA and transferred to water were 1.354 and 1.216 mg/g fresh weight respectively.

With increasing concentrations of ABA, the amount of total chlorophyll, chlorophyll 'a', chlorophyll 'b' retained in the leaf discs reduced markedly. The amount of chlorophyll 'a' retained in the leaves pretreated with water, 10^{-5} and 10^{-4} M ABA were 1.642, 1.045 and 0.869 mg/g fresh wight. The corresponding values for chlorophyll 'b' were 0.764, 0.409 and 0.337 respectively.

In benzoic acid 100 µM total chlorophyll content retained was 2.619, 2.852, 2.422 mg/g fresh weight in leaf discs pretreated with water, ABA 10^{-5} and ABA 10^{-4} M respectively. Same trend was observed in case of chlorophyll 'a' and chlorophyll 'b' content.

Cobalt, an ethylene synthesis inhibitor also retarded senescence significantly both under normal and ABA induced conditions. Total chlorophyll content values were 3.284, 2.246, 2.164 mg/g fresh weight

Table 5. Chlorophyll content of soybean leaf discs pre-treated with abscissic acid and transferred to benzoic acid, STS and cobalt

Incubated in	Transferred to	Chl.'a'	Chl.'b'	Total Chl.
		(mg/g fresh wt.of leaf discs)		
H_2O	H_2O	1.642	0.764	2.406(49)*
H_2O	Benzoic acid (100 µM)	1.718	0.910	2.619(45)
H_2O	Co (100 µM)	2.077	1.207	3.284(31)
H_2O	STS (100 µM)	2.464	1.284	3.748(21)
ABA 10^{-5} M	H_2O	1.045	0.409	1.354(71)
ABA 10^{-5} M	Benzoic acid (100 µM)	1.653	1.199	2.852(40)
ABA 10^{-5} M	Co (100 µM)	1.561	0.685	2.246(53)
ABA 10^{-5} M	STS (100 µM)	1.927	0.933	2.860(40)
ABA 10^{-4} M	H_2O	0.869	0.337	1.216(74)
ABA 10^{-4} M	Benzoic acid (100 µM)	1.816	0.606	2.422(49)
ABA 10^{-4} M	Co (100 µM)	1.461	0.703	2.164(54)
ABA 10^{-4} M	STS (100 µM)	1.736	1.001	2.737(42)
C.D.		0.610	0.561	1.224

*Per cent reduction in total chlorophyll in relation to initial values.

Initial chlorophyll content - 4.74 mg/g fresh weight of leaf tissue.

in leaf discs pretreated with water, ABA 10^{-5} and ABA 10^{-4} M solutions respectively.

Among the senescence inhibitors used in this experiment, STS proved to be more effective. Highest total chlorophyll values 3.748, 2.860 and 2.737 mg/g fresh weight were observed in leaf discs floated over water, ABA 10^{-5}M and ABA 10^{-4}M solutions and then transferred to STS 100 µM. Highest chlorophyll 'a' and chlorophyll 'b' values were also observed in these treatments.

STS 100 µM retarded senescence significantly both under normal and induced conditions. This was followed by cobalt and benzoic acid.

Abscisic acid accelerated total chlorophyll degradation markedly at all the concentrations. However, in the presence of different ethylene synthesis/action inhibitors, total chlorophyll degradation was less.

Transferring leaf discs pretreated with ABA to medium containing different ethylene synthesis/action inhibitors reduced the per cent leakage of solutes considerably (Table 6). Benzoic acid was found to be

Table 6. Per cent leakage of solutes from soybean leaf discs pre-treated with abscissic acid and transferred to benzoic acid, STS and cobalt

	Incubated in	Transferred to	Per cent leakage of solutes
1.	H_2O	H_2O	19.3 (26)*
2.	H_2O	Benzoic acid 100 uM	12.8 (21)
3.	H_2O	Co 100 uM	19.7 (26.3)
4.	H_2O	STS 100 uM	18.1 (25.3)
5.	ABA 10^{-5}	H_2O	45.2 (42.2)
6.	ABA 10^{-5}	Benzoic acid 100 uM	15.5 (23.1)
7.	ABA 10^{-5}	Co 100 uM	25.0 (30)
8.	ABA 10^{-5}	STS 100 uM	20.5 (26.5)
9.	ABA 10^{-4}	H_2O	44.2 (41.5)
10.	ABA 10^{-4}	Benzoic acid 100 uM	30.1 (33.2)
11.	ABA 10^{-4}	Co 100 uM	32.7 (34.6)
12.	ABA 10^{-5}	STS 100 uM	26 (30.5)
	C.D.		N.S.

*Angular transformed values.
NS : Non-significant

effective in maintaining integrity both under induced and non-induced conditions. Transferring to ABA pre-treated leaves to STS and cobalt chloride solution reduced ABA induced leakage of solutes.

4.1.6 Effect of calcium on senescence of soybean leaf discs

Incubating the leaf discs of soybean over calcium medium inhibited the process of senescence significantly (Table 7).

Total chlorophyll content of leaf discs incubated in water was 0.776 mg/g fresh weight whereas the initial total chlorophyll content was 3.82 mg/g fresh weight. Leaf discs incubated in a medium having calcium, retained more chlorophyll content. The amount of chlorophyll retained varied between from 1.087 mg/g fresh weight and 2.885 mg/g fresh weight when the calcium concentration in the medium was between 250 μM to 3 mM. Leaf discs treated with calcium concentration in the range of 1 to 3 mM retained higher chlorophyll content with the highest quantity of 2.885 mg/g fresh weight. Further increase in calcium concentrations to 4 mM and 5 mM reduced the

Table 7. Chlorophyll content in senescing soybean leaf discs in the presence of calcium in the incubation medium

Treatments (Concentration in uM)	Chl. 'a'	Chl. 'b'	Total Chl.
	(mg/g fresh weight of leaf discs)		
Water	0.538	0.238	0.776(80)*
Ca 100 µM	0.651	0.436	1.087(72)
Ca 250 µM	1.016	0.698	1.714(55)
Ca 500 µM	1.068	0.723	1.791(53)
Ca 750 µM	1.[illegible]05	0.485	1.590(58)
Ca 1 mM	1.437	0.905	2.342(39)
Ca 2 mM	1.553	0.954	2.507(34)
Ca 3 mM	1.722	1.163	2.885(24)
Ca 4 mM	1.718	0.891	2.609(32)
Ca 5 mM	1.796	1.002	2.798(27)
C.D. at 5%	0.1973	0.2063	0.411

* Per cent reduction in total chlorophyll in relation to initial values

Initial chlorophyll content - 3.82 mg/g fresh weight

amount of chlorophyll retained in the leaf discs when compared to 3 mM concentration.

Same trend was observed in case of chlorophyll 'a' content which showed an increase from 0.651 mg/g fresh weight to 1.553 mg/g fresh weight in the calcium concentrations of 100 μM and 1 mM respectively. In water treated leaf discs chlorophyll 'a' content was 0.538 mg/g fresh weight. Chlorophyll 'b' content also increased with increase in concentration of calcium.

4.1.7 Effect of calcium on senescence of soybean leaf discs under normal conditions and in the presence of EGTA

This experiment was conducted with an objective to study the involvement of calcium in senescence process. EGTA was used to sequester the calcium ion. Its effect with different levels of calcium in the medium on senescence process was tested.

Leaf discs from soybean leaves were incubated in different treatment solutions taken in petridishes for 48 hours under dark conditions at 35°C. Chlorophyll content and membrane integrity were measured at

Table 8. Chlorophyll content of senescing soybean leaf discs in the presence of calcium and EGTA in the incubation medium

Treatments (Concentration in mM)	Chl.'a'	Chl.'b'	Total Chl.
	(mg/g fresh weight)		
H_2O	1.022	0.814	1.836(46)*
Ca 1	1.538	0.869	2.407(31)
Ca 2	1.821	0.813	2.634(25)
Ca 5	1.405	0.765	2.17 (38)
EGTA 1	1.211	0.604	1.615(54)
Ca 1 + EGTA	1.101	0.443	1.544(56)
Ca 2 + EGTA	1.195	0.892	2.087(40)
Ca 5 + EGTA	1.184	0.508	1.692(52)
C.D. at 5%	0.0871	0.2056	0.0566

* Indicate the per cent reduction in total chlorophyll content in relation to original total chlorophyll content.

Original chlorophyll content - 3.5 mg/g fresh weight.

the end of incubation period. Data are presented in Table 8 and Table 9.

Total chlorophyll content reduced from 3.5 mg/g fresh weight to 1.836 mg/g fresh weight in leaf discs treated with water. Calcium in the medium retarded chlorophyll degradation significantly. Highest chlorophyll content was observed in leaf discs treated with Ca 2 mM. Increasing the calcium concentration in incubation medium to 5 mM decreased the amount of chlorophyll retained by the leaves. The total chlorophyll content in leaf discs incubated in 5 mM was only 2.17 mg compared to 2.634 mg in calcium 2 mM.

EGTA in the medium reduced the chlorophyll retained by the leaf discs significantly at all the levels of calcium in the medium.

Highest chlorophyll 'a' content was observed in leaf discs treated with calcium 2 mM followed by calcium 1 mM. Among combination treatments, EGTA 1 mM in combination with Ca 2 mM showed the highest chlorophyll content of 1.195 mg/g fresh weight (Table 8).

