ROLE OF AUXIN IN CORRELATIVE CONTROL OF ABSCISSION AND APICAL DOMINANCE

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Certificate

This is to certify that the thesis entitled "**ROLE OF AUXIN IN CORRELATIVE CONTROL OF ABSCISSION AND APICAL DOMINANCE**" submitted in partial fulfilment of the requirement 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 Miss. **DHANALAKSHMI. R** 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 any other similar titles.

Bangalore
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LIST OF ABBREVIATIONS USED

ABA	Abscissic acid
ATI	Auxin transport inhibitor
BA	Benzyl adenine
cm	Centimetre
g	gram
h	hours
HPLC	High performance liquid chromatography
IAA	Indole - 3 - acetic acid
l	litre
LSC	Liquid Scintillation Counter
M	Molar
mg	milligram
mm	millimetre
min	minute
ng	nanogram
ppm	Part per million
TIBA	2,3,5 - Tri iodo benzoic acid
ul	Micro litre
10E4M	Read as 10^{-4} M

INTRODUCTION

I. INTRODUCTION

The life cycle of a plant begins with the onset of germination of a seed, after which it undergoes a series of developmental changes. The trigger for this comes from intercellular communication between cells constituting the organism. The co-ordination of metabolism, growth and morphogenesis is dependent on signals that move from one plant part to another.

Intercellular communication in plants is mediated by chemical messengers - Hormones. The idea of hormones being the chemical messenger was first proposed by Went and Thimann (1937).

Auxin was the first hormone to be identified in plants (Went, 1928). Thimann and other workers identified that the active auxin in plants is indole-3 acetic acid (IAA).

Auxin plays a major role in regulation of various plant processes. Auxin regulates a number of growth and developmental processes in plants like, initiation of rooting from stem cuttings, maintenance of apical dominance, stimulation of cell elongation, leaf expansion, fruit growth and retardation of abscission of plant parts. The role of auxin in apical dominance (Cline, 1991; Phillips, 1975) and abscission has been studied extensively.

Initial reports suggested that the auxin gradient between the proximal and distal end is the cause for abscission. Auxin gradient theory of abscission proposed by Addicott and Lyon as early as 1950's suggested that the abscission of an organ is deferred until the auxin concentration in the distal end is more than that of the proximal end to the abscission zone and as long as there is continuous movement of auxin from distal to proximal end.

Abscission of flowers is a very common phenomenon, especially in a racemose inflorescence. Experiments conducted by various workers suggest that the abscission is influenced by both temporal and spatial factors. For eg., abscission of flowers in a racemose inflorescence (Tamas et al., 1979; Huff and Dybing, 1980).

Competition for assimilate could have been part of the explanation for this phenomenon. However, in many flowering plants maximum abscission of flowers was observed at very early stages of reproductive growth when competition for assimilates is relatively less. This indicates that competition for assimilate may not be the only cause for premature abscission of organs as well as for apical dominance phenomena.

Dominance phenomenon is wide spread in plant kingdom and this influences apical dominance as well as premature abscission of reproductive organs (Michael and Beringer, 1980). Dominance is seen not only in vegetative organs but also among fruits and between fruits and vegetative sinks. A new theory - Primigenic Dominance was proposed to explain apical dominance and premature abscission of young reproductive structures (Bangerth, 1989).

The theory of Primigenic Dominance emphasizes the sequence of sink development and the basipetal transport of auxin in regulation of dominance leading to maintenance of dominance by apical bud as well as abscission of young developing reproductive organs. The theory proposes that the basipetal transport of auxin from the first formed or dominant sink inhibits the basipetal transport of auxin from later developed or subordinate sinks thus leading to the abscission or restricted growth of subordinate sinks.

The role of auxin in this regard seems to be relevant. As yet there is no conclusive evidence.

Hence, to study the importance of auxin in these phenomena the present investigation was conducted with the following objectives :

1. To study the role of auxin in apical dominance and premature abscission of organs.
2. To study the influence of translocation of auxin from dominant sinks on the abscission/growth of subordinate sinks.

REVIEW OF LITERATURE

II. REVIEW OF LITERATURE

The phenomenon of abscission and apical dominance and the role of auxin in these has been well documented. Other plant hormones like ethylene, cytokinins and abscissic acid also have some role in these phenomena .

The present study is an attempt to investigate the importance of auxin transport in abscission of plant parts and also its involvement in apical dominance. This section emphasizes the earlier work done in this area of research.

Role of auxin in premature abscission of organs

Abscission of leaves and immature reproductive units (flowers, developing pods) involves highly coordinated biochemical changes which lead to cell wall breakdown and cell separation at the abscission layer. Extensive work has been carried out to characterise the role of plant hormones on the abscission of plant parts (Osborne, 1968, Addicott, 1970, Jackson and Osborne, 1972). The general view is that auxin delays abscission and ethylene accelerates it. Many scientists feel that ethylene induced abscission is mediated by reducing endogenous auxin concentrations (Blake, *et al.*,1983).

It has been suggested that the auxin levels in the abscission zone are critical in delaying abscission (Beyer and Morgan,1971; Jackson and Osborne, 1972; Morgan and Durham, 1972). Auxin actually delays the onset of abscission of plant parts (Osborne, 1968)

In earlier works conducted to understand the role of auxin in delaying abscission, debladed petiole explants of cotton, bean or coleus are generally used. In general when the leaf blade was removed from the petiole explant, the petiole abscises. This abscission could be delayed by application of IAA to the cut end of the petiole. It is suggested that the IAA is supplied continuously from the leaf lamina and this prevents abscission (Shoji et al ., 1951; Hall, 1951).

Auxin induced acceleration of abscission

A number of research findings show that auxin at relatively high concentration induces abscission of flowers and young fruits. Leopold and Rubinstein as early as 1963 showed that auxin application either promoted or inhibited leaf abscission depending on the concentration and time between deblading and application of auxin to petiole explants. They have concluded that initially abscission of debladed petioles is inhibited by the auxin concentration applied and it is further promoted by the same concentration over a period of time.

Carns (1966), has reported that IAA applied to petiole stumps of explants retarded abscission and increasing concentration of IAA resulted in accelerated abscission.

In cotton it has been shown (Morris, 1993) that debladed cotyledonary petioles abscised in intact plants. When an exogenous application of IAA was given to the cut apex there was acceleration in the abscission of the cotyledonary petioles. Even exogenous application of auxin to intact system was shown to induce abscission particularly when the auxin concentration applied was high.

In a recent study in rose (Goszczynska, 1993) it has been reported that IAA application to the site of bud removal postponed abscission of decapitated peduncles than when compared to untreated decapitated peduncles.

In experiments conducted with *Melia azederach*, IAA when applied to the fruits at varying concentrations fruit drop increased by 91.3% (Sharma and Paliwal, 1989).

These results indicate that auxin at relatively high concentration accelerates abscission. At high concentration auxin was shown to induce ethylene synthesis and this was said to be the trigger for acceleration of abscission process.

Auxin transport and its relation to abscission of organs

Earlier work conducted between 1957 to 1965 emphasized the importance of auxin transport from distal end to proximal end of the abscission zone for growth, development and maintenance of organs on the plant (Addicott and Lyon, 1953).

The auxin gradient theory also emphasized the need for maintenance of continuous flow of high concentration of auxin from distal to the proximal end. When once the concentration is reversed, for eg., if the auxin concentration in the distal end is less than that of the proximal end of the abscission zone it induces processes which accelerate abscission. During abscission, there is a decrease in the level of auxin in the abscission zone (Beyer and Morgan, 1971)

It has been shown that stress reduced basipetal auxin transport from cotton leaves inducing their abscission. Leaf abscission did not occur in well watered seedlings. With increased level of stress abscission increased although the percent auxin transport remained constant. It was said that stress inhibits auxin synthesis and decreased levels of auxin reaching the abscission zone from leaf blade influences abscission process (Davenport et al., 1977).

Morgan et al., (1977) showed that both ethephon and auxin transport inhibitors (ATI) induced rapid abscission. Further, water stress or ATI was shown to replace stress effect but not the ethephon, indicating that water stress or ATI influences initial events in abscission. From these studies Morgan et al., concluded that initial events which triggers abscission is influenced by inhibition of auxin transport.

The mechanism by which auxin delays abscission is not known. In earlier work auxin was shown to increase the activity of cellulase in the abscission zone (Osborne, 1968). Jackson and Osborne (1972), Osborne (1968), showed that auxins extend the period of insensitivity to ethylene, so that the tissue does not recognize the ethylene concentration or not activated by ethylene. Auxin is also known to delay senescence .

Bangerth (1989), suggested that auxin moving from sinks influences growth and development of sinks from which it moves as well as to other competitive sinks. Tamas et al., (1985) reported that the dominance effect of proximal (dominant) flowers in soybean is inhibiting growth and abscission of distal flowers because of high amount of IAA moving from dominant sink.

Tamas et al.,(1989) made the assumption that the IAA translocated from the dominant to inhibited organs was possibly the decisive signal. They showed the involvement of IAA by using ^{14}C-IAA in which application of ^{14}C-IAA to dominant organs inhibited the growth and development of subordinate organs. However, the direct effect of IAA inhibiting the growth and development of subordinate organ is questioned by Huff and Dybing (1980) and Bangerth (1984).

The two important points argued against the role of IAA transport is that:

(1) Only a very small quantity of the applied ^{14}C activity was found in the inhibited organ

(2) There was no evidence that the translocated radioactivity was still in the unmetabolised IAA molecule. Since IAA moves only in basipetal direction

its involvement in inhibiting late formed flowers from upper node of a racemose inflorescence remains questioned.

Gruber and Bangerth (1990), have concentrated on the diffusable IAA and have reported that dominant fruits have more diffusable IAA than subordinate fruits. This diffusable IAA is involved in the correlative signal regulating the dominance relationship.

It has been shown that the sequence of induction of fruits determined the import rate of assimilates. Fruits induced first were heavier and they imported more ^{14}C assimilates than fruits that were induced later. They further said that this could be due to the IAA produced by the first induced fruit which prevented later induced fruits from obtaining metabolites. The position of fruits in a truss is said to modify the primigenic dominance effect (Bangerth and Ho, 1984).

Bangerth (1989) gave a number of experimental proofs to prove the involvement of IAA in transport of dominant signal. The results that support his hypothesis include;

(1) The rate of diffusable IAA from dominant tomato, bean and cucumber fruits were higher than those from inhibited fruits of the same plant (Bangerth, 1984, 1989).

(2) Manipulating the dominance relationship changed the rate of IAA diffusion (Bangerth, 1989). When shoot tips of an apple, grape or bean plant is removed, nearby inflorescence shows fruit set. Since the IAA moving from the shoot tip is removed the dominance effect is removed resulting in high fruit set in the nearby inflorescence.

(3) The possible role of IAA export rate in dominance also can be seen in a few cases. For example, in some species parthenocarpic fruit set can be obtained only when competition with vegetative sinks is eliminated (Chapman et al., 1979; Carbonell and Garcia-Martinez, 1980).

Exogenous application of IAA induces parthenocarpic fruit set in many plant species. However, simultaneous presence of competing seeded fruits restricts or even prevents the development of parthenocarpic fruits (Goldwin and Schwabe, 1975; Retamales and Bukovac, 1986). These results indicate the dominance effect of seeded fruit on the growth of parthenocarpic fruit. In addition, the rate of export of IAA from the seeded fruit is shown to be closely related to the presence and number of seeds per fruit (Sjut and Bangerth, 1984).

The removal of seeded dominant fruit was shown to greatly enhance the IAA export out from the remaining seeded or seedless fruits and presumably prevents or reduces abscission of the remaining fruit (Chapman, 1979).

Bangerth (1989) argues that the highest IAA export of the first induced or otherwise dominant organ inhibits the IAA export from the later induced inhibited organs. Bangerth also showed some experimental proof for his argument in which he showed high IAA concentration at the base of an excised segment or at the "junction" of dominant and inhibited organ.

Similar observations were made by Morris (1977), with pea seedlings. Using these results Bangerth (1989), proposed that the auto inhibition of polar IAA transport at junctions represents the inhibiting correlative signal. The assumption made by Bangerth (1989) include that the polar IAA export is essential for a growing organ. If the IAA export does not occur or is inhibited, for instance, because of the proposed autoinhibition, the growth of the respective organ may be reduced or it may abscise.

Auxin transport inhibitors in auxin induced abscission

Basipetal transport of auxin is inhibited by use of inhibitors like 2,3,5-tri iodo benzoic acid (TIBA). TIBA has been used in many systems to study the role of auxin in apical dominance and abscission. This section emphasizes the use of TIBA in regulating auxin induced abscission phenomena.

In a very early work it has been reported (Thimann and Bonner, 1948) that in TIBA applied plants the concentration of auxin required to bring about a particular formative effect is much higher than in control plants. Higher concentrations of TIBA inhibited auxin induced growth.

Reports by Morgan and Durham (1972), suggest that TIBA application promoted abscission of petioles and this is mediated through a higher ethylene level. They have also suggested that a decrease in auxin content in the abscission zone could increase sensitivity to ethylene thus increasing abscission.

Application of TIBA resulted in shorter internodes, reduced apical dominance increased pod set and also helped in early maturity. TIBA had little effect on the flower number, seeds/pod or seed size (Newaz and Lawes, 1980).

It has been shown that TIBA application to three cultivars of Mango suppressed apical dominance and increased the number of flower buds. The levels of ABA increased but, auxin levels decreased (Sivagami *et al*., 1989).

Wang *et al*., (1991) in experiments conducted in Sunlite nectarine trees have shown that TIBA treated fruits showed higher accumulation of IAA than in untreated fruits.

In a recent report on TIBA induced abscission, Wein *et al*., (1992) have reported that TIBA application to pedicels increased flower abscission. The position of TIBA application has also been looked into and they have reported that TIBA applied to the stem below the floral abscission zone caused higher abscission.

Auxin and apical dominance

The role of auxin in apical dominance has been extensively studied and documented. Snow (1925) was the first to propose hormonal signals to explain apical dominance. Later, Laibach (1933), and Thimann and Skoog (1933), suggested the involvement of "growth substances" which was later identified as IAA as being the main signal. Apical dominance is explained in terms of direct auxin action. Indole acetic acid produced in the terminal bud migrates down the stem and into the lateral buds where it directly suppresses the lateral bud growth. Removal of the apical bud involves removal of the auxin source and hence depletion of auxin in the inhibited lateral bud and its subsequent outgrowth. The role of auxin as a correlative signal of the shoot apex that inhibits axillary bud growth has been discussed in depth by Sachs (1991).

It is well documented that in growing plants high concentration of auxin is produced in the shoot tip (White et al, 1975) and it moves basipetally down the stem (Brown et al, 1979; Brown and Phillips, 1982; Everat - Bourbouloux, 1981; Everat-Bourbouloux and Bonnemain, 1980; Lim and Tamas, 1989; Morris, 1977; Morris and Thomas, 1979; Saltveit and Fonteno, 1983). Few reports argued against the basipetal movement of IAA using radiolabelled IAA (Hall and Hillman, 1975; Hillman et al ., 1977).

Exogenous application of auxins to the stumps or stems of decapitated shoots was shown to suppress at least partially or temporatively the lateral bud outgrowth (Blake et al., 1983; Leaky and Longman, 1986; Saltveit and Fonteno, 1983; Prasad et al, 1989; Tamas et al., 1989). One of the strong evidence against this theory is that when apical bud is removed the auxin concentration in the lateral inhibited bud does not decrease. Actually, in a few instances the auxin concentration in the bud increased (Hillman, et al., 1977; Gocal, et al., 1991). This increase in concentration of auxin in the lateral bud immediately after removal of apical bud could be due to the enhanced synthesis of IAA by the lateral bud.