Table 9. Per cent leakage of solutes from soybean leaf discs incubated in medium containing calcium and EGTA

Treatments (Concentration in uM)	Per cent leakage of solutes
H_2O	20.6 (26.88)*
Ca 1	10.1 (18.56)
Ca 2	3.1 (9.46)
Ca 5	3.18(10.28)
EGTA	31.9 (34.26)
Ca 1 + EGTA	33.7 (35.5)
Ca 2 + EGTA	16.1 (23.61)
Ca 5 + EGTA	10.4 (18.54)
C.D. at 5%	8.746

* Angular transformed values.

Calcium at 2 mM concentration was found effective in maintaining membrane integrityl Per cent leakage observed was only 3.1 per cent. This treatment was followed by Ca 5 mM where the per cent leakage was 3.18 per cent.

Per cent leakage of solutes was highest (33.7 per cent) in leaf discs treated with calcium 1 mM + EGTA 1 mM solution (Table 9).

4.1.8 Effect of calcium pre-treatment on senescence under normal condition and in the presence of EGTA

Effect of calcium pretreatment on chlorophyll degradation was studied in leaf discs of soybean. Trifoliate leaves of soybean were excised from soybean plants aged 30 days. Cut ends of petioles of leaves were kept immersed in treatment solutions of H_2O, calcium 1 mM, Ca 2 mM, and Ca 5 mM for 6 hrs under high light intensities.

Leaf discs taken from these leaves were incubated in the respective treatment solutions with which they were pretreated. After 24 hrs leaf discs

from each treatment were transferred to petridishes containing H_2O, EGTA, calcium and calcium + EGTA treatment solutions. Petridishes were kept under dark conditions at 35°C for 72 hrs. Chlorophyll content and membrane integrity were estimated at the end of incubation period. Data are presented in Table 10.

Pretreatment of soybean leaf discs with calcium significantly inhibited the total chlorophyll degradation. Highest total chlorophyll values were 2.5323, 2.4548 mg/g fresh weight observed in the treatments pretreated with calcium 2 mM and transferred to water and Ca 2 mM respectively.

The total chlorophyll content retained was 2.3985, 2.2106 mg/g fresh weight in the leaf discs which were pretreated with calcium (1 mM) and transferred to Ca (1 mM) and water.

A significant reduction in total chlorophyll content was observed, when the leaf discs were incubated in EGTA. Total chlorophyll values were 1.3403, 1.4644, 1.0543 and 1.1395 mg/g fresh weight in leaf discs pretreated with Ca-0, Ca-1 mM, Ca-2mM and Ca 5 mM and then transferred to EGTA.

Table 10. Chlorophyll content of soybean leaf discs in the presence of calcium and EGTA in the incubation medium

Incubated in (24 hrs)	Transferred to (48 hrs)	Chl.'a'	Chl.'b'	Total chlorophyll
		(mg/g leaf fresh weight)		
Ca 0 mM	H_2O	1.007	0.7336	1.7406 (54)*
	EGTA 1 mM	0.851	0.4893	1.3403 (64)
Ca 1 mM	H_2O	1.402	0.8086	2.2106 (41)
	EGTA 1 mM	0.919	0.5454	1.4644 (61)
	Ca 1 mM	1.481	0.9175	2.3985 (36)
	Ca 1 mM+ EGTA 1 mM	0.924	0.3029	1.2269 (68)
Ca 2 uM	H_2O	1.928	0.6043	2.5323 (33)
	EGTA 1 mM	0.589	0.4653	1.0543 (72)
	Ca 2 mM	1.679	0.7758	2.4548 (35)
	Ca 2 mM + EGTA 1 mM	0.981	0.2516	1.2326 (67)
Ca 5 mM	H_2O	0.753	0.6311	1.384 (63)
	EGTA 1 mM	0.662	0.4775	1.1395 (70)
	Ca 5 mM	1.210	0.5287	1.7387 (54)
	Ca 5 + EGTA 1 mM	1.337	0.8718	2.2088 (41)
	C.D.	0.2560	0.0507	0.2636

* Per cent reduction in total chlorophyll in relation to initial chlorophyll content.

Initial total chlorophyll content - 3.76 mg/g fresh weight of leaf tissue

Table 11. Percent leakage of solutes from soybean leaf discs incubated with calcium and EGTA.

Treatments		Per cent leakage
Incubated in (24 hrs)	Transferred to (48 hrs)	
H_2O,	H_2O	25,22 (30.11)*
	EGTA 1 mM	36.26 (37.02)
	H_2O	11.75 (19.49)
Ca 1 mM	EGTA 1 mM	32.35 (34.62)
	Ca 1 mM	6.97 (15.30)
	Ca 1 mM+EGTA 1 mM	17.64 (24.83)
Ca 2 mM	H_2O	4.74 (12.44)
	EGTA 1 mM	15.85 (23.37)
	Ca 2 mM	1.60 (7.26)
	Ca 2 mM+EGTA 1 mM	7.65 (15.84)
Ca 5 mM	H_2O	3.18 (10.28)
	EGTA 1 mM	10.40 (18.54)
	Ca 5 mM	3.16 (10.22)
	Ca 5 mM+EGTA 1 mM	12.19 (20.42)
CD at 5%		5.348

* Values in the parenthesis indicate the angular transformation value.

Incubating calcium pretreated leaves in EGTA solution reduced the concentration of chlorophyll retained by the leaf discs. EGTA reduced the availability of calcium thereby causing more chlorophyll degradation.

Highest chlorophyll 'a' content 1.928 mg/g fresh weight was observed in leaf discs pretreated with calcium and transferred to water. In the presence of EGTA chlorophyll 'a' content retained by the leaves was significantly less.

Chlorophyll degradation was associated with loss of membrane integrity. Per cent leakage was 25.22 per cent in leaf discs treated with water (Table 11). This was increased to 36.26 per cent in case of leaf discs treated with EGTA, presence of calcium in the incubation medium, reduced the leakage considerably. Highest level of membrane integrity was observed in leaf discs pre-treated with calcium 2 mM and transferred to calcium 2 mM. Per cent leakage observed in this treatment was 1.6 per cent.

4.1.9 Effect of calcium and aluminium on senescence of soybean leaf discs

In this experiment, effect of combination of

Table 12. Chlorophyll content of soybean leaf discs incubated with calcium and aluminium

Treatments (Concentrations in uM)	Chl.'a'	Chl.'b'	Total chlorophyll
	(mg/g fresh wt.of leaf discs)		
H_2O	1.4128	0.7635	2.1763(55)*
Ca 0.01	1.2424	0.8495	2.0919(56)
Ca 0.1	1.4869	0.8418	2.3287(52)
Ca 1	1.5381	1.4202	2. 958(39)
Al 1	1.7935	1.0199	2.8134(42)
Al 1 + Ca 0.01 mM	1.6670	0.6985	2.3655(51)
Ca 0.1 + Al 1	2.1853	1.4559	3.6412(24)
Ca 1.0 + Al 1	2.1716	1.555	3.7271(23)
CD at 5%	0.0308	0.0165	0.1609

* Per cent reduction in total chlorophyll content in relation to initial chlorophyll content.

Initial total chlorophyll content: 4.8 mg/g fresh weight.

aluminium and calcium on senescence was studied in soybean leaf discs.

Calcium and aluminium retarded senescence significantly. Total chlorophyll values at Ca 1 mM and Al 1 mM were 2.9583 and 2.8133 mg/g fresh weight respectively. Lower concentrations of calcium were found not effective in reducing the chlorphyll degradation. Highest total chlorophyll content 3.7271 mg/g was observed in leaf discs incubated with Ca 1 mM in combination with Al 1 mM solution. This was followed by the treatment calcium 0.1 mM + Al 1 mM where the total chlorophyll content observed was 3.6412 mg/g fresh weight.

4.2 Effect of ethylene synthesis and action inhibitors on floral abscission in field beans

In field beans, a large number of flowers are produced and only a few of them develop into mature pods. When once pod set occurs in the few basal nodes of first formed inflorescence, all the flowers which formed later in the basal nodes as well as flowers and flower buds from the upper nodes abscise. This results in very low pod set percentage in field beans. Ethylene

involvement was shown in the abscission of floral organs (Young pods, flowers and flower buds). Experiments were conducted to study the effect of ethylene action inhibitor STS and ethylene synthesis inhibitor cobalt on abscission of floral organs. With an objective to determine concentrations of chemicals required to inhibit abscission, ethylene synthesis and action inhibitors were first tested on coleus petiolar explants. The effective concentrations which inhibited coleus petiolar abscission were used to see their effedt on floral abscission in field bean inflorescence under laboratory conditions.

Excised inflorescence were treated with different concentration of STS and cobalt chloride and their effect on absèission of floral organs was studied under normal conditions and induced conditions.

4.2.1 Effect of STS on abscission of coleus petiolar explants

Coleus petiolar explants were immersed in water or STS 10, 50 and 100 µM concentrations for 16 hrs. Later, explants were washed with water and planted on agar medium. The number of petioles abscised were

Plate 1: Extent of abscission of petioles from coleus explants at the end of 24 hrs. Explants were pre-treated with STS

Treatments:

1. Control
2. STS 10 µM
3. STS 25 µM
4. STS 50 µM

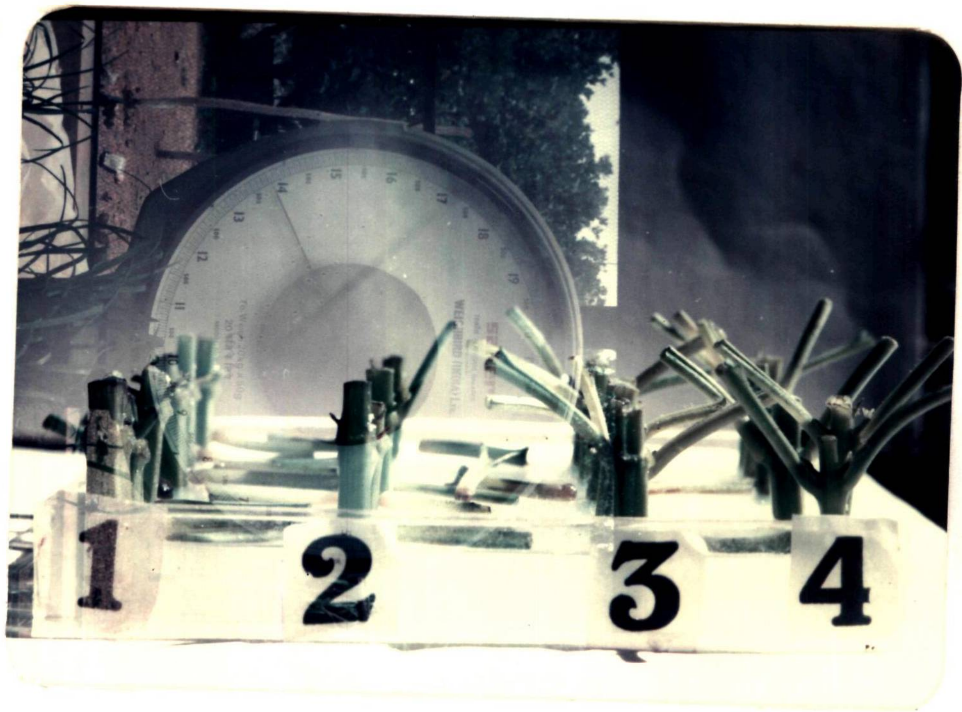

1
2
3
4

Plate 2: Extent of abscission of petioles from coleus explants at the end of 48 hrs. Explants were pre-treated with STS

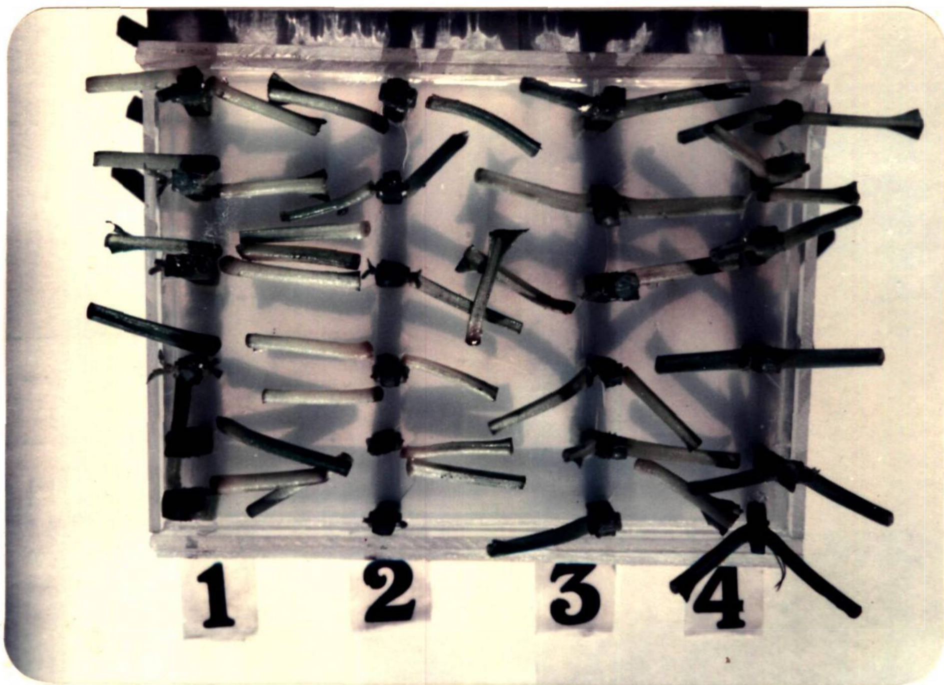
Treatments:

1. Control
2. STS 10 uM
3. STS 25 uM
4. STS 50 uM

Table 13. Cumulative number of petioles abscised at different time intervals following pre-treatment of explants with silver thiosulphate under ethylene induced conditions

	Treatment	Initial No.	Drop of petioles after			
			18 hrs	24 hrs	36 hrs	48 hrs
1.	H_2O	16	16 (100)	16 (100)	16 (100)	16 (100)
2.	STS 10 uM	16	13.34 (83.3)	13.34 (83.34)	13.36 (83.5)	16 (100)
3.	STS 25 uM	16	0	6.64 (41.5)	6.64 (41.5)	14.64 (91.5)
4.	STS 50 uM	16	0	1.36 (8.5)	1.36 (8.5)	13.28 (83.00)
	C.D. at 5%		1.96	2.09	2.09	2.34

Values in the parenthesis indicate the per cent abscission of petioles.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recorded at 18, 24, 36 and 48 hours after planting on agar. Explants treated with water showed 100 per cent abscission within 18 hours after planting on agar medium. STS pre-treatment reduced the number of petioles abscised. Per cent of petioles abscised at the end of 18, 36 and 48 hours were 83.3 per cent, 83.5 per cent and 10 per cent in STS 10 µM concentration. Corresponding values in the treatment where STS 50 µM used were 0, 8.5 and 83 per cent respectively suggesting that STS 50 µM treatment significantly inhibited the abscission of petiolar explants.

Standardization of pretreatment time with STS required to delay abscission of coleus petiolar explants

In another experiment, the minimum pre-treatment time required to get significant difference in abscission was studied.

Coleus petiolar explants were immersed in STS 50 µM solution for 0, 8, 16 and 24 hours and planted over agar medium. Number of petioles abscised at the end of 16, 20, 24 and 48 hrs were 37.5, 50, 100 and 100 respectively in water treated petiolar explants.

Plate 3: Extent of abscission of petioles from coleus explants at the end of 16 hrs. Explants were pretreated with STS 50 µM

Treatments

Explants were treated for -

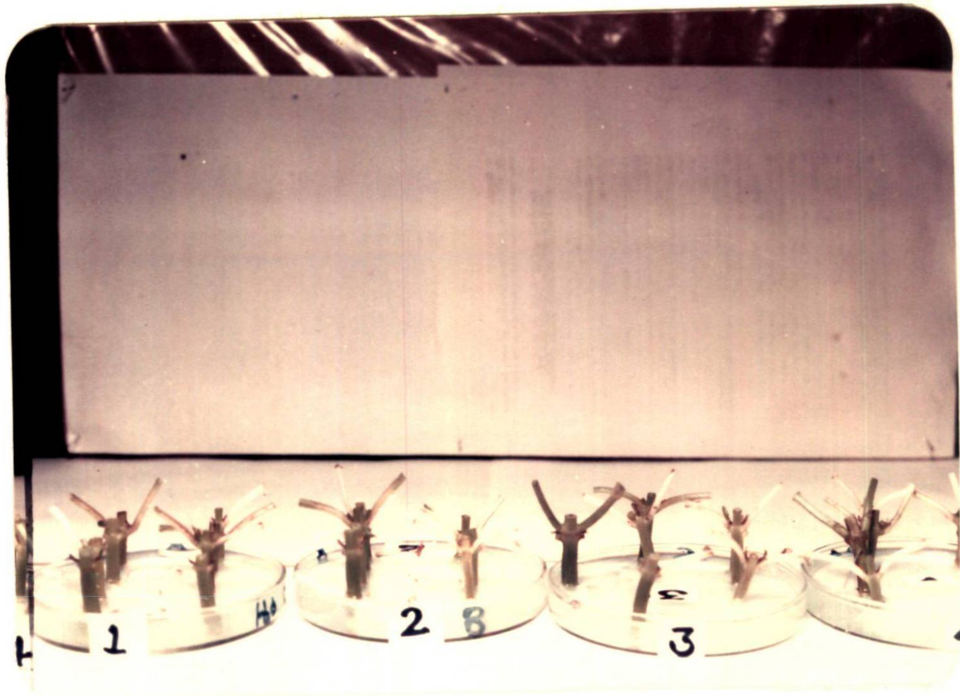
1. 0 hrs
2. 8 hrs
3. 16 hrs
4. 24 hrs

Plate 4: Extent of abscission of petioles from coleus explants at the end of 20 hrs. Explants were pretreated with STS 50 µM.