Gocal et al., (1991) have measured the IAA and ABA concentrations in the lateral bud following decapitation. They found a five fold increase in IAA concentration and a 70% decrease in the ABA content of the lateral buds in decapitated plants in comparison with control.

Hillman et al., (1977) using Phaseolus have measured the level of IAA in intact and decapitated seedlings. Their studies have shown an increase in IAA content in the lateral bud of decapitated plants than in intact plants which suggests that IAA synthesis and export out of an organ is necessary for development of that organ.

The involvement of auxin induced ABA and ethylene synthesis was implicated in auxin induced inhibition of lateral bud outgrowth. Blake et al., (1983) suggested an hypothesis saying that auxin induced bud inhibition could be through increased ethylene synthesis rather than the action of auxin alone. Their study also revealed that in decapitated seedlings bud outgrowth was better and when IAA was applied exogenously bud growth was inhibited.

Tamas and Engels (1981) also showed similar results in which deseeding the filled pods increased the adjacent vegetative bud growth. Exogenous IAA application inhibited the bud growth with the inhibition being highest at higher levels of IAA. It has been suggested that the IAA released by the seeds may act as the correlative signal moving from fruit to the target organ. When ABA synthesis is stimulated abscission of the target organ may occur.

Tamas *et al*., (1989) have shown that the apical end when exposed to IAA caused bud abscission and prevented appearance of new lateral buds. They have also said that IAA is to be transported for the phenomena to occur.

IAA is known to move only in one direction ie., basipetally. How this IAA coming from the apical bud moves acropetally into the lateral bud to inhibit its outgrowth is difficult to explain. There has been little or no evidence that polarly transported labelled auxin is able to move into lateral buds (Hall and Hillman, 1975; Morris, 1977). In following [^{3}H] -IAA transport in broad beans Everat-Bourbouloux and Bonnemain (1980) found accumulation of label in the nodes rather than in the buds, with the highest concentration of label in those nodes adjacent to the most inhibited node.

Hall and Hillman (1975) showed that when ^{3}H-IAA was applied to the stumps of a decapitated Phaseolus vulgaris, shoot only little label moves down the stem and none to the lateral buds. However, the lateral bud growth was being repressed. These results indicate that this IAA need not have to be translocated to the buds to inhibit growth. High accumulation of auxin in the node from which the buds are developing may be enough to inhibit the outgrowth of lateral buds.

Auxin transport inhibitors were often used to study the need of auxin transport for inhibiting lateral bud growth. Use of TIBA in many systems induced lateral bud growth (Ackerson and Chilcote, 1978; Maheshwari and SriKrishna, 1982; Prasad et al., 1989). Similarly few other potent auxin transport inhibitors like N-1-Naphthyl phthalamic acid and 2-(1-Pyrenol)benzoic acid were also shown to induce lateral bud growth (Tamas et al., 1989; Katekar and Geissler, 1977, 1980; Prasad et al, 1989).

Bangerth (1989), on the basis of work on the sequence of sink development in fruits which presumably extrapolated to vegetative growth, recently proposed that "highest IAA export of the first induced or otherwise dominating organ inhibits the IAA export from the later induced, inhibited organs". This inhibition of polar IAA transport at the junction of dominant (mainshoot) and inhibited organs (lateral buds) preclude IAA transport out of the subordinate organs (lateral buds) and represent the inhibitory correlative signal. The important assumption here is that auxin export from a lateral bud is necessary for its outgrowth. The auxin coming from the shoot apex need not enter the lateral bud in order to function as the correlative signal. Bangerth (1989) describes this type of inhibition as "primigenic dominance".

Lim and Tamas (1989) opined that it is the basipetal transport of IAA in the stem and not the accumulation of IAA in the axillary but that inhibits bud growth.

It is also argued that auxin may be indirectly inducing the production of secondary messengers like ethylene (Burg and Burg, 1968) or ABA (Tucker, 1975) that inhibits lateral bud outgrowth. However, confirming evidences are not available to implicate a role for the secondary messengers. Martin (1987), suggested oligosaccharides as possible secondary inhibitory signals. IAA induces the production of oligosaccharides via endo-B-glucanase action in the shoot tip cell walls that are transported to the axillary bud when out growth is inhibited. However this hypothesis is yet to be tested.

Auxin induced transport of metabolites and its effect on abscission of organs and apical dominance

Auxin is known to move basipetally in a plant system. The positive effect of IAA in mobilising metabolites is also well understood. Radiolabelled metabolites have proved to be a useful tool in studying the pattern of translocation of metabolites. Radiolabelled IAA has aided in studying the zones of accumulation of IAA and its possible role in abscission and apical dominance. This section gives a brief insight to this aspect.

Seth and Wareing, (1966) have reported that IAA applied to the peduncles from which fruits were removed showed higher retention of ^{32}P in the region of hormone application. The retention was enhanced when kinetin was applied along with IAA.

Morris and Thomas (1968), have studied the movement of ^{14}C Sucrose as influenced by IAA in decapitated and intact plants. Accumulation of the label was seen in the lateral buds of decapitated plants. When an exogenous application of IAA was given to the decapitated internode it was noticed that the movement of the label was similar to that in an intact plant and this exogenous IAA prevented lateral bud growth.

Ernest and Valdovinos (1971), studied the effect of ethylene application on auxin levels in Coleus blumei. Their study reveals a 50% inhibition in the transport of radiolabelled IAA. Extractable IAA level from the apical buds of treated tissues was about half of that of untreated controls. This could be the reason for ethylene induced abscission.

Hall and Hillman (1975) have reported that movement of ^{14}C assimilates from the subtending primary leaf to the lateral bud which increased following decapitation. When IAA was applied to the decapitated apex the ability of the buds to import IAA from the primary leaf decreased.

Croxdale (1977) has shown that decapitation shifted the point of accumulation of the label from the apex to the lateral buds. Decapitated plants when treated with IAA label accumulation was seen in the stem. When auxin transport inhibitors were used accumulation of the labelled compound decreased in the apex.

In a recent study Petzold *et al*, (1992) studied the export of ^{14}C Sucrose in *Vicia faba* plants treated with IAA. They have observed increased retention of Sucrose in the source leaf when IAA is applied to the source leaf. Sink treatment with IAA increased ^{14} C Sucrose translocation to the sink.

Auxin induces mobilisation of the metabolites to the organ where the concentration of auxin is maintained high. This hypothesis proposes that auxin originating in the fruit or apical bud directs nutrient transport to the growing fruit/apical bud and away from the inactive sinks. Although, direct evidence for this hypothesis is lacking it explains the role of both auxin and nutrient in dominance effect of the sink.

In many test systems the influence of IAA in nutrient transport is demonstrated using ^{14}C- metabolites or ^{32} P. Application of IAA to decapitated stumps was shown to increase the movement of metabolites to the region of application of auxin.

However, the nature of the mechanism of auxin directed transport of metabolites has not been understood. This tempted many scientists to ask a few questions on auxin directed movement of metabolites. Does auxin act directly on phloem transport mechanism via movement down the stem and affect the loading/unloading process? or does auxin act directly to generate an acropetal nutrient gradient to the terminal bud by promoting its growth in the apical meristem?

Using auxin transport inhibitors between the source and auxin applied sink tissue many scientists have proved the direct action of auxin (Patrick, 1979; Patrick and Wareing, 1978). However, there are other evidences which indicate an indirect role of auxin in auxin induced movement of metabolites via increasing sink activity (Da Cruz and Audus, 1978; Morris, 1982)

Cline (1991), suggests that auxin directed nutrient transport remains a viable theory for the mechanism of apical dominance control.

Recently, Ganeshiah and Uma Shaanker proposed a hypothesis indicating that differential development of sinks and hence their abortion might occur purely as a consequence of the autocatalytic or feedback movement of the resource molecules to the developing sinks. They also showed the differential development can lead to abortion even under resource abundant conditions. The resource limitation may further aggravate the extent of abortion, which in turn is shown to be highly dependent on the sink drawing ability of the species. They argue that self organization amongst the sinks could be the basic process regulating resource flow to sinks and factors such as resource limitation and chemical inhibition might be additionally aggravating the asymmetry among developing sinks.

MATERIAL AND METHODS

III. MATERIAL AND METHODS

Abscission is a widespread phenomenon. There is a competition between vegetative and reproductive sinks and also between reproductive sinks for development. Only the dominant sinks are able to develop while the subordinate sinks abscise. Emphasis is now given to the concentration of auxin in the dominant sinks and this seems to be the signal that is the trigger for inhibited development of subordinate sinks.

An effort was made to understand the role of auxin in abscission and apical dominance. A series of experiments were conducted to study the role of auxin in these phenomena. Experiments were conducted both in the field and in the lab.

In initial experiments studies were conducted relating to the pattern of abscission of reproductive units in inflorescences of cowpea. In the next experiment, the source to sink ratio was manipulated and the effect of this on the pattern of abscission and pod set was studied. For these studies, the test material used was cowpea -variety C-152.

In another set of studies conducted in the lab the test crop used was cotton All these experiments were conducted in both explant systems and in intact systems. Depending on the experimental requirement modification in the explant was done.

The materials and methods used for conducting these experiments is discussed below

Location

The experimental material was developed in the Green house, and field unit of the Department of Crop Physiology, GKVK, Bangalore

Experimental details

3.1 Experiments with cowpea

3.1.1 Pattern of flower abscission and pod set at various nodal positions in cowpea

The pot culture experiment was conducted in the Green house, Department of Crop Physiology from Jan'94 to March 94 The pattern of flower abscission at various nodal positions was looked into. Observations were recorded from the day of anthesis

Observations recorded

1. Time taken for anthesis of first flowers produced at second / third raceme from that at first raceme.
2. Abscission of reproductive units in the form of flowers, pods or buds from different racemes in an inflorescence.

The observations were taken in 40 plants. Each plant bore 7-8 inflorescences out of which 2 or 3 inflorescences were selected. Selected inflorescences were tagged and numbered accordingly. On the whole 140 inflorescences were selected and observations recorded from these inflorescences.

Experimental Layout

As it was a pot culture experiment large sized pots were used for the experiment. The pot mixture used was 2:1:1 part of soil:sand:FYM respectively. The pots were filled with pot mixture and each pot held about 12.5 kg soil.

Seed material and Sowing

The plant material used for the study was Cowpea variety C-152. The seeds were obtained from National Seed Project, GKVK, Bangalore. Seeds were germinated in a germinator at 30^{0}C. These pregerminated seeds were used for sowing. Seeds were sown in the pots to a depth of

1cm. 3-4 seeds were sown at either end of the pot. One week after germination plants were thinned out to maintain 2 plants per pot.

Irrigation

Light irrigation was given to the plants twice a day. Care was taken to see that water logging did not occur as cowpea is susceptible to water logging.

Fertilizers

At the time of sowing the basal dose of fertilizers was applied. Recommended dosage for cowpea is 25:50:25 kg N:P:K respectively. The quantity of fertilizer required for each pot was calculated and applied to each pot separately.

Fertilizer	Quantity recommended (g/pot)
Urea	0.67
Single Super Phosphate	3.90
Muriate of Potash	0.52

Top dressing with Urea of same dose was given 40 days after sowing and earthing up was done.

Observations recorded

1. Time taken for anthesis of flowers at second and third raceme.

Fruiting points were tagged on the day of anthesis at the first node. Time taken for anthesis at the second and third node from that at the first node was noted.

2. Pattern of abscission

The number of flowers which set pods in each raceme of the inflorescence was noted. The number of reproductive structures abscised as pods, flowers and buds was recorded.

3.1.2 FIELD EXPERIMENT

Location

The study was conducted in the field at GKVK.

Soil and Climate

The soil was laterite and the crop was irrigated once a week.

Experimental Layout

The study was conducted with 4 replications. The plot size was 30m x 5m. This was divided into 2 strips each measuring 15m x 5m.

Seed material and Planting

Seeds of cowpea were obtained from NSP, GKVK. The variety used was C-152. The land was prepared previously and seeds were sown in rows. In each strip there were 30 rows. Spacing maintained was 30cm between the rows and 15cm between plants within a row. Ten days after germination the plants were thinned out to maintain uniform plant population.

3.1.2.1 Effect of removal of flowers at various racemes in an inflorescence on the pattern of abscission of reproductive structures

Experimental Details

The experiment was conducted in the field at GKVK. The investigation was carried out to study the effect of removal of flowers in an inflorescence on the production or abscission of reproductive structures in the upper racemes.

Treatment Details

T_1 - Removal of flowers at the first raceme of each inflorescence

T_2 - Removal of flowers at the first and second raceme of a inflorescence

T_3 - Control

The flowers at raceme one or the flowers at raceme one and two were removed. The pattern of abscission of reproductive structures was noted. In each plant about five inflorescences were tagged. Twenty such plants were selected and observations recorded from these plants. At the end of the observation period, average number of reproductive structures produced or abscised was computed.

Observations recorded

The observations recorded were;

1. Total number of flowers and pods produced in the inflorescence.
2. The number of reproductive structures that abscised in the form of flowers, buds or pods.
3. The total number of pods set in an inflorescence at the time of harvest.

3.1.2.1 Effect of source and sink manipulation on the pattern of abscission of reproductive structures

Under normal conditions in a inflorescence of cowpea only the flowers at raceme one set into pods. Those flowers at raceme two and onwards abscise. Manipulation is made either by reducing sink or source size in a plant. The aim of the experiment was to investigate the effect of manipulation of source or sink on the pattern of abscission of reproductive structures.

Experiment I

In the first experiment the source to sink ratio was increased by retaining only one or two inflorescences per plant. The main objective of this experiment was to note the change in the pattern of abscission and fruit set after the manipulation. The plant material was raised as explained for the previous experiment.

Treatment details

T_1 _ Maintaining only one inflorescence per plant

T_2 - Maintaining two inflorescences per plant

T_3 - Control

After tagging the inflorescences in the plants, the other inflorescences were removed just after they initiated. Twenty plants were selected and observations recorded in these twenty plants.

Observations recorded

The observations recorded were;

1. Total number of reproductive structures produced in the inflorescence.
2. The total number of pods set in an inflorescence at the time of harvest.

Experiment II

In this experiment the source to sink ratio was decreased by removing the leaflets which supply photosynthates to the inflorescence axis subtended by it. The objective here was to see the effect of decrease in source to sink ratio on the pattern of abscission and pod set in the inflorescences. The plant material was raised as explained in the previous experiment.

Treatment details

T_1 _ Removal of the terminal leaflet subtending an inflorescence

T_2 - Removal of the terminal and one lateral leaflet subtending an inflorescence

T_3 - Control

The treatments were imposed when the flowers at the first raceme of that particular inflorescence opened. Twenty plants were selected and in each plant five inflorescences were tagged and observations recorded from these five inflorescences.

Observations recorded

1. Total number of reproductive units produced in an inflorescence
2. The total number of pods in an inflorescence at the time of harvest.

3.1.2.3 Effect of application of TIBA on flower abscission and pod set

Experimental details

For this experiment field grown plants were selected. On a random basis 40 plants were selected and tagged. Fruiting points were selected such that they were uniform and the flowers and buds at all nodal positions were intact (Fig 1).