Treatments

Explants were treated for -

1. 0 hrs
2. 8 hrs
3. 16 hrs
4. 24 hrs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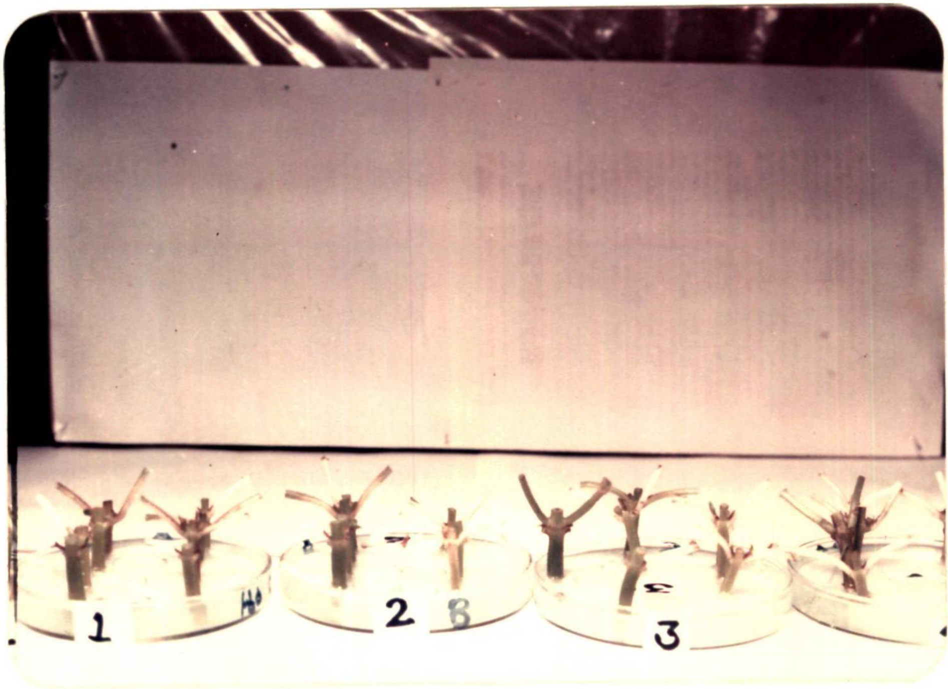
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Table 14. Cumulative number of petioles abscised at different time intervals following pretreatment of coleus explants with STS 50 uM for different periods

Treatment	Initial No.	Drop after			
		16 hrs	20 hrs	24 hrs	48 hrs
0 hrs	16	6 (37.5)	8 (50)	16 (100)	16 (100)
8 hrs	16	1 (6.2)	1 (6.2)	10 (62)	13.02 (81.4)
16 hrs	16	1 (6.2)	1 (6.2)	11 (68)	13.06 (81.6)
24 hrs	16	0	0	0	1 (6.2)
C.D. at 5%		2.810	3.328	4.95	4.48

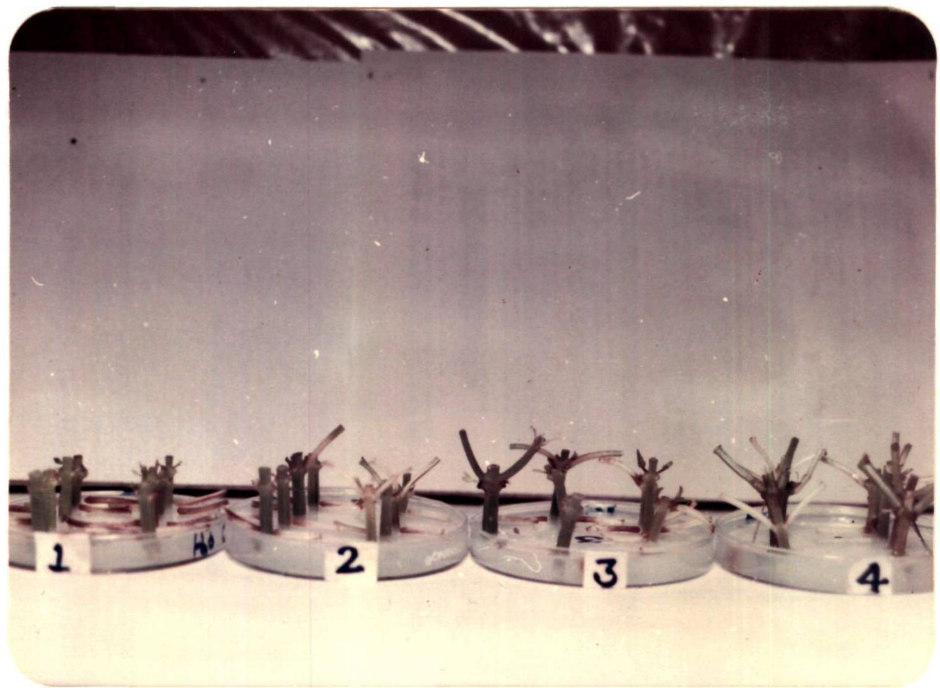
Values in the parenthesis indicate the per cent abscission of petioles.

Plate 5: Extent of abscission of petioles from coleus explants at the end of 48 hrs. Explants were pre-treated with STS 50 uM

Treatments

Explants were treated for -

1. 0 hrs
2. 8 hrs
3. 16 hrs
4. 24 hrs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Treating the petioles for 8 hrs with 50 µM STS significantly reduced the abscission. Petioles abscised were 6.2, 62 and 8.4 per cent at the end of 16, 24 and 48 hrs respectively. Pretreating the petiolar explants continuously for 24 hrs with 50 µM STS completely inhibited abscission.

4.2.3. Effect of STS and cobalt on abscission of petiolar explants

In another experiment, the relative efficiency of STS and cobalt on abscission of petiolar explants were studied. Petiolar explants were treated by immersing in water and 50 µM each of STS and Co for 16 hrs. Petiolar explants were planted on agar and number of petioles abscised were recorded at the end of 16, 36 and 48 hrs after planting on agar. Number of petioles abscised increased with time in water pre-treated explants reaching 100 per cent within 48 hrs after planting over agar. Data are given in Table 14.

Per cent abscission of petiolar explants pretreated with 50 µM Co were 12.5, 23.4, 34.3 and 34.3 per cent respectively at the end of 16, 24, 36 and 48 hrs respectively.

Table 15. Number of petioles abscised at different time intervals following the pre-treatment of peteolar explants with STS and Co (50 uM)

Treatment	Initial No.	Drop after petioles after			
		16 hrs	24 hrs	36 hrs	48 hrs
1. H_2O	16	4.5 (28.13)	6 (37.5)	12 (75.0))	16 (100.0)
2. STS 50 uM	16	0	0	0	0
3. Co 50 uM	16	2 (12.5)	3.75 (23.4)	3.5 (34.3)	5.5 (34.3)
C.D. at 5%		1.251	2.262	2.413	0.461

Values in the parenthesis indicate per cent abscission of petioles.

STS pre-treated explants did not show abscission at the end of 48 hrs suggesting that amongst STS and cobalt, STS is more effective in inhibiting the abscission of coleus petiolar explants.

4.2.4. Effect of ethylene synthesis/action inhibitors and free radical scavengers on abscission of coleus petiolar explants

Coleus petiolar explants were treated with different ethylene synthesis/action inhibitors and free radical scavenger for 16 hrs. Explants were planted on agar media and the number of petioles abscised at the end of 16, 36, 48 and 64 hrs were counted.

Explants treated with water showed 18.75, 41.6, 75 and 100 per cent abscission at the end of 16, 36, 48 and 64 hrs respectively. All the chemicals used markedly reduced the abscission of petiolar explants. Explants treated with $CoCl_2$ showed 6.2, 15.75 50 and 81 per cent abscission at the end of 16, 36, 48 and 64 hrs. Explants treated with Hydroxyquinoline and sodium benzoate showed absicssion only at the end of 48 hrs. However, in explants treated with

STS 50 µM and Ca 1 mM the abscission was observed only at the end of 64 hrs.

At the end of 64 hrs, untreated explants showed 100 per cent abscission whereas per cent abscission at the end of 64 hrs were 37.5, 52, 75, 81.2 and 83.25 in the treatments in which the explants were treated with STS 50 µM, Ca 1 mM, hydroxyquinoline 10^{-4}M (0.1 mM) Co 50 µM Sodium benzoate 500 µM respectively.

In another experiment the effect of pretreating petiolar explants with different ethylene synthesis and action inhibitors on ethylene induced abscission was studied. Explants were treated with different chemicals for 16 hrs and planted on agar and then the explants were exposed to ethylene. Observations were recorded at different time intervals. Data are given in Table 17.

Explants treated only with water showed 50 per cent abscission within 16 hrs. At the end of 36 and 48 hrs the per cent abscission in water treated explants were 87.5 and 100. All the chemicals used markedly reduced the abscission of petiolar explants.

Table 16. Cumulative number of petioles dropped at different time intervala after pretreating the coleus explants with different ethylene synthesis/action inhibitors under normal conditions

	Treatments	Initial No.	Drop of petioles after			
			16 hrs	36 hrs	48 hrs	64 hrs
1.	H_2O	16	3 (18.75	6.68 (41.63)	12 (75.0)	16 (100.0)
2.	STS 50 uM	16	0	0	0	6 (37.5)
3.	Co 50 uM	16	1.00 (6.25)	2.52 (15.75)	8.04 (50.0)	13 (81.25)
4.	Sodium benzoate (500 uM)	16	0	0	5.32 (33.25)	13.32 (83.25)
5.	Ca 1 mM	16	0	0	0	8.32 (52.0)
6.	Hydroxy quinoline (0.1 mM)	16	0	0	2.0 (12.5)	12 (75.0)
	C.D. at 5%	NS	NS	1.68	3.88	5.08

Values in the parenthesis indicate per cent abscission of petioles.

Table 17. Cumulative number of petioles abscissed at different time intervals following the pre-treatment of coleus explants with different ethylene synthesis/action inhibitors under ethylene induced conditions.

Treatments	Initial number	Petioles dropped after		
		16 hrs	36 hrs	48 hrs
1. H_2O	16	8 (50)	14 (87.5)	16 (100)
2. STS 50 uM	16	2 (12.5)	3 (18.75)	5 (31.25)
3. Co 50 uM	16	12 (75.0)	13 (81.25)	15 (93.75)
4. Sodium benzoate (500 uM)	16	4 (25.0)	6 (37.5)	14 (87.5)
5. Ca 1 mM	16	0	1 (6.25)	11 (68.75)
6. Hydroxy quinoline (0.1 mM)	16	9 (56.25)	11 (68.75)	13.04 (81.5)
CD at 5%		5.36	5.44	4.92

Values in the parenthesis indicate per cent abscission of petioles.

Explants treated with different chemicals showed abscission only at the end of 36 hrs. In case of calcium (1 mM) treated explants, the abscission was noticed only at the end of 48 hrs. Amongst the different chemicals used to inhibit abscission in petiolar explants calcium and STS were found to be effective. The per cent abscission at the end of 48 hrs was 31.25, 68.75, 81.5, 87.5, 93.75 and 100 in STS 50 µM, Ca 1 mM. Hydroxy quninoline 10^{-4} M (0.1 mM), Co 50 µM and water respectively.

Effect of STS on normal and ethylene induced abscission of floral organs

Experiment 1

First formed inflorescence from the plants of field bean were excised and ten inflorescences for each treatment were sprayed with different concentrations of STS. Cut ends of the inflorescences were placed in water or ethrel (50 ppm). Number of floral organs abscised were counted at 24, 30 and 48 hrs. (Data are given in Table 18). Abscission per cent increased with increase in duration of treatment. Inflorescences treated with water and kept in water

Table 18. Cumulative number of floral organs abscised at different time intervals following pre-treatment with silver thiosulphate under inductive and normal conditions.

Treatment	Initial number	Abscission of floral organs after		
		24 hrs	30 hrs	48 hrs
H_2O, H_2O	41.0	3.75(9)	4.75(11.6)	10.5(25.6)
STS 50 µM	41.8	2 (4.8)	2.5 (6)	8.5(20.3)
STS 100 µM	39.8	1.5(3.3)	2.25(6)	5.75(14.4)
STS 200 µM	35.8	0.75(2.1)	1.5(4.2)	3.75(10.47)
Ethrel, H_2O	46.8	8.00(17)	14(30)	20.75(44)
STS 50 µM	46.5	3.75(8)	6.5(14)	16.75(36)
STS 100 µM	43.00	5.5(13)	9.5(22)	12.5(29)
STS 200 µM	39.8	3.25(8)	4.5(11)	8.75(21.9)
CD at 5%	10.526	2.714	2.987	4.156

Values in the parenthesis indicate the per cent of floral organ abscission.

(control) showed 9.1, 11.6 and 25.6 per cent of abscission at the end of 24, 30 and 48 hrs respectively. STS treatment reduced the number of floral organs abscised. In inflorescences sprayed with different concentrations of STS and kept in water percentage abscission of floral organs at the end of 48 hrs were 20.3, 14.4 and 10.47 in 50, 100 and 200 uM of STS treatment respectively compared to 25.6 per cent in water treated inflorescences.

Placing the inflorescence in ethrel solution enhanced the number of floral organs abscised compared to the inflorescences placed in water. STS treatment reduced even the ethrel induced abscission of floral organs. At the end of 48 hrs, percentage abscission of floral organs in water treated inflorescence kept in ethrel was 44 per cent. Pre-treating the inflorescence with STS before keeping in ethrel reduced the percentage of abscission to 36, 29 and 21.9 per cent in STS 50, 100 and 200 uM respectively at the end of 48 hrs (Table 18). This suggests that STS pre-treatment inhibits normal abscission as well as ethylene induced abscission of floral organs in excised inflorescence of field beans.

Experiment 2

A similar experiment conducted using STS also showed same trend as Experiment-1. Observations of the number of floral organs abscised at different time intervals showed that STS pre-treatment reduced abscission of floral organs in excised inflorescences. Data are given in Table 19. Percentages of floral organs abscised at the end of 24, 36 and 48 hrs were 11.5, 37.5 and 47.4 in water treated inflorescence. The corresponding values in STS 100 µM were 4.73, 15.44 and 22.94 and in STS 200 µM were 4.02, 13.77 and 22.84 per cent, respectively.

Immersing the cut end of inflorescence in ethrel stimulated the number of floral organs abscised. At the end of 48 hrs, the per cent of floral organs abscised were 51.96 (in control). Inflorescences pre-treated with STS 50, 100 and 200 µM showed the abscission percentages 33.23, 33.17 and 27.15 respectively.

The result of these two experiments confirmed the fact that STS, an ethylene action inhibitor reduces abscission of floral buds and flowers under induced as well as under non-induced conditions.

Table 19. Cumulative number of floral organs abscised at different time intervals following pre-treatement with silver thiosulphate under normal and inductive conditions.

Treatment	Initial number	Abscission of floral organs after		
		24 hours	36 hours	48 hours
Water:				
1. H_2O	48	5.53(11.52)	18.00(37.5)	22.75(47.4)
2. STS 50 µM	47	2.00(4.25)	12.25(26.06)	14.75(31.4)
3. STS 100 µM	53	2.53(4.73)	8.25(15.44)	12.25(22.94)
4. STS 200 µM	47	1.9(4.02)	6.5(13.77)	10.78(22.84)
Ethrel:				
5. H_2O	48	14(29.16)	17.36(16)	24.94(51.6)
6. STS 50 µM	52	7(13.46)	13.75(26.4)	17.28(33.23)
7. STS 100 µM	52	4.25(8.17)	10.25(19.71)	17.25(33.17)
8. STS 200 µM	48	3.25(6.77)	12.25(25.52)	13.03(27.15)
CD at 5%	6.209	7.47	6.95	3.060

Values in the parenthesis indicate per cent of floral abscission.

Experiment 3:

With an objective to see the extent of abscission of different reproductive organs, the number of pods, flowers and buds abscised at different time intervals in inflorescences treated with different concentrations of STS were studied.

Amongst the reproductive organs, flowers were very sensitive and showed highest per cent abscission compared to pods and buds. At the end of 48 hrs percentage of flowers abscised in the inflorescences kept in water were 83 per cent whereas the per cent of pods and buds abscised were only 50 and 30 per cent respectively. Same trend was observed in the case of inflorescences kept in ethrel also.

Per cent of pods, flowers and buds abscised at the end of 48 hrs in the inflorescence kept in water were 50, 83 and 30 in water treated inflorescences. The corresponding per cents in STS 50, 100 and 200 µM pre-treated inflorescence were 25, 97 and 30 per cent: 50, 100 and 19: 100, 68 and 11 per cent respectively.

Table 20. Cumulative number of floral organs abscissed at different time intervals following pretreatment of inflorescence with silver thiosulphate under inductive and normal conditions.

Treatments	Initial number			Abscission of floral organs after 24 hrs			Abscission of floral organs after 36 hrs			Abscission of floral organs after 48 hrs		
	Pods	Flowers	Buds	Pods	Flowers	Buds	Pods	Flowers	Buds	Pods	Flower	Buds
Water:												
1. H_2O	2.0	6	35.5	0	1.2 (20)	1 (2.8)	1 (50)	4 (66)	10 (28)	1 (50)	5 (83)	10.5 (30)
2. STS 50 uM	2.0	6	30	0	0.9 (15)	1.1 (4)	0.5 (25)	4.7 (78)	7.03 (23.43)	0.5 (25)	5.8 (97)	9.18 (30.6)
3. STS 100 uM	2.0	6	30	0	1.7 (28)	1 (3.3)	1 (50)	5.7 (95)	3.25 (11)	1 (50)	6 (100)	5.75 (19.17)
4. STS 200 uM	2.0	6	34	0	2 (32)	0.25 (0.74)	2 (100)	4.25 (68)	3.5 (10)	2 (100)	4.25 (68)	3.75 (11.11)
Ethrel:												
5. H_2O	1.5	6.5	36	0.25	4.26 (65)	6.5 (18)	2 (100%)	6.25 (96)	13.25 (38)	2 (100)	6.25 (96)	20.75 (57)
6. STS 50 uM	2.0	6	30	0	2.5 (41.66)	4 (13.3)	2 (100)	3.73 (62)	9 (30)	2 (100)	4.4 (74)	17.5 (58)
7. STS 100 uM	2.0	6	30	0.4 (20%)	3.1 (51.66)	0.26 (0.86)	2 (100)	4.35 (72)	8.38 (28)	2 (100)	4.8 (80)	13 (43)
8. STS 200 uM	1.5	6	38	0	2.4 (40)	0.25 (0.66)	0.25 (16.66)	4.6 (76.5)	6.4 (17)	0.75 (50)	5.2 (87)	8.75 (23)
CD at 5%	0.682 NS	0.684 NS	11.76 NS	-	2.325	2.79	3.826 NS	2.03	4.68	3.826 NS	3.16 NS	5.015

Values in the parenthesis indicate the per cent of floral organ abscission.

Keeping the inflorescence in ethrel increased percent abscission of floral organs. Number of pods, flowers, buds abscised at the end of 48 hrs were 100, 96 and 57 per cent in the inflorescence pre-treated with water under inductive conditions. STS pre-treatment reduced even the ethylene induced abscission of pods, flowers and buds. The per cent of pods, flowers and buds abscised in inflorescence pre-treated with 200 µM STS at the end of 48 hrs were 50, 87 and 23 per cent.

The results of these experiments suggest that STS pre-treatment reduced the number of floral organs abscised from the inflorescence both under normal and inductive conditions. STS pre-treatment reduced the abscission of pods, flowers as well as flower buds.

Effect of cobalt on ethylene induced abscission of floral organs

Excised inflorescence of field beans were sprayed with 0, 50, 100, 200 and 500 µM $CoCl_2$ solution. Immediately after spraying, the cut ends of inflorescence were immersed in water and the number of floral

organs abscised were counted at the end of 48 hrs. In the case of water pre-treated inflorescence, number of floral organs abscised were 19 at the end of 48 hrs. The number of floral organs abscised in the corresponding period in the inflorescence treated with cobalt 50, 100, 200 and 500 µM were 13, 13.4, 12.8 and 12.01 respectively. Cobalt pre-treatment reduced the per cent of floral organs abscised significantly from 38 per cent in water to 25 per cent in cobalt pre-treated inflorescence. Cobalt pre-treatment at concentration 50 µM was effective in significantly reducing abscission of floral organs. However, all the concentrations of $CoCl_2$ used showed almost same per cent of abscission at the end of 52 hrs.