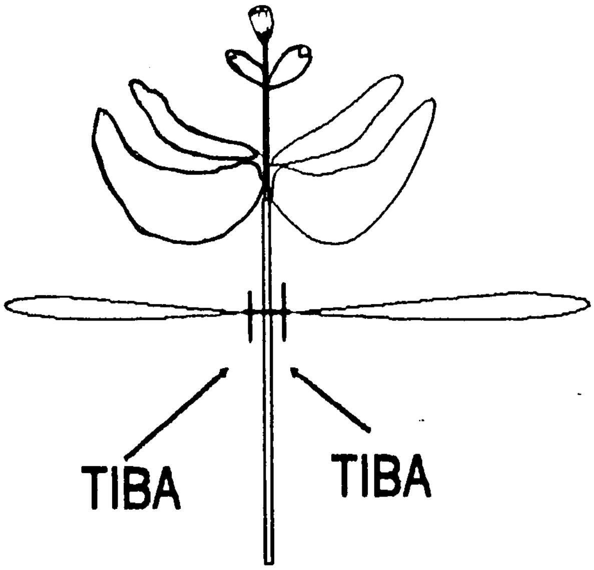


Fig 1: Cowpea inflorescence showing point of application of TIBA

Treatment Details

T_1 - Control

T_2 - TIBA at 10^{-4}M

TIBA was applied to the pedicels of pods at the first raceme when they had attained a length of 3-5cm. A small cotton wick was placed at the pedicel of the pod and TIBA was applied using a micropippette. (50ul)

Observations recorded

1. The pattern of abscission of the flowers/pods/buds at first second and third raceme.

LABORATORY EXPERIMENTS

3.2 Studies on abscission process in test systems

A series of experiments were conducted with the main purpose of investigating the effect of auxin on abscission. The plant material used for all the experiments was cotton. Seedlings were raised in bread boxes. Seeds presoaked for six hours were used for sowing. Seedlings aged three weeks were used for all the experiments. The studies were conducted in intact and explant system.

For experiments where explants were used the explants were prepared in the lab . These explants were planted in 8% agar which was previously prepared and allowed to solidify. Modification in the explants were done depending on the experimental requirement.

Preparation of the explant

Cotton seedlings were raised in bread boxes in the green house, Dept. of Crop physiology. In all the experiments seedlings aged three weeks were used. The explants were prepared by cutting the stem just above soil surface and further preparation of the explant was done in the lab. In the lab the cotyledonary leaves were debladed so as to retain about 1 cm length of the petiole. The main stem above the cotyledonary leaves was also removed. From the junction of the cotyledonary petioles about 3cm length of the stem below the lateral petiole was retained (Fig 2). The explant was planted in 8% solidified Agar in such a manner that about 5mm of the cut end of the stem was immersed in Agar gel. Five explants were used in each petridish of 10 cm diameter and the petridishes with the explants were kept in plastic trays and relative humidity was maintained in the range of 95-100%. This material was kept under diffused light.

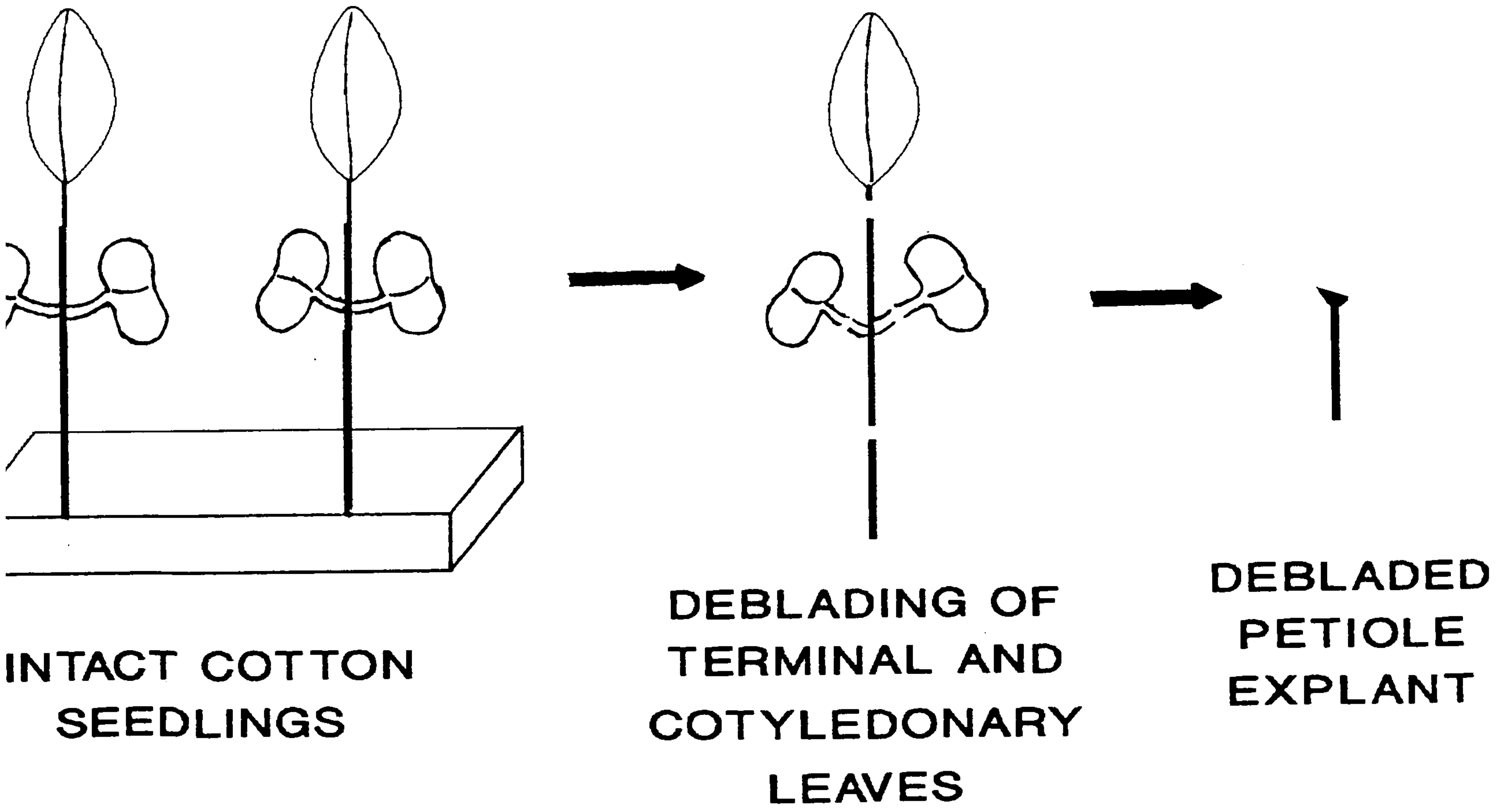


FIG 2: PREPARATION OF COTTON PETIOLE EXPLANTS

3.2.1 Effect of application of varying concentrations of IAA to the lateral petioles on the abscission of the same

In a debladed cotton double petiole explant system varying concentrations of IAA was applied to the lateral petioles (Fig 3). Cotton swab was placed on both the petioles and IAA was applied (10 ul) using a micro pippette. Two applications were given at an interval of 24 hours. Three replications were maintained in each treatment, with ten explants in each replication.

Treatment Details

T_1 - 10^{-6} M IAA
T_2 - 10^{-4} M IAA
T_3 - 10^{-2} M IAA

Observations recorded

Observations on the number of treated petioles that abscised was counted, the first observation taken 72 h after treatment.

3.2.2 Effect of application of varying concentrations of IAA to one of the lateral petioles on the abscission of the other petiole

In debladed cotton double petiole explants varying concentrations of IAA was applied to the cut end of one of the lateral petioles (Fig 4). A cotton swab was placed on the distal cut end of the lateral petiole and 10ul of the

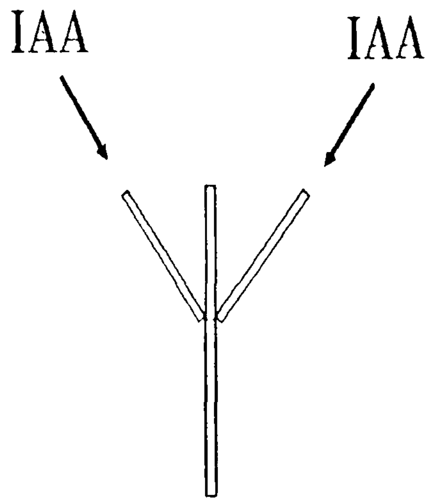


Fig 3: Test system used for expt. 3.2.1

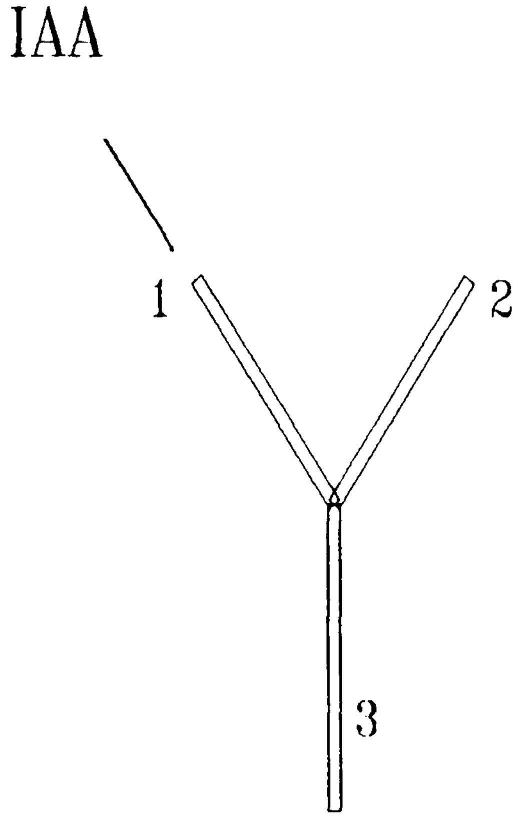


Fig 4: Test system used for expt. 3.2.2

required concentration of IAA was applied using a micro pippette. Three replications were maintained and in each replication ten plants were maintained.

Treatment Details

T_1 - 10^{-8} M IAA
T_2 - 10^{-6} M IAA
T_3 - 10^{-4} M IAA
T_4 - 10^{-2} M IAA

Observations recorded

The number of untreated petioles that abscised with time were counted, the first observation taken 24h after treatment.

3.2.3 Effect of application of varying concentrations of IAA to the cut end of the stem on the abscission of lateral petioles

This experiment was conducted in debladed cotton explants where in along with two lateral petioles, the terminal petiole was also maintained (Fig 5). IAA of varying concentrations was applied to the terminal petiole. Three replications were maintained in each treatment and ten explants in each replication.

A cotton swab was placed at the distal cut end of the terminal petiole. 10 ul of the required concentration of IAA was applied using a micro pippette. Two applications were given at an interval of 24 hours between the applications.

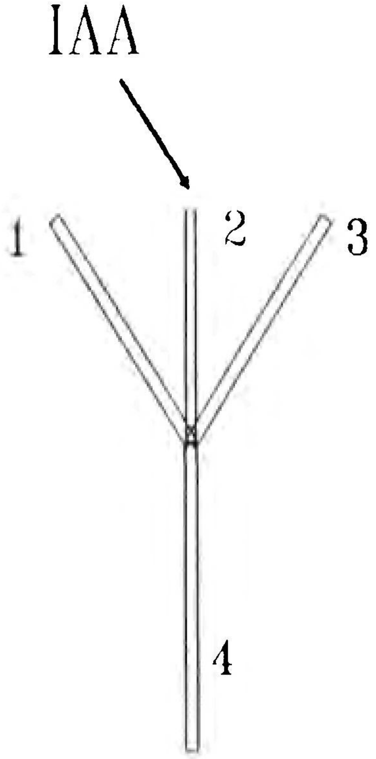


Fig 5: Test system used for expt. 3.2.3

Treatment Details

T_1 - 10^{-8} M IAA
T_2 - 10^{-6} M IAA
T_3 - 10^{-4} M IAA
T_4 - 10^{-2} M IAA

Observations recorded

Observations were taken starting from 48 hours after treatment. The number of lateral petioles that abscised was counted and the percentage calculated.

3.2.4 Effect of application of IAA to the terminal petiole on the abscission of the lateral petioles

In a modified cotton explant system IAA was applied to the terminal petiole (Fig 6). A cotton swab was placed at the cut end of the petiole and 10 ul of IAA was applied using a micro pippette. Two applications were given at an interval of 24 h. Three replications were maintained with ten explants in each replication.

Treatment Details

T_1 - 10^{-6} M IAA
T_2 - 10^{-4} M IAA
T_3 - 10^{-2} M IAA

Observations recorded

The number of untreated petioles that abscised with time were counted with the first observation being taken 24 h after treatment.

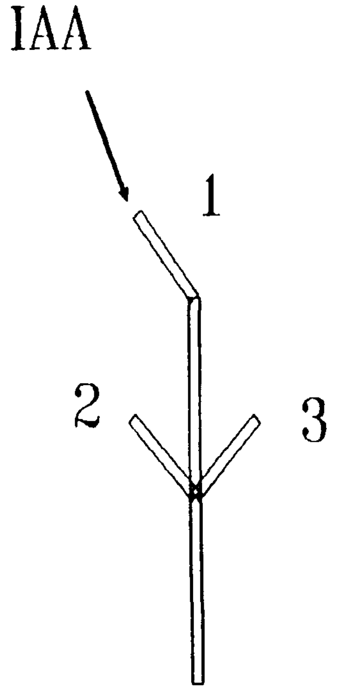


Fig 6: Test system used for expt.3.2.4

3.2.5 Effect of application of IAA to various petioles on the abscission of lateral petioles

In a modified cotton explant system IAA of 10^{-4} M concentration was applied either to one of the lateral petioles, to both the lateral petioles or to the terminal petiole (Fig 7). The abscission of the lateral petioles with time was noted. Cotton swabs were placed and IAA (10 ul) was applied using a micro pippette. Two applications of 10 ul each were given with an interval of 24 h. Three replications were maintained in each treatment with ten explants in each replication.

Treatment details

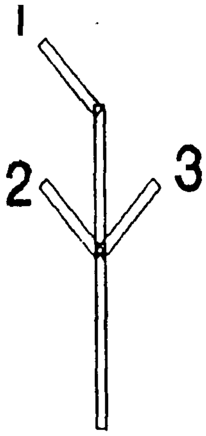
T_1 - IAA applied to both the lateral petioles
T_2 - IAA applied to one of the lateral petioles
T_3 - IAA applied to the terminal petiole

Observations recorded

In all the treatments the number of lateral petioles that abscised with time was noted and the first observation was taken 48 h after treatment.

3.2.6 Effect of application of IAA to the leaf lamina on abscission of the lateral petioles

Debladed cotton petiole explants were prepared as explained previously (Fig 8). IAA of varying concentrations was applied to the leaf lamina and the number of lateral petioles that abscised with time was noted.



T1: IAA applied to 2 & 3

T2: IAA applied to 2

T3: IAA applied to 1

Fig 7: Test system used for expt. 3.2.5

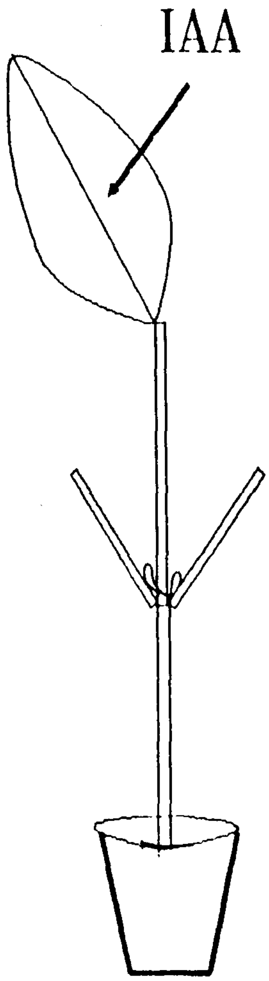


Fig 8: Test system used for expt. 3.2.6

Treatment details

T_1 - 10^{-8}M IAA
T_2 - 10^{-6}M IAA
T_3 - 10^{-4}M IAA
T_4 - 10^{-2}M IAA

Observations recorded

The number of lateral petioles that abscised with time was recorded and the percent abscission computed.

3.2.7 Effect of application of TIBA on IAA induced abscission

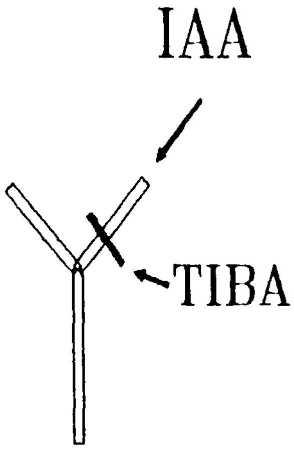
Debladed cotton petiole explants were used to study the need for translocation of IAA in inducing abscission. Debladed petiole explants were prepared and TIBA was applied to one of the lateral petioles (as shown in Fig. 9). TIBA (10^{-4} M) was applied first depending on the treatment and six hours later IAA was applied. In this case IAA (concentration of 10^{-4}M) application of 10 ul was given only once. Three replications were maintained in each treatment with ten explants in each replication.

Treatment details

T_1 - Only IAA applied to one of the lateral petiole

T_2 - Only TIBA applied to one of the lateral petiole

T_3 - TIBA and IAA applied to the same lateral petiole



T1: Only IAA

T2: Only TIBA

T3: IAA + TIBA

Fig 9: Test system used for expt. 3.2.7

Observations recorded

The treatments were imposed as mentioned and the number of untreated lateral petioles that abscised was recorded. The first observation was taken 24 h after treatment.

3.3 INFLUENCE OF IAA ON THE PHENOMENON OF APICAL DOMINANCE

3.3.1 Effect of application of IAA to the lateral petioles on the initiation of growth of axillary buds

In a debladed cotton plant IAA of varying concentrations was applied to both the lateral petioles (Fig 10). The main aim of the experiment was to see the initiation of growth of buds. IAA was applied twice at an interval of 24h.

Treatment Details

T_1 - 10^{-8} M IAA
T_2 - 10^{-6} M IAA
T_3 - 10^{-4} M IAA
T_4 - 10^{-2} M IAA

Observations recorded

The number of lateral buds that initiated growth from the axil of the lateral petioles was noted. Observations were taken from the day of application of IAA upto 15 days after application.

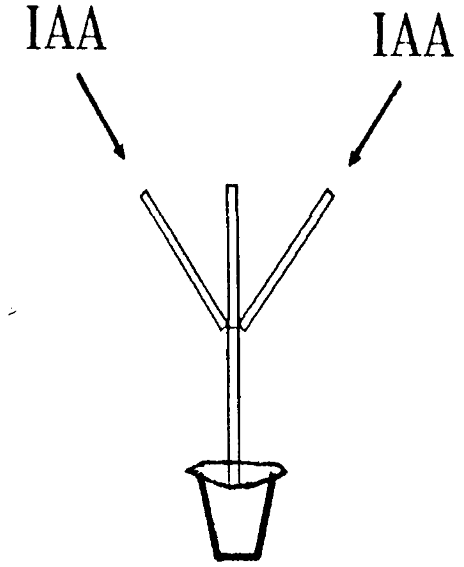


Fig 10: Test system used for expt. 3.3.1

3.3.2 Effect of application of varying concentrations of IAA on the extent of growth of axillary buds

In debladed cotton plants (Fig 11) IAA of varying concentrations was applied to the cut end of both the lateral petioles and the growth of the buds in the axils of these petioles was observed. Two applications of IAA was given at an interval of 24h.

Treatment Details

T_1 - 10^{-8} M IAA
T_2 - 10^{-6} M IAA
T_3 - 10^{-4} M IAA
T_4 - 10^{-2} M IAA

Observations recorded

The extent of growth of the axillary bud was noted. This was arrived at by measuring the length of the axillary buds every alternate day for a period of 15 days. The increase in growth of the axillary buds over a period of 15 days was calculated.

3.3.3 Effect of application of varying concentrations of IAA to the terminal petiole on the initiation of growth of the bud present at different position in an explant system.

In an intact cotton plant but with the leaves debladed (Fig 12) IAA of varying concentrations was applied to the apical petiole and the effect of this on the initiation of buds from the axil of the lateral petioles and the terminal petiole was looked into. IAA was applied twice at an interval of 24 h.

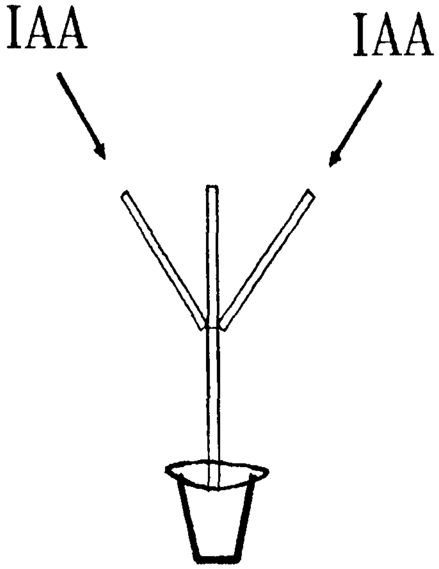


Fig 11: Test system used for expt. 3.3.2

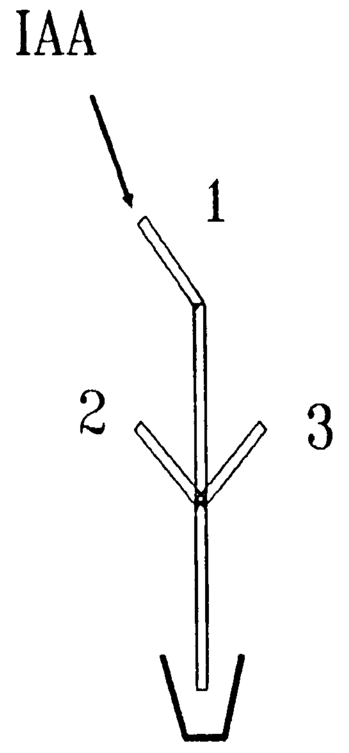


Fig 12: Test system used for expt.3.3.3

Treatment Details

T_1 - 10^{-6} M IAA

T_2 - 10^{-4} M IAA

T_3 - 10^{-2} M IAA

Observations recorded

The number of buds that initiated growth was counted. Observations were recorded on the axillary bud of the apical petiole as well as from the axil of the lateral petiole. Observations were recorded from the day of application of IAA upto 15 days after application.

3.3.4 Effect of application of IAA to the leaf lamina on the initiation of growth of axillary buds

In an intact system the cotyledonary leaf lamina was debladed and the terminal leaf retained (Fig 13). IAA of varying concentrations was smeared to the leaf lamina. Three applications each of 50 ul were given on alternate days. Ten plants were maintained in each treatment.

Treatment Details

T_1 - 10^{-8} M IAA
T_2 - 10^{-6} M IAA
T_3 - 10^{-4} M IAA
T_4 - 10^{-2} M IAA

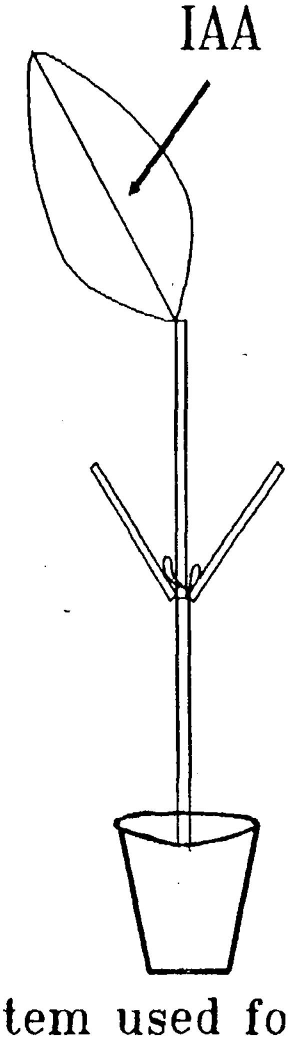


Fig 13: Test system used for expt. 3.3.4 to 3.3.8

Observations recorded

The number of lateral buds that sprouted from the axil of the lateral petioles was counted, the observations taken from the day of bud initiation.

3.3.5 Effect of application of IAA to the leaf lamina on the extent of lateral bud growth

In intact cotton plants wherein the cotyledonary leaf lamina was debladed and the terminal leaf retained intact (Fig 13), IAA of varying concentrations was smeared to the leaf lamina. Three applications of IAA (50 ul each) were given every alternate day.

Treatment Details

T_1 - 10^{-8} M IAA
T_2 - 10^{-6} M IAA
T_3 - 10^{-4} M IAA
T_4 - 10^{-2} M IAA

Observations recorded

The growth of the lateral bud arising from the axil of the lateral petiole was recorded, by measuring the growth of the bud every alternate day. The extent of growth of the bud over a period of 15 days was calculated.

3.3.6 Effect of application of IAA on initiation of buds at the apical region

In intact cotton plants wherein the cotyledonary leaf lamina was debladed and the terminal leaf retained intact (Fig 13), IAA of varying concentrations was smeared to the leaf lamina. Three applications of IAA (50 ul each) were given every alternate day.

Treatment Details

T_1 - 10^{-8} M IAA
T_2 - 10^{-6} M IAA
T_3 - 10^{-4} M IAA
T_4 - 10^{-2} M IAA

Observations recorded

The number of apical buds arising from the axil of the apical petiole was recorded. The observations were taken from the day of bud initiation for a period of 15 days. The number of buds iunitiated upto 15 days are given in the results.

3.3.7 Effect of application of IAA on the growth of the apical bud

In intact cotton plants wherein the cotyledonary leaf lamina was debladed and the terminal leaf retained intact (Fig 13), IAA of varying concentrations was smeared to the leaf lamina. Three applications of IAA (50 ul each) were given every alternate day.

Treatment Details

T_1 - 10^{-8} M IAA
T_2 - 10^{-6} M IAA
T_3 - 10^{-4} M IAA
T_4 - 10^{-2} M IAA

Observations recorded

The growth of the apical bud arising from the axil of the apical petiole was recorded, by measuring the growth of the bud every alternate day. The extent of growth of the bud over a period of 15 days was calculated.

3.3.8 Effect of application of IAA on the internodal length

In intact cotton plants wherein the cotyledonary leaf lamina was debladed and the terminal leaf retained intact (Fig 13), IAA of varying concentrations was smeared to the leaf lamina. Three applications of IAA (50 ul each) were given every alternate day. Ten plants were maintained in each treatment.

Treatment Details

T_1 - 10^{-8} M IAA
T_2 - 10^{-6} M IAA
T_3 - 10^{-4} M IAA
T_4 - 10^{-2} M IAA

Observations recorded

The internodal length of the plants were measured, by using a scale. Observations were recorded from 2 days after treatment upto 15 days. The change in internodal length was calculated over a period of 15 days.

3.3.9 EFFECT OF REPEATED APPLICATION OF GROWTH HORMONES ON GROWTH AND DEVELOPMENT OF PLANTS

The experiment aimed to study the effect of repeated application of hormones on the growth and branching pattern of Mestha and Cowpea.

A. Mestha

Planting Material

Seeds of Mestha (variety M-17) were sown about a cm deep in soil and 2 seeds were sown in each corner of the pot. A week after germination the seedlings were thinned out to maintain 4 plants per pot. Each plant was considered as one replication.

Treatment details

The Statistical Design used for the study was Completely Randomised Design. There were four treatments.

T_1 - Control
T_2 - IAA 10^{-4}M
T_3 - IAA 10^{-4}M + BA 0.2 ppm
T_4 - TIBA 10^{-3}M

Plants were irrigated twice a day.

Hormone application

Required concentrations of the hormones were prepared. Cotton swabs were placed at the apex of the plants and the hormones were applied twice a day continuously from ten days after germination upto the final day of observation . During each application 50 ul was applied.

Observations were recorded from the tenth day of germination and at an interval of every three days.The observations recorded during the one month study period were:

1. Plant height
2. Leaf area
3. Number of branches

Observations recorded

Plant height

Plant height was measured using a measuring tape and expressed in cm. Height was measured from the base to the apex.

Leaf area

For calculation of leaf area young fully expanded leaves were selected. The selected leaves were tagged and the length and width of the leaf was measured. The leaf area was recorded every third day. While computing the leaf area the length and width were multiplied and the area expressed in cm^2.

Number of primary branches

In case of Mestha primary branches arise from the axil of the leaf. They were considered as branches after they had attained a length of 1 cm.

B Cowpea

Experimental Layout

A pot culture experiment with 2 replications. The Statistical Design used was Completely Randomised design.

Seed material and Planting

Seeds of cowpea (cv. C-152) were obtained from NSP, GKVK. Pre-germinated seeds were sown. 3-4 seeds were sown in soil at either end of the pot. Ten days after germination thinning was done to maintain two plants per pot. Each plant was considered as one replication.

Irrigation

Light irrigation was given to the plants twice a day.

Fertilizers

At the time of sowing the basal dose of fertilizers was applied. Recommended dosage for cowpea is 25:50:25 kg N:P:K respectively. The quantity of fertilizer required for each pot was calculated and applied to each pot separately.

Fertilizer	Quantity recommended (g/pot)
Urea	0.67
Single Super Phosphate	3.90
Muriate of Potash	0.52

Top dressing with Urea was given 40 days after sowing and earthing up was done.

Treatment details

Two replications were maintained and treatments were the same as explained for Mestha.

Hormone application

Required concentrations of the hormones were prepared and applied as explained for the experiment conducted on Mestha.

Observations recorded

Observations were recorded from the 10th day of germination and at an interval of every three days.The observations recorded during the one month study period were:

1. Plant height
2. Leaf area
3. Number of branches

Observations recorded

Observations were recorded as explained for Mestha.

3.4 ESTIMATION OF ENDOGENOUS CONCENTRATION OF IAA IN OVARIES OF COWPEA AT THE TIME OF ANTHESIS AT DIFFERENT RACEMES

From field grown cowpea plants the flowers were collected from different racemes on the day of anthesis. In the lab the flowers were dissected and the ovaries were removed. The fresh weight of the ovaries was taken, The ovaries were stored in liquid nitrogen till the time of quantification.

Extraction of IAA from the ovaries for estimation

The stored ovaries were ground in 10 ml of 80% methanol. This was done thrice and the samples were centrifuged at 10,000 rpm for 10 mts. The supernatant was collected. The collected supernatant was passed through a Sep Pak cartridge (C-18).

The Sep Pak cartridge was pre wetted with 5 ml of 80% methanol and then with 5 ml of distilled water. Excess moisture was removed by blowing air. The samples were then passed through the cartridge and the purified samples were collected. The samples were totally dried by vacuum desiccation. The dry samples were extracted in 1 ml of Phosphate buffer saline (PBS) and stored at -20^{0}C.