Though Co reduced abscission amongst the two chemicals used, STS is more effective in preventing abscission both under non-inductive and inductive conditions.

Field experiments

Effect of foliar application of ethylene synthesis inhibitor, cobalt and ethylene action

Table 21. Cumulative number of reproductive organs abscissed at the end of 48 hrs following pretreatment of inflorescence with different concentrations of cobalt chloride

Treatments	Initial number	Abscission of floral organs after 48 hrs
1. H_2O	50	18.94(38)*
2. Co 50 uM	53	13.03(24.26)
3. Co 100 uM	54	13.40(24.8)
4. Co 200 uM	50	12.87(25.74)
5. Co 500 uM	50	12.01(24.02)
C.D. at 5%	6	1.36

Values in the parenthesis indicate per cent of floral abscission

inhibitor, silver on pod setting was studied in determinate field bean under field conditions. Two separate field experiments were conducted between July 1985 to November 1985 and November 1985 to February 1986. Aqueous solutions of different concentrations of silver thiosulphate (STS) and $CoCl_2$ were sprayed to the inflorescence only when flowers started opening in the basal nodes of inflorescence.

4.2.6. Experiment 1

Plants were sprayed with different concentrations of the chemicals when flower buds started opening in the basal nodes of first formed inflorescences. At the time of harvest, pod number, pod dry weight, leaf and stem dry weight of the plant and also the total dry matter/plant were assessed. In the first field experiment, 3 concentrations each of silver thiosulphate and cobalt chloride were used (Table 22).

Total dry matter accumulated per plant showed significant differences among the treatments. Plants sprayed with 200 µM concentration of STS showed the highest dry matter accumulation at the time of harvest.

It was least in the treatment where Co 50 µM was applied to the plants. Leaf dry weight at the time of harvest did not show any significant difference among the treatments. No significant difference was observed in stem weight at the time of harvest. The dry weight of plant was highest in the plants treated with STS 200 µM followed by Co 100 µM. However, the difference between the various concentrations of STS, Co and untreated plants were not significant (Table 22).

The number of pods produced per plant were highest in the treatment in which the plants were treated with Co 100 µM followed by STS 100 uM.

The dry weight of the plant was highest in the plants treated with STS 200 µM followed by Co 100 µM. However, the difference between the various concentrations of STS and Co and untreated plants were not significant.

4.2.7. Experiment 2

In this field experiment 2, concentrations of STS and $CoCl_2$ and their combinations were sprayed to

Table 22. Pod number, dry weights of pods, leaf, stem and dry matter accumulated at the time of harvest following spraying inflorescence with different ethylene synthesis and action inhibitors

	Treatments	Pod number	Pod dry weight (gms)	Leaf dry weight (gms)	Stem dry weight (gms)	Dry matter (gms)
1.	Control	17.25	14.11	4.14	8.63	26.88
2.	STS 50 uM	18.04	14.64	3.22	5.58	23.44
3.	STS 100 uM	19.37	14.66	3.87	6.87	25.40
4.	STS 200 uM	18.70	17.09	4.86	8.85	30.8
5.	Co 50 uM	14.95	14.86	3.3	5.71	23.87
6.	Co 100 uM	20.42	16.13	4.07	6.99	27.19
7.	Co 200 uM	18.33	13.42	4.86	7.95	26.23
	C.D. at 5%	2.83	NS	2.06	2.88	NS

plants immediately after flowers started opening. Observations on total dry matter accumulated in leaves, stem, pods and number of pods produced per plant were calculated (Table 23).

Total dry matter accumulated per plant ranged from 43.87 to 61.7 g/plant. The dry matter per plant was highest in the treatment in which the plants were treated with STS 50 + $CoCl_2$ 100 µM followed by Co 100µM. Dry matter accumulated in the leaf was 5.07 in the water treated plants whereas it was 9.43 g in STS 50 + Co 100 µM treated plants (Table 23).

Stem weight at the time of harvest was highest in the treatments in which STS 25 µM and STS 50 µM were sprayed to plants. Plants treated with STS 50 + Co 100 µM also showed higher dry matter accumulation.

Number of pods produced per plant ranged from 31.4 to 50.15 pods per plant. Both STS and $CoCl_2$ treatments resulted in increased number of pods. An increase in number of pods per plant was observed with increasing concentration of cobalt chloride. Plants treated with STS also showed an increasing trend in

Table 23. Pod number, dry weights of leaf, stem, pods and total dry matter accumulated at the time of harvest following the spraying the inflorescence with different ethylene synthesis and action inhibitors

	Treatments	Pod No.	Pod dry wt./plant (gms)	Leaf dry wt/plant (gms)	Stem dry weight/plant (gms)	Dry matter/ plant (gms)
1.	Control	31.4	26.17	5.07	12.63	43.87
2.	STS 25 uM	36.22	25.26	9.00	20.37	54.63
3.	STS 50 uM	36.93	30.33	8.79	21.47	60.59
4.	Co 50 uM	50.15	25.86	9.04	14.10	49.00
5.	Co 100 uM	48.69	32.37	8.66	14.47	55.50
6.	STS 25 + Co 50 uM	33.80	29.39	7.03	13.90	50.32
7.	STS 25 + Co 100 uM	34.03	31.27	7.61	16.63	55.51
8.	STS 50 + Co 50 uM	38.18	25.87	8.70	15.46	50.03
9.	STS 50 + Co 100 uM	45.28	31.57	9.43	20.70	61.7
	C.D. at 5%	4.66	5.08	2.65	3.53	NS

NS : Non-significant

the number of pods per plant. All the concentrations of STS and $CoCl_2$ from 25 - 100 µM concentrations significantly increased the number of pods. Amongst the different treatments Co 50 µM aqueous spray to inflorescence resulted in highest pod number followed by Co 100 µM and STS 50 + Co 100 µM.

Weight of pods at the time of harvest ranged from 25.26 g to 32.37 g/plant treated with Co 100 µM and STS 50 + Co 100 µM showed significantly higher pod weight.

Field experiments conducted to study the effect of application of different concentrations of STS and CoCl suggeste that application of STS 50 µM + Co 100 µM significantly enhanced the pod number and pod weight/plant.

DISCUSSION

V. DISCUSSION

In many crop plants, a direct relationship between source size and bioproductivity has been shown. A positive relationship between leaf area duration and biological yield and economic yield has been shown in paddy (Devendra *et al*., 1980), groundnut (Choudhuri, 1982) cowpea (Mekhri, 1979) finger millet (Gurumurthy, 1982, Sashidhar *et al*., 1985) setaria (Sashidhar *et al*., 1986) and in field beans (Prasad, 1983).

Importance of higher source size and its capacity for establishment of more number of viable reproductive sinks and post fertilization development of reproductive sinks has been emphasized. Higher source size during pre-anthesis and post-anthesis development period in many crops result in higher grain yield (Johnston *et al*., 1969; Peet *et al*., 1977). Premature senescence of leaves is the major factor limiting seed yield in many crops - particularly in pulses. Increasing the source size by agronomical or chemical manipulations is a means of increasing crop productivity.

Premature abscission of buds, flowers and young pods is another major constraint for productivity in many horticultural crops and pulses. In many pulse crops, abscission of buds, flowers and pods constitute more than 80 to 90 per cent of total reproductive organs initiated. Kombal (1969) while studying pod development in field beans estimated that only 84 to 90 per cent of flower buds opened into flowers and 39 to 63 per cent of flowers produced young pods. Thirteen per cent to fifteen per cent pods developed into matured fruits.

Eblong (1968) reported abnormal number of flower drop in cowpea and soybean due to unknown physiological reasons, Kaul (1976) reported that flower drop in cowpea, greengram and redgram was about 60, 50 and 45 per cent, respectively. High percentage of flower drop was also reported in field bean, greengram, blackgram, chickpea and soybean etc. (Chinnaswamy, 1979; Powar and Bhatia, 1980; Savitri et al.,1978; Huff and Dybing, 1980; Sheldrake and Saxena, 1979).

Endogenous hormones regulate senescence and

abscission process. Auxins are found to have major role in controlling senescence and abscission. Senescence retarding property of auxins was showed recently by Nooden _et al_. (1979). GA was shown to retard chlorophyll degradation during senescence (Hsia _et al_., 1978).

Cytokinins retard senescence and thus have an inhibitory effect on abscission. The leaf cytokinin content decreases drastically during senescence (Engelbrecht, 1971) Exogenous application of kinetin was shown to retard senescence and stimulate metabolism of sugars in maize leaf discs (Tanaki, 1982); Lesham _et al_. (1984) observed that cytokinin resulted in reduced lipoxygenase and superoxide dismutase activity in senescing foliage.

Abscissic acid accelerates senescence, ABA levels increase sharply during senescence (Dumbroff _et al_., 1977; Gepstein and Thimann, 1980, 1981). In Lupinus albus ABA promotes senescence by speeding up the translocation of nutrients from cotyledons to axis (Elkinsway, 1983).

Ethylene promotes senescence at all stages of plant growth. Ethylene production during senescence of leaves was reported in many crops like tobacco, pinto bean (Aharoni and Lieberman, 1979) melia leaves (Morgan and Durham, 1980) oat leaves (Gepstein and Thimann, 1981) and in rice leaves (Kao and Yang, 1983). Legge and Thompson (1983) studied ethylene biosynthesis and membrane changes that occur during senescence.