The purified sample was then injected into HPLC column for estimation (model LC - 10A). The partially purified plant samples were injected on RP-HPLC column system (Shimadzu Instruments, Japan) at room temperature (25^0C). The dimensions of the column were 25 cm in length and 8 mm diameter (Shimpak CLS-ODS column). The solvent used for the mobile phase was 100% methanol. The column was equilibrated with 100% methanol for 30 minutes, at a flow rate of 2 ml/min. The partially purified samples were diluted 1:1 with 100% methanol. 20 ul of the sample was injected onto the HPLC column. The concentration of IAA present in the sample was calculated by comparing with the standard obtained for pure IAA (Sigma chemicals, USA)

Composition of Phosphate Buffer Saline (PBS) (pH 7.4)

Sodium Chloride (NaCl)	8.00 g
Potassium Chloride (KCl)	0.20 g
Potassium dihydrogen phosphate (KH_2PO_4)	0.20 g
Disodium hydrogen phosphate (Na_2HPO_4)	

Volume was made to 1 l using distilled water

3.5 INFLUENCE OF IAA ON THE UPTAKE OF RADIOLABELLED SUCROSE

3.5.1 Effect of IAA on the uptake of radiolabelled sucrose

This experiment was conducted in debladed cotton explants The explants were planted in 8% Agar. 10ul of IAA (10^{-2}M) was applied to the petioles depending on the treatment. 12 hours after IAA application radiolabelled Sucrose was given from the base. Samples were taken 24 hours after treatment.

For analysis each explant was divided into four parts. Each part was taken separately for analysis. The samples were first extracted in Methanol. This methanol extract was used for taking radioactive counts. Paper discs of 1cm diameter were cut and the extract put on the discs and allowed to evaporate. The dried discs were put into scintillation vials containing 2ml Bray's solution and counts taken in a Liquid Scintillation Counter (LSC).

Treatment Details

T_1 - IAA applied to both lateral petioles (1 & 3)
T_2 - IAA applied to one lateral petiole (1)
T_3 - IAA applied to the terminal petiole (2)
T_4 - Control- Water applied to both the lateral petioles.

Ten explants were maintained in each treatment.

Observations recorded

The radioactivity in each sample was analysed in a LSC.

3.5.2 Effect of IAA on the mobilisation of metabolites

This experiment was conducted initially in the green house and later the plants were treated in the lab. In the lab explants were used. Plants aged 3 weeks were selected for this experiment. Enhanced CO_2 was given in the form of radiolabelled Sodium Carbonate. Due to this the photosynthesis was enhanced and the amount of radioactivity could be traced.

The plants selected were covered by a hood and radiolabelled CO_2 was given to these plants. The plants were kept for half an hour after which the hood was removed and the plants left in field condition for 30 hours.

After 30 hours the plants were debladed and the explants planted in Agar and IAA treatment was given. Samples were taken for analysis 12 h after treatment. Each plant sample was taken as explained earlier. The samples were oven dried, ground and put into scintillation vials with 2ml Bray's solution and counts taken in LSC..

Treatment Details

T_1 - IAA applied to both lateral petioles (1 & 3)

T_2 - IAA applied to one lateral petiole (1)

T_3 - IAA applied to the terminal petiole (2)

T_4 - Water applied to both the lateral petioles.

3.5.3 Effect of application of hormones on the uptake of radiolabelled Sucrose

This experiment was conducted using inflorescences of Cowpea. For this purpose, inflorescences where in all the nodal positions were intact were selected. A cotton wick was placed round the peduncle of the pods that had formed at the first node. and the various treatments were imposed. After 24 hours these treated inflorescences were detached from the plants and kept in small vials containing 10ul of radiolabelled Sucrose. This set up was left for 24 hours under light. After the time period the inflorescences were removed and the various regions were assayed for radioactivity. The samples were dried, and put into Scintillation vials containing 2 ml Bray's solution and counts taken using a LSC.

Treatment details

T_1 - Control

T_2 - TIBA 10^{-4} M applied to the peduncles of pods at the first raceme

T_3 - IAA (10^{-4}M) + BA (2 ppm) applied to the peduncles of pods at the first raceme

3.5.4 Translocation of radiolabelled IAA in cotton explants

Cotton explants were prepared and various treatments imposed. To the explants which were treated with TIBA first TIBA application was given. Eight hours after application of TIBA, IAA was applied to the petioles depending on the treatment requirements. After four hours radiolabelled IAA was applied to the opposite petiole. All the explants were maintained at a relative humidity of 100%. The explants were placed in eppendof tubes which contained 0.2 ml water, the reason being to collect the translocated radioactivity in this water. After 48 hours, the explants were removed and divided into various parts as indicated and these individual samples were assessed for radioactivity. The samples were oven dried and then put into Scintillation vials containing 2 ml of Bray's solution and counts taken using a LSC.

Treatment details

T_1 - Control

T_2 - 10^{-5} M IAA applied to petiole 3 and ^{14}C IAA applied to petiole 1

T_3 - 10^{-3} M IAA applied to petiole 3 and ^{14}C IAA applied to 1

T_4 - 10^{-5} M IAA and TIBA 10^{-4} M applied to 3 and ^{14}C IAA applied to 1

Composition of Bray's solution

Primary Scintillator (POP) (2,5 diphenyl oxazole)	4.0 g
Secondary Scintillator (POPOP) 1,4bis 2(5 phenyl oxazolyl benzene)	0.2 g

Solvents

Naphthalene	60.0 g
Methanol	100.0 ml
Ethylene glycol	20.0 ml

Volume made up to 1 l with 1,4 Dioxane

RESULTS

IV. RESULTS

In the present investigation experiments were conducted to study the relevance of auxin produced by the developing dominant sinks on abscission and apical dominance phenomena.

In the first series of experiments the pattern of flower opening and pod set was studied in a racemose inflorescence using cowpea as a test crop, and the pattern of establishment of dominant sinks was assessed. The effect of inhibiting auxin transport from the dominant sinks on flower abscission from the upper nodes was studied.

In a well defined test system using cotton petiole explants the influence of varying concentrations of IAA applied to both the lateral petioles or to only one of the lateral petioles on abscission was studied. The involvement of auxin transport in influencing abscission was also tested. In another set of experiments, the influence of IAA in the establishment of dominance to the IAA applied source and its effect on mobilization of metabolites were looked into. The transport of IAA from the dominant sinks and its influence on transport of IAA from subordinate sinks was studied using ^{14}C labelled IAA.

4.1 Experiments with cowpea

4.1.1 Pattern of flower opening and pod set within an inflorescence in cowpea

The phenomenon of abscission of reproductive units is of common occurrence in a racemose inflorescence, in which flowers open in acropetal succession. There seems to be a particular pattern of abscission of reproductive structures at various racemes within an inflorescence and the time taken for anthesis of flowers at different racemes.Investigations were carried out to understand the pattern of flower opening, abscission of reproductive units and pod set with in an inflorescence. The relevance of auxin production and transport from the early set dominant pods on abscission of reproductive units in the upper racemes in a inflorescence also was determined.

The inflorescence of cowpea is a raceme. In a inflorescence the flowers come to anthesis starting from the first raceme and proceeds upwards. Flowers at the first raceme come to anthesis first. Two flowers open simultaneously in the first raceme and two more flowers in the second raceme opens next (Table 1).

Table 1 : The total number of flowers opened and abscised at raceme I and II in cowpea inflorescences (observations recorded on 140 inflorescences upto 15 days after opening of the first flower within an inflorescence)

Position of raceme on inflorescence	Number of flowers produced	Number of flowers abscised	Per cent abscission of flowers
Raceme I	280	48	17.14
Raceme II	244	194	79.5
Raceme III	--	--	--
Above raceme III	--	--	--

It was observed that the flowers at the second raceme come to anthesis between 48 hours and 120 hours after opening of flowers in the first raceme in different inflorescences (Fig 15,15a,15b). As the time taken for flower opening at the second raceme increased from that at the first raceme it was observed that pod set at the second raceme decreased. As the time taken for anthesis at the second raceme increased, the abscission potential of flowers increased and they abscised early (Fig 14).

It was further noted that all the reproductive units produced in the third raceme or the racemes above abscised as buds even before opening of flowers (Fig 16).

In cowpea only the flowers at the first raceme develop into pods and a very small per cent of flowers at the second raceme form pods.

From these results we can say that as the time interval between anthesis of flowers in raceme one and two increases there is decreased pod set and increased abscission of flowers produced in the second raceme.

4.1.2 Effect of removal of flowers at various racemes in an inflorescence on the pattern of abscission of reproductive structures

Once the flowers set pods in the first raceme this may be acting as the dominant sink, influencing the abscission

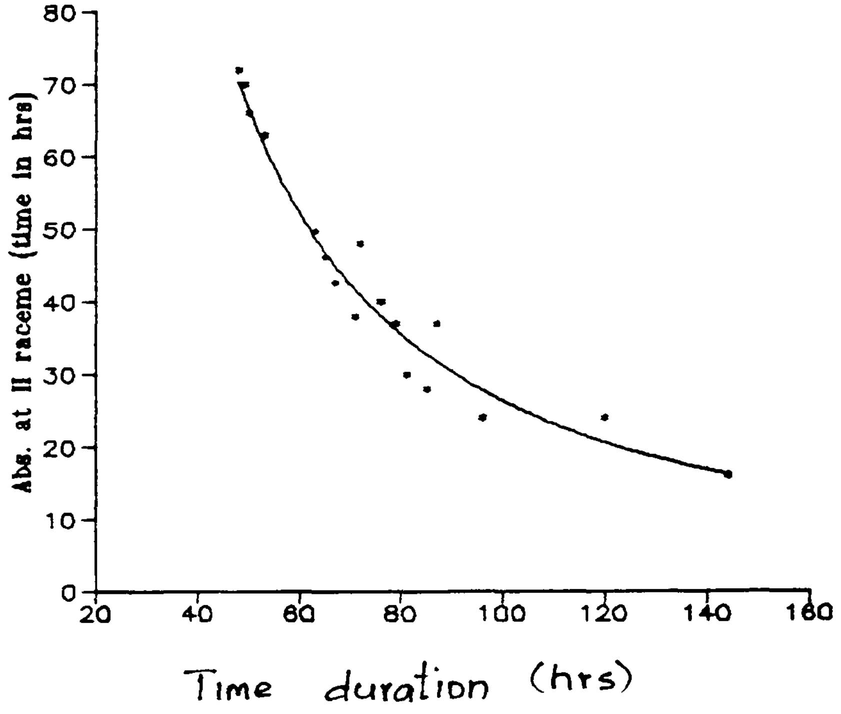


Fig 14: Time duration between opening of flowers on successive racemes (I & II) and its relationship to abscission of flowers in raceme II

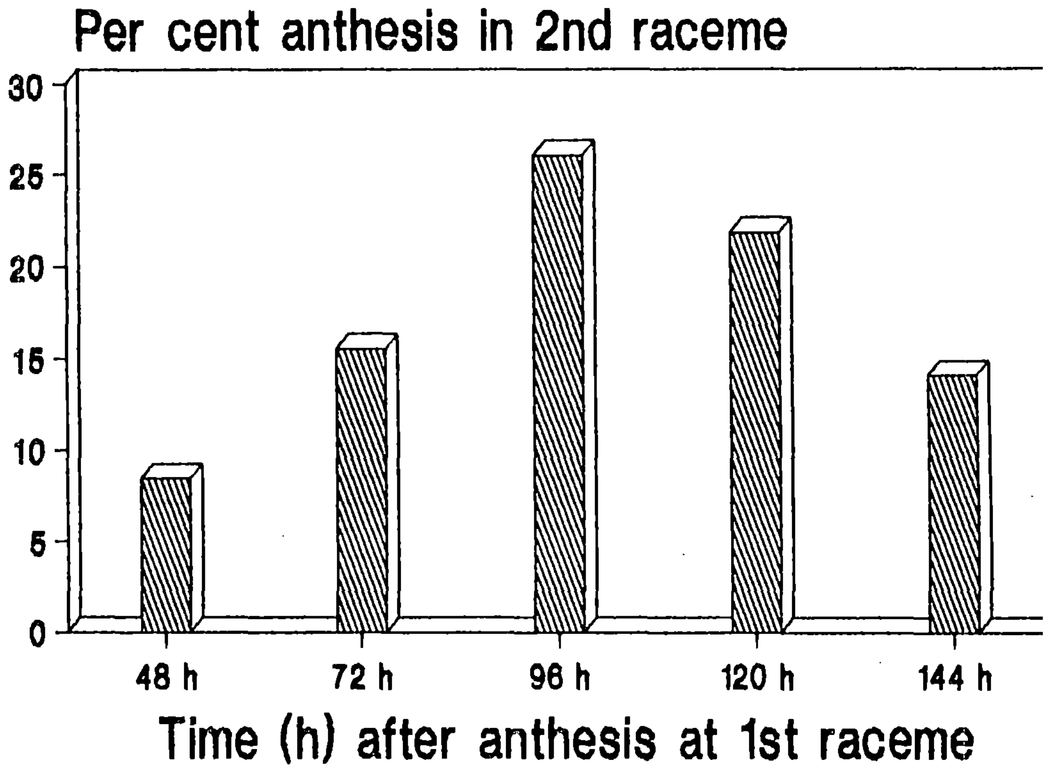


Fig 15: Pattern of anthesis in second raceme in inflorescences of cowpea

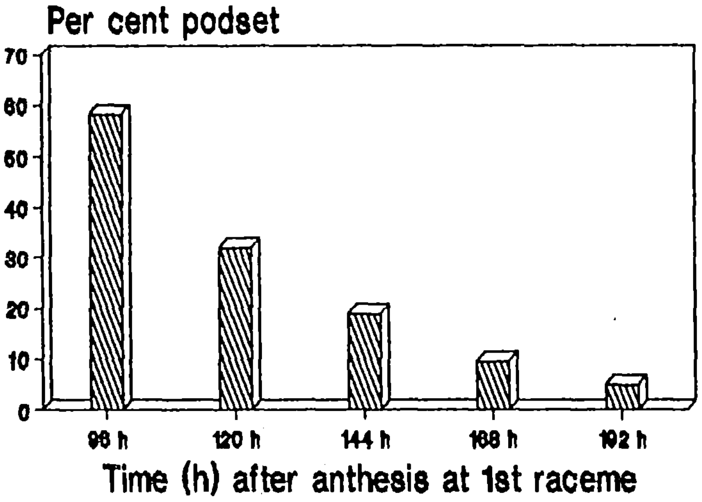


Fig 15a: Pod set in 2nd raceme in inflorescences of cowpea

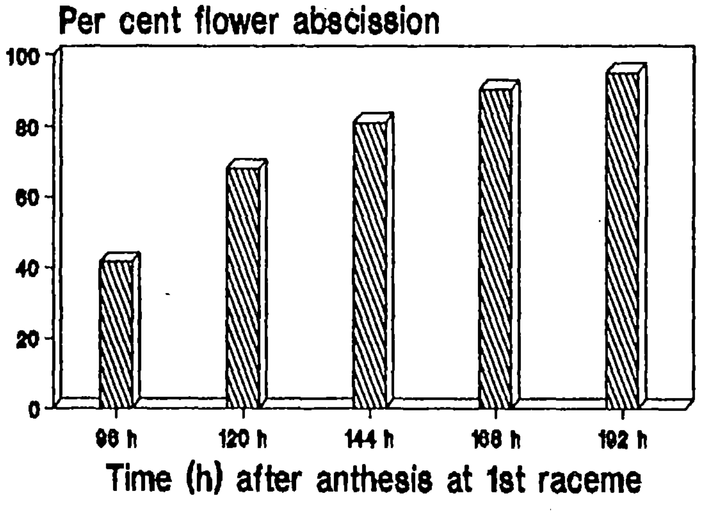


Fig 15b: Abscission of flowers at 2nd raceme in inflorescences of cowpea

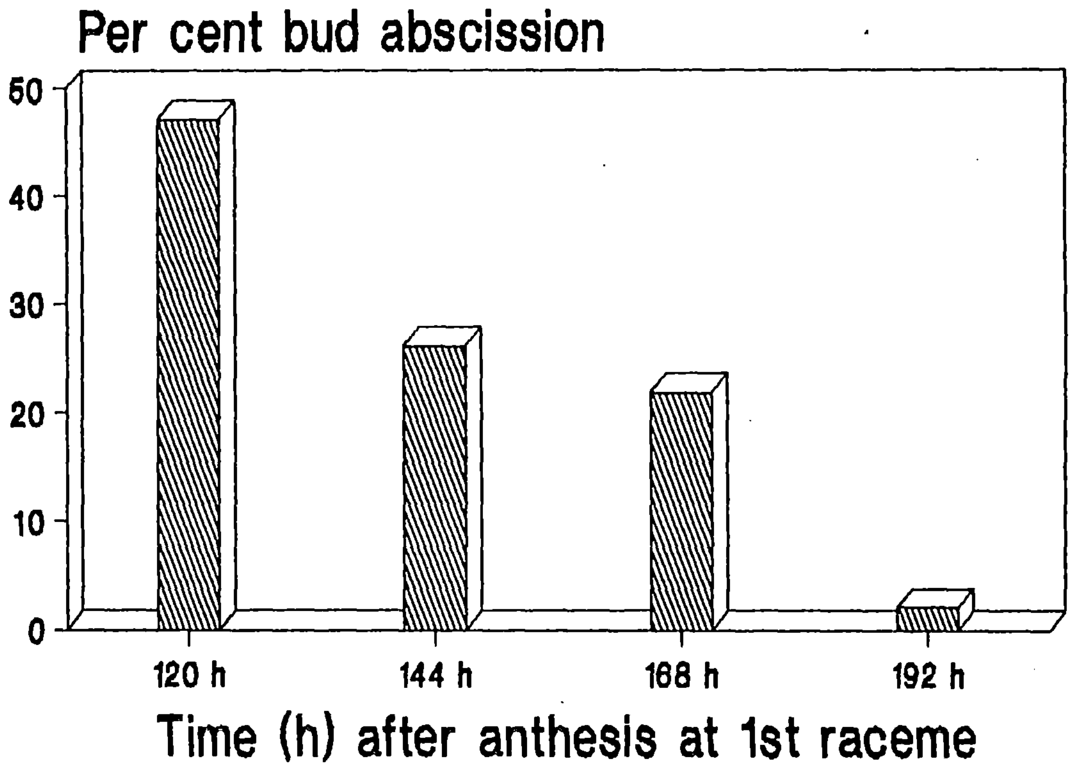


Fig 16: Abscission of buds at 3rd raceme in inflorescences of cowpea

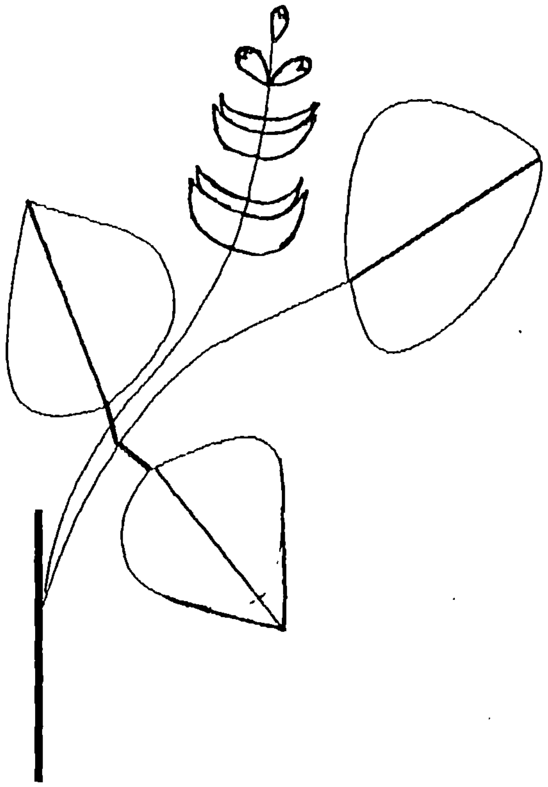
of flowers and other reproductive units produced in the second raceme. To test this hypothesis experiments were conducted in which the flowers opened in the first raceme or in the first and second racemes were removed immediately after opening and the pod set in upper nodes was studied (Fig 17).

Removal of flowers from the first raceme increased the total number of reproductive units produced as well as abscised in the upper nodes. However, removal of flowers from the first two basal racemes increased total number of reproductive units produced from the upper nodes (Fig17a).

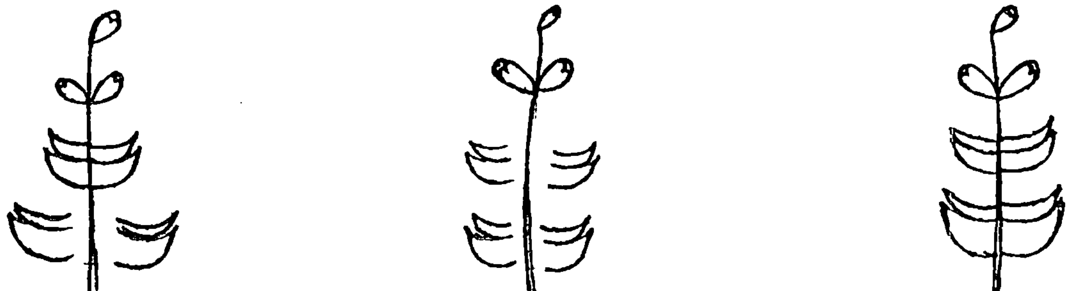
The total number of pods set per inflorescence however remained in the range of 1.40 to 2.20 , indicating that once about two pods set per inflorescence all the other reproductive units abscises (Fig 17b).

4.1.2.1 Influence of Source-Sink manipulation on pod set and abscission of reproductive units in cowpea

In cowpea each inflorescence is subtended by a leaf and the leaf acts as a source for photosynthates for the developing pods. The size and photosynthetic activity of this subtending leaf may be enough only for development of about two pods per inflorescence.



INFLORESCENCE OF COWPEA WITH
A SUBTENDING TRIFOLIATE LEAF



FLOWERS AT
RACEME I
REMOVED

FLOWERS AT
RACEME I & II
REMOVED

FLOWERS AT ALL
RACEMES RETAINED

FIG 17: SINK MANIPULATION IN
COWPEA INFLORESCENCE

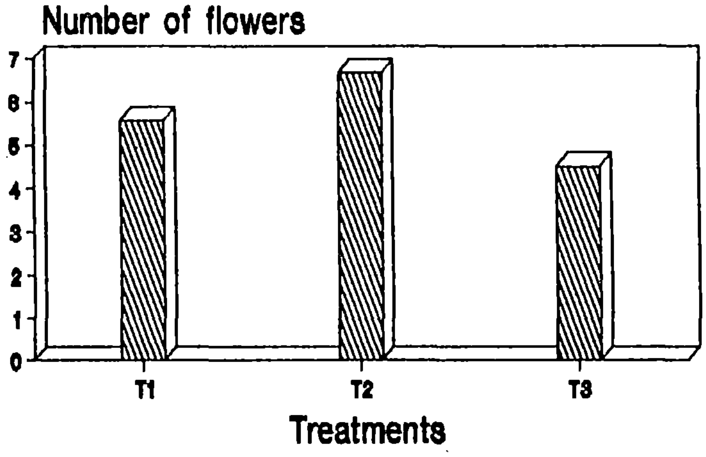


Fig 17a: Influence of removal of flowers from basal racemes on total number of flowers produced/inflorescence of cowpea

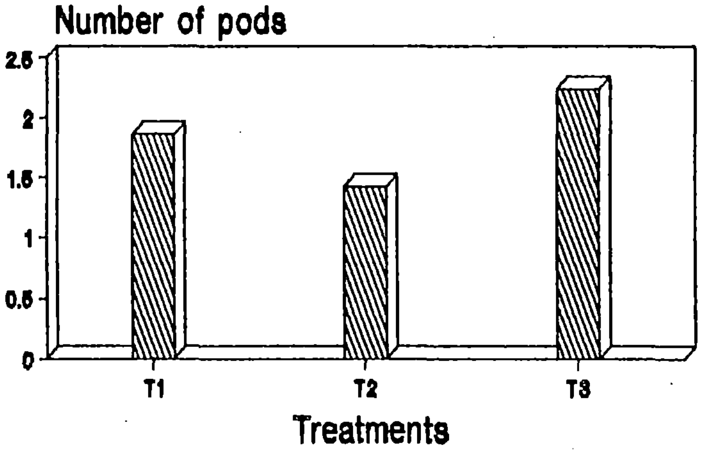


Fig 17b: Influence of removal of flowers from basal racemes on total number of pods at harvest/inflorescence of cowpea

TREATMENT DETAILS

T1: REMOVAL OF FLOWERS AT RACEME I
T2: REMOVAL OF FLOWERS AT RACEME I & II
T3: FLOWERS RETAINED AT ALL RACEMES

To study this in another experiment, source-sink manipulation was done by increasing or decreasing the source to sink ratio. With an objective to increase the source to sink ratio, only one or two inflorescences were maintained per plant and other inflorescences were removed just after they initiated. To decrease the source to sink ratio the terminal leaflet or the terminal + one lateral leaflet was removed from the subtending leaf of all the inflorescences at the time of anthesis at that inflorescence and observation on abscission of reproductive units was recorded

Maintaining only one or two inflorescences per plant increased the total number of reproductive units that were produced and abscised. The total number of reproductive structures that developed in the inflorescence increased when only one or two inflorescences were maintained per plant. The total number of pods set and developing to maturity increased per inflorescence when only one or two inflorescences were maintained per plant respectively (Fig 18, 18a).

Reduction in the source to sink ratio by removal of leaves either the terminal leaflet or the terminal and lateral leaflet led to a decrease in the production of reproductive units indicating that this could be due to the lack of translocation of photosynthates. Due to the reduced

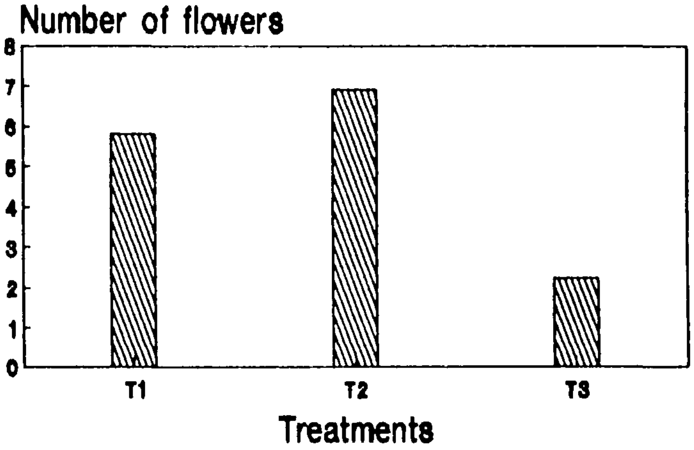


Fig 18: Effect of sink manipulation on the total number of flowers produced per inflorescence of cowpea

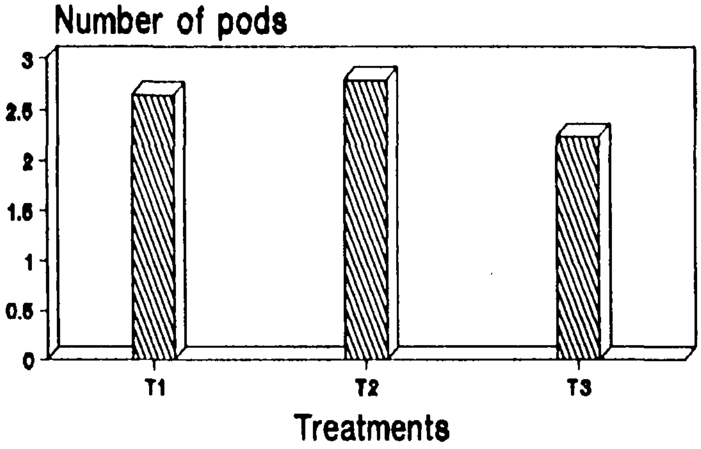


Fig 18a: Effect of sink manipulation on total number of pods at harvest per inflorescence of cowpea

TREATMENT DETAILS

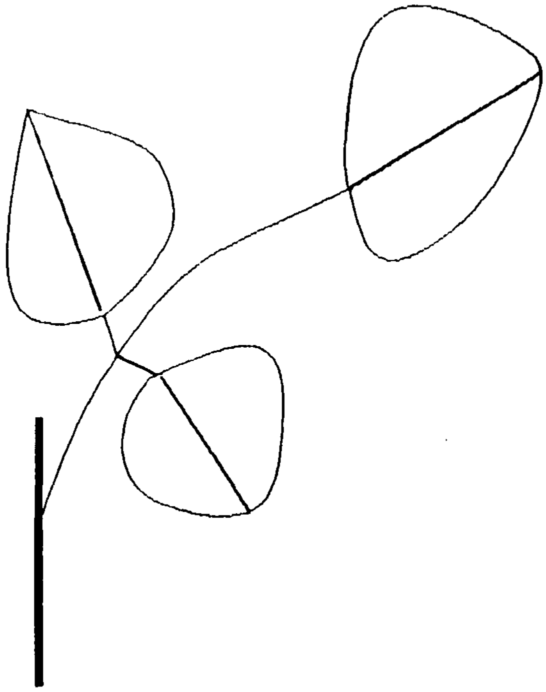
T1: MAINTAINING 1 INFLORESCENCE /PLANT
T2: MAINTAINING 2 INFLORESCENCES /PLANT
T3: RETAINING ALL INFLORESCENCES OF PLANT

supply of photosynthates to the developing flowers there is restricted development of the flowers and hence they abscise and this leads to a decrease in pod set. The number of pods produced was 1.72 and 1.32 when the source was reduced by 33% or 66% respectively compared to 2.2 in non defoliated treatment.(Fig 19,19a,19b).

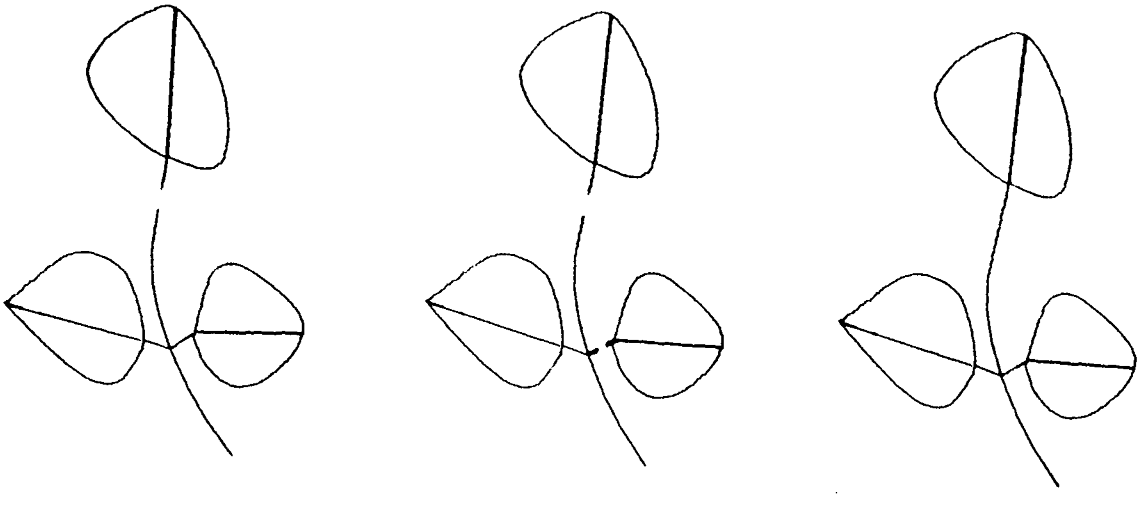
4.1.2.2 Influence of application of TIBA on the pattern of abscission of reproductive structures and pod set in cowpea

The basipetal transport of auxin from the dominant sink (developing pods from the first raceme) and its accumulation in the peduncle is said to be the trigger that inhibits flowers at other racemes from developing to mature pods. If the auxin transport is inhibited from the developing pods at the first raceme more reproductive units may be produced at the upper racemes and may develop into pods.