Ethylene plays a predominant role in abscission of floral organs also. During senescence and abscission, higher concentrations of ethylene was shown to be released in many organs. A close relationship between amount of ethylene released and senescence and abscission of plant organs has been reported (Mayak and Kofranek, 1976; Suttle and Kende, 1978; Nichols, 1977; Whitehead *et al*., 1984).

It has been recognised that ethylene is the dominant hormone having a direct role in initiating senescence and thus leads to abscission of flower buds. Exogenous application of ethylene releasing substances to plants leads to senescence which is associated with increased chlorophyll degradation, loss in membrane

function, degradation of stored carbohydrates, proteins and nucleic acids.

Regulation of ethylene biosynthesis in plants has a great advantage in agricultural production. Manipulation of ethylene synthesis in plants is possible now through application of knowledge obtained from studies conducted to understand the biosynthetic pathway of ethylene.

The key synthetic enzymes in the pathway of ethylene biosynthesis and regulation of their activity by applying number of chemicals has been shown recently. 1-Amino-cyclopropane-1-carboxylic acid synthase seems to be the key intermediate enzyme in the ethylene biosynthesis.

A number of environmental factors influence ACC synthase activity. Chemical injury, wounding, flooding, drought and chilling increases ethylene production by increasing the synthesis of ACC. The current knowledge in the field of ethylene biosynthesis and identification of number of substances which inhibits ethylene biosynthesis provides scope for manipulation

of some physiological processes in plants to achieve desired effects.

In this study, the effect of number of ethylene synthesis and action inhibitors on senescence and abscission processes were studied. The influence of ethylene synthesis and action inhibitors on senescence of excised soybean leaf discs were studied. Chlorophyll degradation during senescence was used as a parameter to assess the effectiveness of these compounds.

Incubation of leaf discs in a medium containing STS inhibited chlorophyll degradation during senescence. STS at concentrations of 50, 100 and 200 µM significantly reduced chlorophyll degradation. Per cent reduction in total chlorophyll contents were 36 and 22 per cent in STS 50 and 100 uM treatments compared to 44 per cent in water treatment.

Cobalt an inhibitor of ethylene biosynthesis was also effective in reducing degradation of chlorophyll during senescence. Cobalt at a concentration of 50 µM was effective in inhibiting chlorophyll degradation. Higher concentrations of cobalt in the medium

resulted in an increased amount of chlorophyll in the leaf tissue at the end of incubation period.

Aluminium was reported to change the growth regulator balance, in plants. Aluminium was shown to inhibit starch hydrolysis (Schnabl and Ziegler, 1975).

Aluminium also effectively inhibited chlorophyll degradation during senescence. When leaf discs were placed in a medium of water or different concentrations of aluminium ranging from 100 uM to 1 mM, significant reduction in chlorophyll degradation was observed. Increasing concentrations of aluminium in the medium upto 1 mM, resulted in corresponding inhibition of chlorophyll degradation. However, aluminium when used at a concentration of 3 mM was not effective in overcoming chlorophyll degradation during senescence.

These results suggested that Ag, Co and Al were effective in inhibiting senescence of leaf discs individually. The concentrations of Al required to inhibit senescence was relatively more compared to Ag and Co.

Ag^+ ion has long been known as a potent inhibitor of ethylene action (Veen and Kwakkenbos, 1983; Gepstein and Thimann, 1981). In soybean leaf discs, Ag^+ ions showed a marked effect on delaying senescence. It has also been proposed that in the presence of Ag^+ ions, ethylene action in the tissue was inhibited since ethylene forms a complex with Ag^+ in plant tissue (Beyer, 1976). It has been proposed that silver ions inhibit ethylene action by binding to receptor sites of ethylene. Aharoni et al. (1979) suggested that Ag^+ CO_2 have same effect of binding to the receptor sites of ethylene and thus inhibit the action of ethylene in plant tissues.

Cobalt a known inhibitor of ethylene biosynthesis, was shown to inhibit the conversion of ACC to ethylene (Yu and Yang, 1979). Cobalt also exerts its inhibitory effect by complexing withsulfhydryl group of proteins. Studies conducted with tomato plants suggested that cobalt effectively inhibited ethylene production (Bradford, 1982). Heavy metals like cobalt, and Manganese have been found to confer some amount of resistance to membranes against loss of permeability on imposition of moisture stress (Young and Kauss, 1983).

Cobalt and Manganese were shown to reduce loss of solutes (Garrod and Humphreys, 1967). Apart from binding to the anionic sites and thus maintaining membrane integrity, cobalt also had an effect on inhibiting the formation of lipid peroxidase (Dupont and Lance, 1980; Tam and Mccay, 1970).

Experiments conducted to study the effect of STS, cobalt and aluminium individually and in combination on senescence showed that, Co, STS and Al. reduced the rate of senescence as measured by chlorophyll degradation. Amongst cobalt, STS and aluminium, aluminium was the most effective in inhibiting chlorophyll degradation. Presence of 1 mM concentration aluminium in the medium resulted in marked reduction in senescence. Al 1 mM with cobalt 200 uM treatment was most effective in delaying chlorophyll degradation during senescence.

Effect of cobalt, STS and free radical scavenger benzoic acid on ABA induced degradation of chlorophyll in soybean leaf discs showed that presence of ABA in the incubation medium increased the rate of degradation of chlorophyll. Amount of chlorophyll reduced in a period of 72 hrs was 47 per cent in control. Presence

of ABA 10^{-5}M and ABA 10^{-4}M increased chlorophyll degradation to 71 and 74 per cent respectively. Ethylene action inhibitor, STS, ethylene synthesis inhibitor cobalt, free radical scavenger benzoic acid were effective in reducing chlorophyll degradation both under induced and non-induced conditions. STS inhibited ABA induced senescence to a greater extent. Benzoic acid and cobalt were also effective in overcoming ABA induced senescence.

In a series of experiments, effect of calcium on senescence of excised leaf discs of soybean was studied. Presence of calcium in the range of 100 µM to 3 mM reduced chlorophyll degradation. With increase of calcium in the medium, the amount of chlorophyll retained increased. However, presence of 3 mM calcium resulted in only 24 per cent reduction in chlorophyll content suggesting that calcium is effectively inhibiting senescence. Role of calcium in senescence was studied by chelating calcium and also by increasing calcium level by giving calcium in the incubation medium. EGTA is a specific chelator of calcium. Presence of EGTA in the incubating medium increased senescence process as measured by chlorophyll degradation.

EGTA was effective in inducing senescence even in the presence of higher concentration of calcium in the medium. Chlorophyll content reduced by 46 per cent when water was in the medium. Presence on 1,2 and 5 mM calcium in the medium reduced degradation of chlorophyll and per cent reduction in chlorophyll during the incubation medium was only 31, 25 and 38 per cent respectively. Presence of EGTA increased degradation to 54 per cent in the presence of 1, 2 and 5 mM calcium respectively. This suggests that calcium is involved in phenomena. When endogenous calcium or added calcium was chelated and made unavailable for physiological processes, there was acceleration of senescence.

Results on per cent leakage of solutes also suggested that calcium inhibits loss of membrane integrity during senescence. Per cent leakage of solutes were only 10, 3 and 3 per cent when calcium 1, 2 and 5 mM was in the medium as against 20.6 per cent in the absence of calcium. Chelating calcium by EGTA increased per cent leakage of solutes. However, at high concentrations of calcium in the presence of EGTA per cent leakage was less.

In another experiment, effect of pre-treatment of calcium and the presence of calcium continuously in the medium on the rate of senescence was studied. In this experiment also leaf discs pre-treated with calcium and transferred to water and leaf discs which were continuously in calcium retained higher chlorophyll content.

Degradation of chlorophyll during senescence was more in the leaf discs which were transferred from water to EGTA. EGTA was effective in inducing senescence even in leaves pre-treated with calcium. When aluminium was present together with calcium in the incubation medium, very low concentration of calcium of 0.1 mM was effective in inhibition of senescence in leaf discs. The degradation of chlorophyll during senescence as well as loss in membrane integrity during senescence was less in the presence of calcium and calcium + aluminium.

Presence of calcium in the medium alone or along with aluminium inhibits senescence of excised leaf discs. Very low concentrations of aluminium was effective in inhibiting senescence when treated along with aluminium.

It has long been known that calcium inhibit senescence of leaves. Calcium was shown to bind to negative charges in membrane structure and forms cross links between membrane structural components. It has been shown that calcium deficiency induces loss in membrane integrity early. Tissues deficient in calcium were shown to loose membrane integrity at very low intensities of moisture stress resulting in membrane damage (Christiansen and Foy, 1979; Legge, 1983). Calcium in the tissue is shown to reduce membrane damage and leakage of tissue (Poovaiah and Leopold, 1973).

In excised leaves, ethylene synthesis inhibitor cobalt and aluminium, ethylene action inhibitor silver ion and free radical scavenger, benzoic acid and calcium which gives ridigity to membrane, inhibits rate of senescence as observed by chlorophyll degradation as well as loss in membrane integrity. Apart from calcium role in membrane integrity, there must be few other possible mechanisms by which calcium is retarding senescence.

In many plant tissues, senescence preceds abscission process. The effect of chemicals which were found effective in inhibiting senescence, were further tested to study their effect on abscission process. In a series of experiments, the inhibitory effect of ethylene synthesis and action inhibitors were tested on coleus petiolar explants to identify the effective concentrations of chemicals in inhibiting abscission.