TIBA was applied to the pedicel of the pod (Fig 1) with an objective to inhibit auxin transport from the developing pods in the first raceme. The results obtained indicate that in the upper racemes, that is above raceme one, more number of reproductive units are produced. Although more number of reproductive units are produced there is still abscission of these reproductive structures



TRIFOLIATE LEAF OF COWPEA



TERMINAL LEAFLET REMOVED

TERMINAL + ONE LATERAL LEAFLET REMOVED

LEAVES RETAINED INTACT

FIG 19: SOURCE MANIPULATION

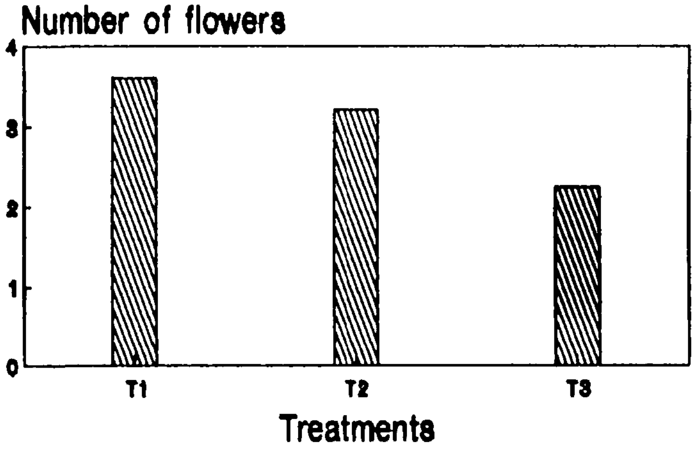


Fig 19a: Effect of source manipulation on number of flowers produced per inflorescence of cowpea

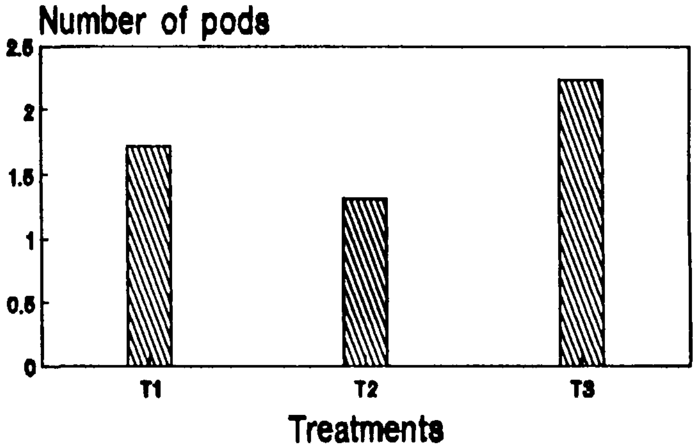


Fig 19b: Effect of source manipulation on total number of pods at harvest per inflorescence of cowpea

TREATMENT DETAILS

T1: REMOVAL OF TERMINAL LEAFLET
T2: REMOVAL OF TERMINAL AND ONE
LATERAL LEAFLET
T3: LEAFLETS NOT REMOVED

with time. However, the number of flowers which set pods was more in the second raceme when auxin transport was inhibited from the developing pods at the first raceme (Fig 20,20a,20b).

Further, it was also noted that the pods treated with TIBA at the first raceme also abscised when compared to pods that were not treated.

These results indicate that auxin produced at raceme one could be the possible signal that prevents pod set of the flowers produced at the upper raceme. Hence, auxin can be said to have a role in the abscission of young immature reproductive units.

4.2 Influence of IAA on abscission of petioles in cotton petiole explants

4.2.1 Effect of application of varying concentrations of IAA to the lateral petioles on the abscission of the same.

Varying concentrations of IAA was applied to the both the petioles (Fig 3). The number of these treated petioles that abscised with time was recorded and the percentage calculated. It was noted that IAA applied to the petiole retarded abscission of treated petioles. With increasing concentration of IAA applied abscission decreased from 60% to none (Fig 21).

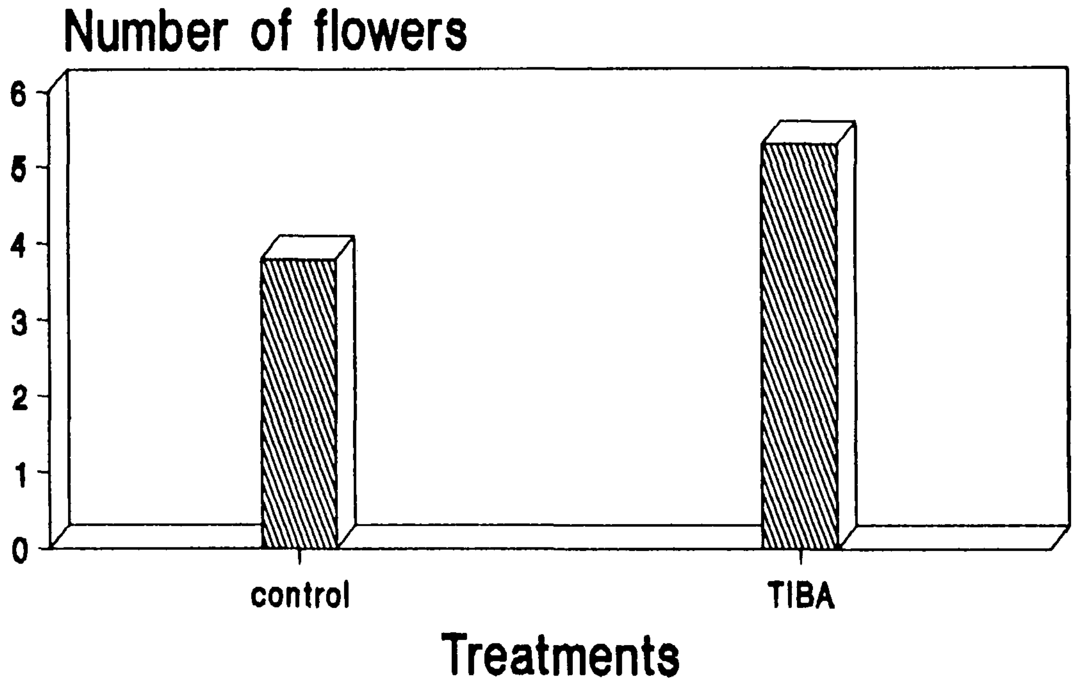


Fig 20: Effect of application of TIBA on total number of flowers produced per inflorescence of cowpea

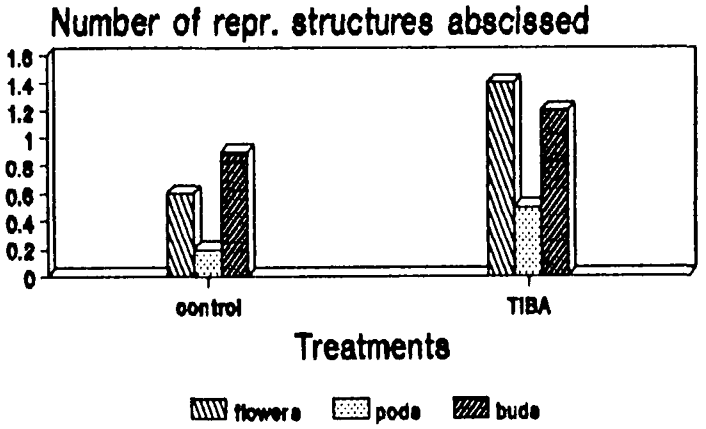


Fig 20a: Effect of application of TIBA on total no. of reproductive structures abscised per inflorescence of cowpea

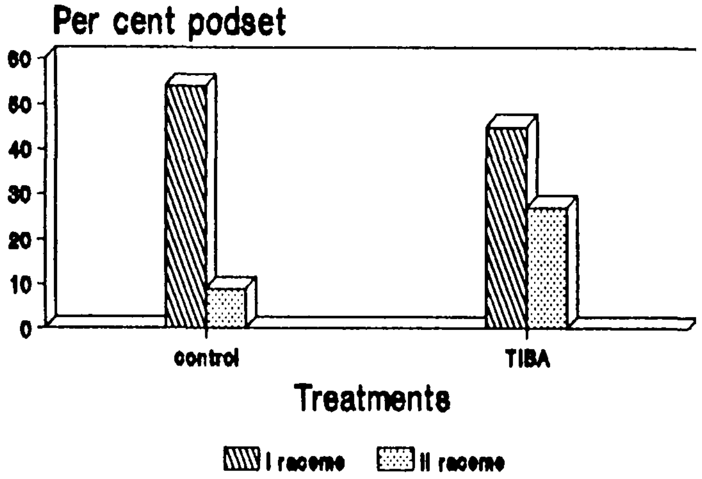


Fig 20b: Effect of TIBA application on pod set per inflorescence of cowpea

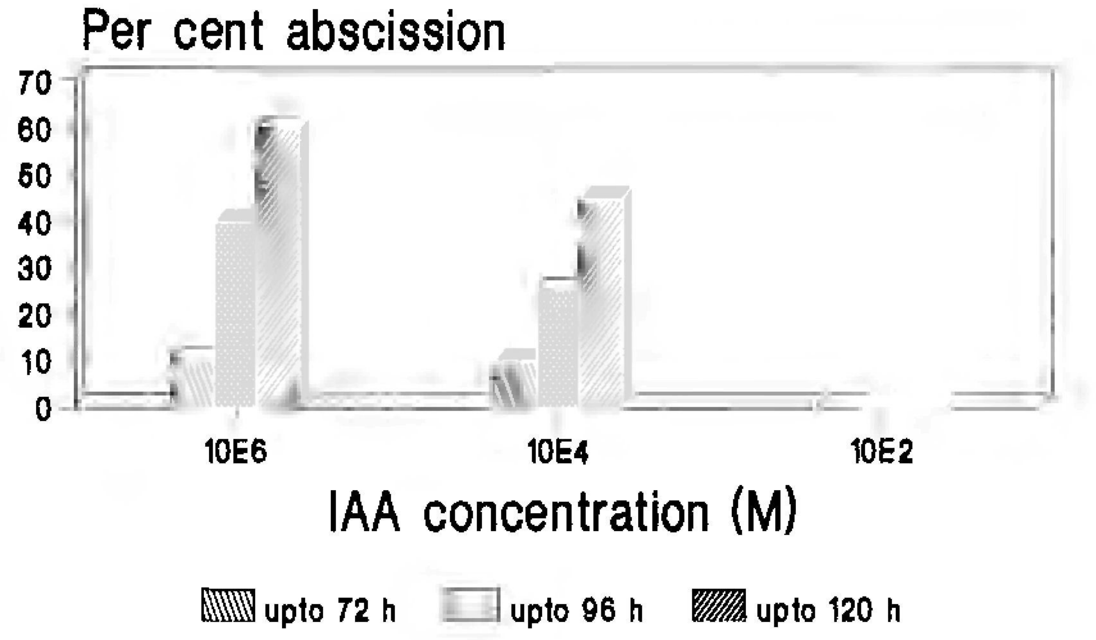


Fig 21: Effect of exogenous appln. of IAA to lateral petiole on abscission of the same

The results indicate that application of auxin to both the petioles defers abscission of these petioles.

4.2.2 Effect of application of varying concentrations of IAA to one of the lateral petioles on the abscission of the other petiole

With an objective to study the influence of various concentrations of IAA on abscission of debladed petioles 3 experiments were conducted. Cotton petiole explants were prepared as described in materials and methods. IAA was applied to one of the 2 lateral petioles in the explant and the number of untreated petioles that abscised at different intervals of time was studied (Fig 4). With time the per cent abscission increased in all the treatments. Increasing IAA concentration resulted in increased per cent abscission of petioles at the end of 48 h. The percent abscission of petioles increased from 20 - 80% when IAA concentration increased from 10^{-8}M to 10^{-2}M (Fig 22,22a,22b).

Although the number of petioles abscised was less for 24-48 hour period, the percent of petioles that abscised increased with increasing concentration of IAA. Similar results were obtained in the second and third experiment.

The results of these three experiments clearly indicates that application of auxin to one of the petioles

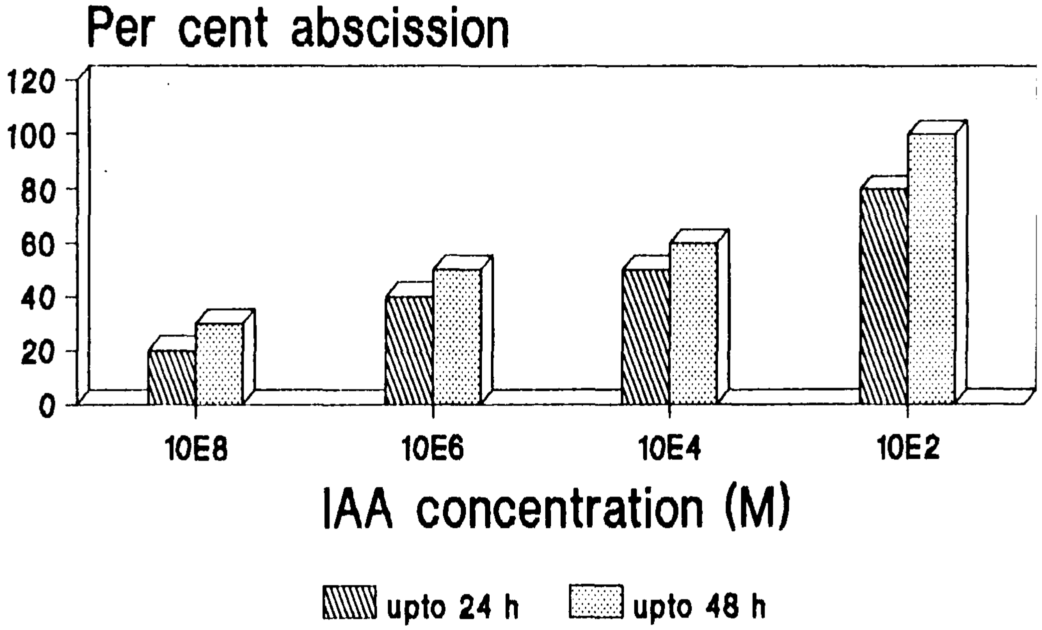


Fig 22: Effect of exogenous appln. of IAA on abscission of petioles in debladed cotton petiole explants

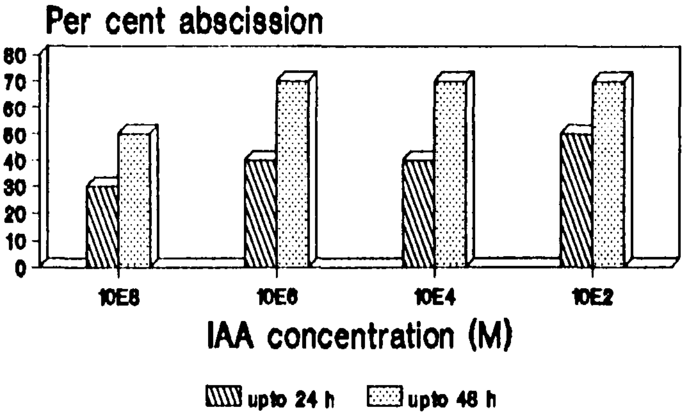


Fig 22a: Effect of exogenous appln. of IAA on abscission of petioles in debladed cotton double petiole explants

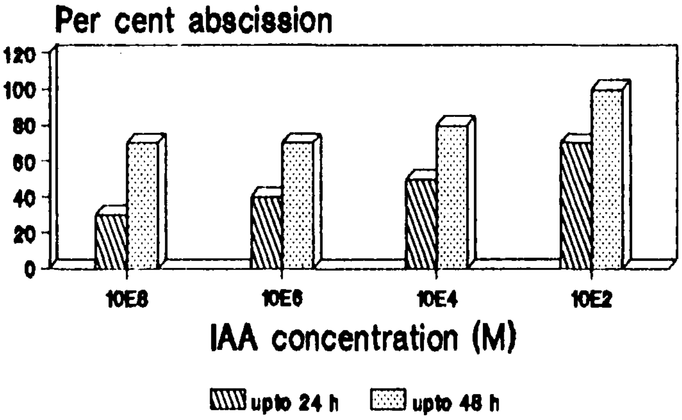


Fig 22b: Effect of exogenous appln. of IAA on abscission of petioles in debladed cotton double petiole explants

induces early abscission of the opposite untreated petiole and per cent lateral petioles abscised increases with increasing concentration of IAA applied.