Silver thiosulphate pretreatment was effective in delaying abscission of coleus petiolar explants. Pre-treating the explants with 10, 25 and 50 uM STS delayed abscission of petioles significantly. Abscission of petioles occurred in water pretreated explants within 17 hrs after planting explants in agar medium. There was no abscission in explants pretreated with STS 25 and 50 mM. At the end of 24 hrs per cent abscission in STS 10, 25 and 50 uM pretreatments were 83.3 per cent, 41.5 and 8.5 whereas it was 100 per cent in water.

The effect of STS, cobalt, sodium benzoate and hydroxy quinoline on abscission of coleus petiolar explants suggested that STS 50 uM and calcium 1 mM were

more effective compared to cobalt, sodium benzoate and hydroxy quinoline. Silver thiosulphate and calcium pretreatment was effective in even delaying ethylene induced abscission. Hydroxy quinoline has cytokinin like activity in retarding senescence (Chua, 1970) was also found effective in inhibiting abscission of coleus petiolar explants. Hydroxy quinoline was shown to inhibit ethylene evolution in rose stamens, apple fruit slices and also in carnation flowers (Parups and Peterson, 1973; Wilkins and Swanson, 1975).

With an objective to see the effect of STS on floral abscission in field bean a number of experiments were conducted in which excised inflorescences were sprayed with different concentrations of STS and the cut ends of inflorescence were kept immersed in water. Number of flowers abscised was counted at regular intervals. In water pretreated inflorescences, per cent of flowers abscised from inflorescence were 11.5, 37.5 and 47.4 at the end of 24,36 and 48 hrs. pretreatment of inflorescence with 50 µM STS markedly reduced the number of floral organs abscised. Per cent floral organs abscised are only 4.02, 13.77 and

22.84 at the end of 24, 36 and 48 hours. When cut end of inforescence were immersed in ethrel solution, there was accelerated abscission of floral organs. STS pre-treatment inhibited even ethylene induced abscission. STS 100 uM and 200 uM pretreatment were effective reducing the abscission of floral organs under normal conditions as well as under ethylene induced conditions.

Amongst the reproductive organs, flowers were very sensitive and showed highest abscission compared to pods and buds.

Experiments conducted with cobalt chloride solutions using coleus petiolar explants showed that higher concentrations of cobalt were effective in delaying abscission. With an objective to identify effective concentration of cobalt to delay floral abscission, excised inflorescences were sprayed with 50, 100, 200 and 500 uM concentrations of cobalt and its effect on abscission of floral organs were studied at the end of 48 hrs. Cobalt was also effective in reducing the number of floral organs abscised, however its effect in reducing the abscission of floral organs was very much less compared to STS.

Abscission of leaves and floral organs is under the control of endogenous levels of hormones in the abscising tissue. Higher levels of auxin reduce abscission. Cytokinins and gibberellins also delay abscission to some extent. Many workers suggest that auxins, cytokinins and ABA regulate abscission process by changing the auxin, gibberellin content in plants. In addition to endogenous auxin level the length and time of duration with which polar transport of auxin is maintained in plants is also important for retarding abscission (Osborne et al., 1968; Beyer and Morgan, 1971).

Ethylene was shown to induce abscission by reducing auxin concentration in the tissue. Ethylene decreases auxin concentration by inhibiting the conversion of auxin precursor treptophan to IAA. Ethylene also has been shownto increase IAA oxidase activity in tissue, which oxidises endogenous IAA. Ethylene is known to inhibit auxin transport in the tissue (Abeles et al., 1971; Beyer and Morgan, 1971).

Since, STS and cobalt are effective in inhibiting ethylene action and synthesis in tissue probably by maintenance of higher concentration of auxin in

tissue ethylene synthesis and action inhibitors are effective in delaying abscission of petiolar explants as well as floral abscission in field beans. Calcium is known to play an important role in abscission of plant parts. Calcium is present in the middle lamella as calcium pectate which is a cementing substance between cells (Leopold, 1964) during abscission and thus affinity for calcium in abscission zone increases; calcium pectate gets converted into pectic acid which is water soluble resulting in separation of cells in abscission zone (Poovaiah and Rasmussen, 1973).

In field experiments, the effect of treating the inflorescence with different concentrations of STS and cobalt during early stages of reproductive growth on pod set and yield was studied. In the first field experiment, inflorescences were sprayed with water, 25, 50, 100 µM of STS or cobalt chloride solution individually on 45th day after sowing. The effect of these treatments on total pods produced at the time of harvest, and on biological yield were recorded.

In this experiment, only cobalt 100 µM treatment was effective in increasing total number of pods

produced/plant. Pod dry weight was more in STS 200 µM and Co 100 µM treatments. However, the difference between the treatments were not significant.

In another field experiment, infloresoence were treated with different concentrations of cobalt and STS and their combinations. Inflorescences were sprayed with treatment solutions on 45th and 50th day after sowing. Co 100, Co 50 and STS 50 + Co 100 uM showed significant increase in the number of pods produced per plant. Significant increase in pod dry weight per plant was also noticed in plants treated with Co 100, STS 25 + Co 100 and STS 50 + Co 100. In some treatments increase in stem dry weight, leaf dry weight and total biological yield w noticed. This increase was mainly due to lesser rate of senescence of leaves.

These results on the effect of ethylene synthesis and action inhibitors suggests that there is scope for inhibiting ethylene synthesis and action. Application of ethylene synthesis and action inhibitors in combination, two times during early stages of reproductive stage showed some interesting trend in increasing productivity.

Studies conducted to see the effect of ethylene synthesis, action inhibitors, free radical scavangers on leaf senescence and abscission of foliar and floral organs suggested that (1) silver thiosulphate 200 µM in the incubating medium inhibits chlorophyll degradation of excised soybean leaf discs during senescence. (2) Ethylene synthesis inhibitor $CoCl_2$ and free radical scavenger benzoic acid were also effective in delaying chlorophyll degradation during senescence of excised soybean. (3) Calcium and aluminium when present in the incubation medium were also effective in delaying chlorophyll degradation during senescence of excised soybean.

A combination treatment of 1 mM Ca, 1 mM aluminium resulted in higher amount of chlorophyll retention in leaves. 2 mM to 3 mM was found effective in retarding senescence. (4) Ethylene action inhibitors STS and synthesis inhibition cobalt were effective in delaying abscission of petiolar explants. (5) STS in the range of 100 uM and 200 uM spray delayed floral abscission in field beans. Cobalt and STS application to the inflorescence at early stages increased pod number in field experiments.

SUMMARY

VI. SUMMARY

Experiments were conducted to study the effect of ethylene synthesis/action inhibitors and free radical scavengers on chlorophyll degradation during senescence of excised soybean leaves and in delaying abscission of coleus petiolar explants and premature floral abscission of field bean inflorescences under *in vitro* and *in vivo* conditions.

Silver thiosulphate (ethylene action inhibitor) at concentration range of 50 µM to 200 µM significantly retarded chlorophyll degradation. Highest chlorophyll contents were observed at 200 µM concentrations of STS (silver thiosulphate).

Experiments conducted with aluminium showed that aluminium retard senescence in the concentration range of 100 µM to 1 mM. Al 1 mM treatment was highly effective in retarding chlorophyll degradation.

Senescence retarding effect of STS, cobalt and aluminium was further studied by incubating the leaf discs in the medium containing combination of these chemicals at different concentrations. Al 1000 µM +

Co 200 µM, Al 1000 µM + Co 100 µM, Al 500 µM + Co 100 µM treatments were found effective in delaying senescence.

Studies conducted to see the effect of STS, cobalt and free radical scavenger, benzoic acid on ABA induced senescence showed that STS at 100 µM and 200 µM were effective in retarding chlorophyll degradation induced by ABA. Cobalt and benzoic acid were also effective in inhibiting ABA induced senescence. This suggests that ABA induces senescence by increased biosynthesis of ethylene.

In a series of experiments effect of calcium on senescence of excised leaf discs of soybean were studied. Calcium ions were found to inhibit chlorophyll degradation in the concentration range of 100 µM to 3 mM. Calcium at 2 mM and 3 mM were observed to be very effective concentrations. Presence of EGTA in the incubation medium induced senescence. EGTA is a specific chelator of calcium ion and makes calcium less available. However, higher concentrations of calcium with EGTA inhibited senescence and membrane damage significantly.

Continuous presence of calcium in the medium significantly inhibited senescence. These observations suggested that calcium is involved in delaying senescence of excised leaves.

Studies conducted with calcium and aluminium have shown that presence of calcium alone or along with Al inhibits senescence in excised leaf discs. Aluminium 1 mM + Calcium 1 mM treatment was found effective.

Experiments were also conducted to see the effect of ethylene synthesis and action inhibitors on abscission process. Silver thiosulphate pre-treatment was effective in delaying the abscission of coleus petiolar explants. Pre-treating the explants for a minimum duration of 8 hrs significantly retarded abscission of coleus petiolar explants. Among the different ethylene synthesis/action inhibitors tested, STS 50 µM and calcium 1 mM were found effective in reducing the petiolar abscission suggesting that calcium plays an important role in abscission of plant parts. Experiments conducted on floral abscission of excised field bean inflorescence suggested that pre-treating the inflorescence with STS 200 µM significantly retarded

the abscission of buds, flowers and young pods. Cobalt was also found effective in reducing the abscission however its inhibitory effect was much less than that of STS.

Field experiments conducted to study the effect of application of silver thiosulphate and cobalt chloride to infloresecence on pod set and yield of field beans showed that application of STS 50 uM, Co 50 uM, STS 50 uM + Co 100 uM treatments were effective in increasing pod yield per plant by increasing the number of pods developed per plant.

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