4.2.3 Effect of application of varying concentrations of IAA to the cut end of the stem on the abscission of lateral petioles

In a debladed cotton explant system IAA of varying concentrations was applied to the cut end of the main stem above the two lateral petioles (Fig 5). The number of lateral petioles that abscised at different intervals of time was recorded and per cent abscission calculated. The results indicate that higher concentrations of IAA applied to the stem induced faster abscission of the lateral petioles. With increasing concentrations of IAA applied to the cut end of the petiole, the abscission per cent of the opposite petiole increased at both the times of observation. The percent abscission computed at 48 h showed an increase from 25% at 10^{-8}M to 45% at 10^{-2}M (Fig 23). Similar trend was recorded in the observations that were recorded 72 h after application of IAA. The results suggest that with increasing concentration of IAA applied to the stem there is increased abscission of the lateral petioles.

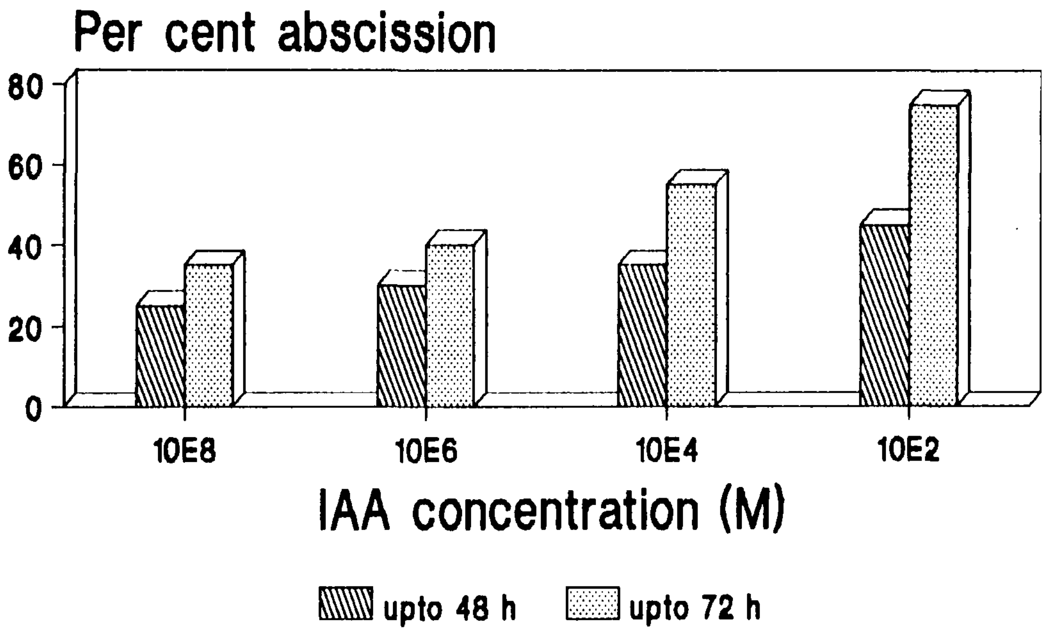


Fig 23: Effect of exogenous appln. of IAA to the cut end of the stem on abscission of lateral petioles

4.2.4 Effect of application of IAA to the terminal petiole on the abscission of lateral petioles

With an aim to study the influence of various concentrations of IAA on abscission of lateral petiole IAA was applied to a debladed apical petiole (Fig 6). Petiole explants were prepared as explained earlier . IAA of three different concentrations were applied to the apical petiole and the number of subapical petioles that abscised with time was recorded and the per cent calculated.

The results clearly indicate that with time there was increased abscission of petioles in the treatments. Percent abscission increased from **30**% at 10^{-6} M to **8**0% at 10^{-2}M IAA (Fig 24).

The results indicate that application of IAA to the terminal petiole induced faster abscission of the subapical petioles and per cent abscission increased with increasing IAA concentration.

4.2.5 Effect of application of IAA to various petioles on the abscission of lateral petioles

With an objective to study the need for translocation of IAA to induce abscission, cotton petiole explants were prepared with three debladed petioles (Fig 7). IAA was

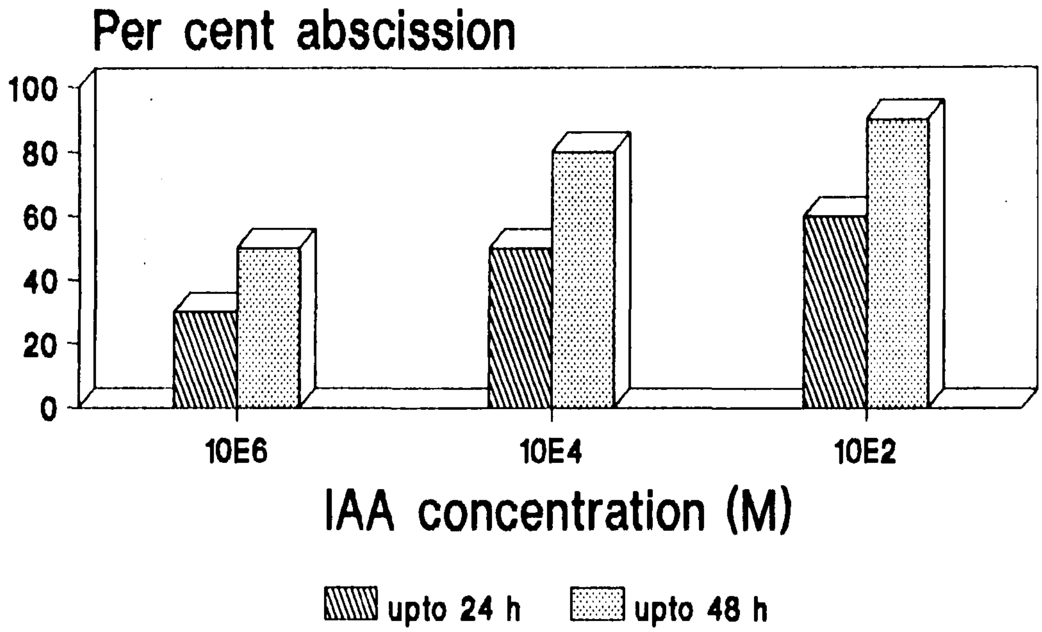


Fig 24: Effect of exogenous appln. of IAA to terminal petiole on lateral petiole abscission

applied to the terminal petiole or one of the lateral petioles or to both the subapical opposite lateral petioles in the explant. The number of subapical lateral petioles that abscised was recorded and per cent abscission was calculated. Application of IAA to the cut end of two lateral petioles did not induce abscission of these petioles upto 72h. Application of IAA to the cut end of a lateral petiole or apical petiole alone induced abscission of untreated subapical petioles. For eg., treating cut end of the apical petiole induced abscission of subapical petioles by 80%. Application of IAA to one of the lateral petioles also induced abscission of untreated petioles (Fig 25).

In explant systems application of auxins to the cut end of the apical petiole induces abscission of the two lateral petioles. These results indicate that high concentration of auxin moving from the apical region or from one of the lateral petioles induces abscission of the untreated lateral petiole.

4.2.6 Effect of application of IAA to the leaf lamina on abscission of lateral petioles

With an objective to study the need for translocation of IAA to induce abscission, an experiment was conducted in intact cotton seedlings where the terminal leaf lamina was retained and both the cotyledonary leaves were debladed

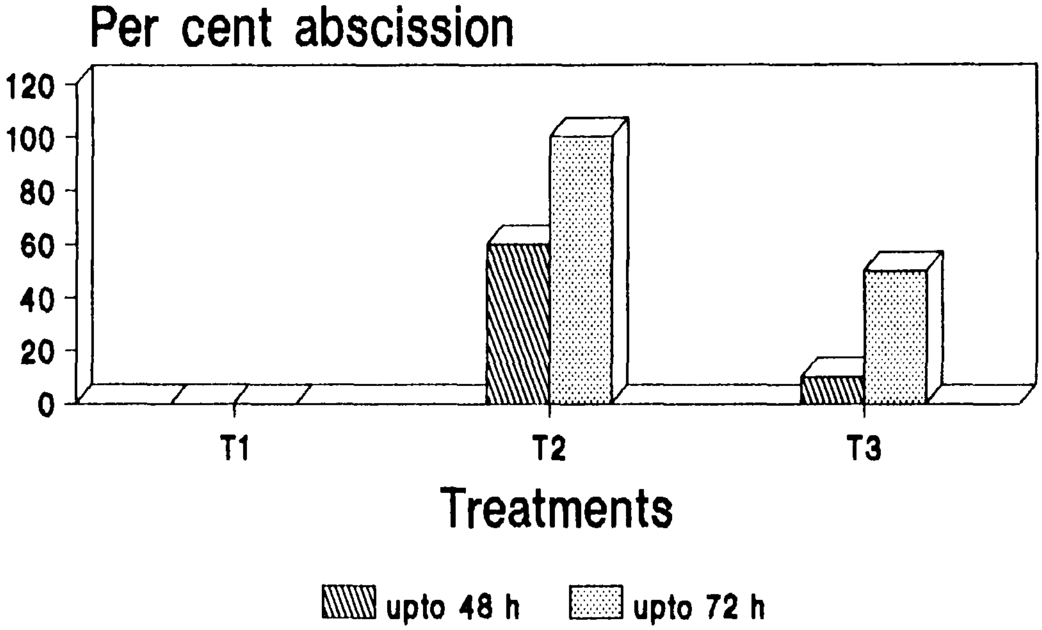


Fig 25: Effect of exogenous appln. of IAA on abscission of lateral petiole in debladed cotton petiole explants

(Fig.8). Varying concentrations of IAA was applied to the leaf lamina and the number of subapical petioles that abscised with time was noted and the per cent abscission was calculated.

The results indicate that higher concentration of IAA applied to the leaf lamina induced faster abscission of petioles. Within 48h, 70% of petioles had abscised at the highest concentration of IAA (10^{-2}M) used. The per cent abscission increased with increasing concentration of IAA (Fig 26)

The results suggest that higher concentrations of IAA moving basipetally from the leaf lamina may induce faster abscission of the subapical petioles.

4.2.7 Effect of application of TIBA on IAA induced abscission

With an objective to study the need for translocation of auxin in inducing abscission of petioles TIBA (an auxin transport inhibitor) was used. The explants were prepared as explained in materials and methods (Fig 9). To one of the lateral petioles only IAA was applied or only TIBA was applied. In another treatment both IAA and TIBA were applied with TIBA being applied as a ring around the petiole six hours prior to application of IAA. The time taken for abscission of the untreated opposite petiole was recorded.

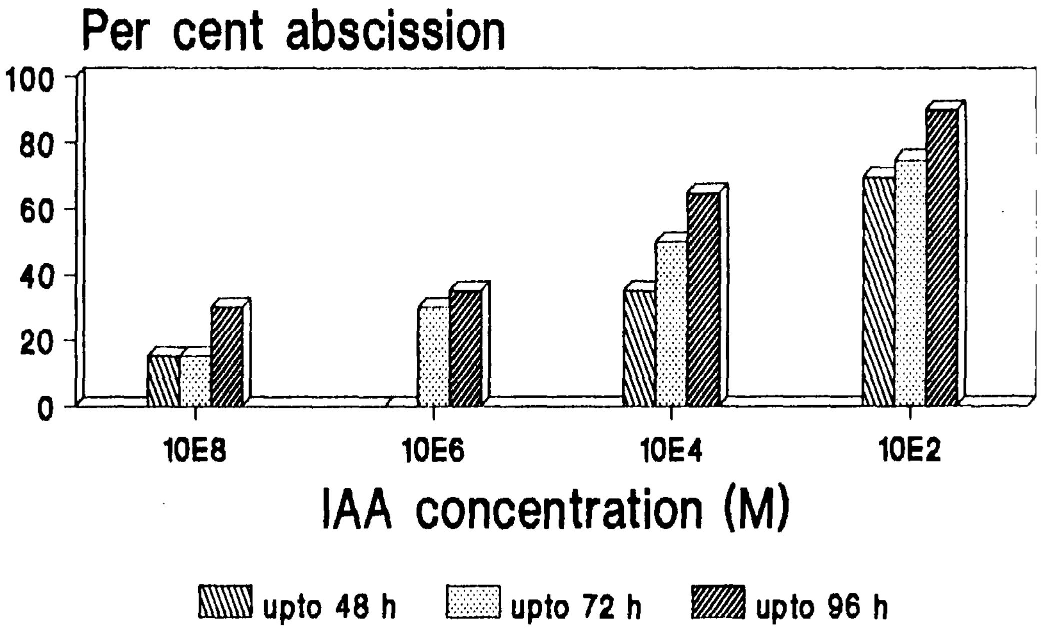


Fig 26: Effect of exogenous appln. of IAA to the terminal leaf on lateral petiole abscission

The results depict that when IAA was applied alone the percent abscission of petioles was very high (70%) (Fig 27) Inhibiting transport of IAA by applying TIBA to the basal part of the petiole reduced abscission of untreated petiole. This clearly indicates that the auxin which would have otherwise moved down and induced abscission of the subapical petioles was restricted due to the presence of TIBA which blocked IAA transport. These results suggest that the auxin that is moving basipetally is responsible for the abscission of the untreated petiole.

4.3 Influence of auxin on the maintenance of apical dominance

Experiment I

An experiment was conducted in cotton plants to study the role of auxin in apical dominance. Cotton petiole explants were prepared as described earlier. Varying concentrations of IAA was applied to both the petioles (Fig 10). The number of buds that sprouted from the axil of these petioles was counted and percent initiation was calculated. This system is comparable to an intact system wherein auxin moving from the apex downwards was shown to inhibit bud outgrowth.

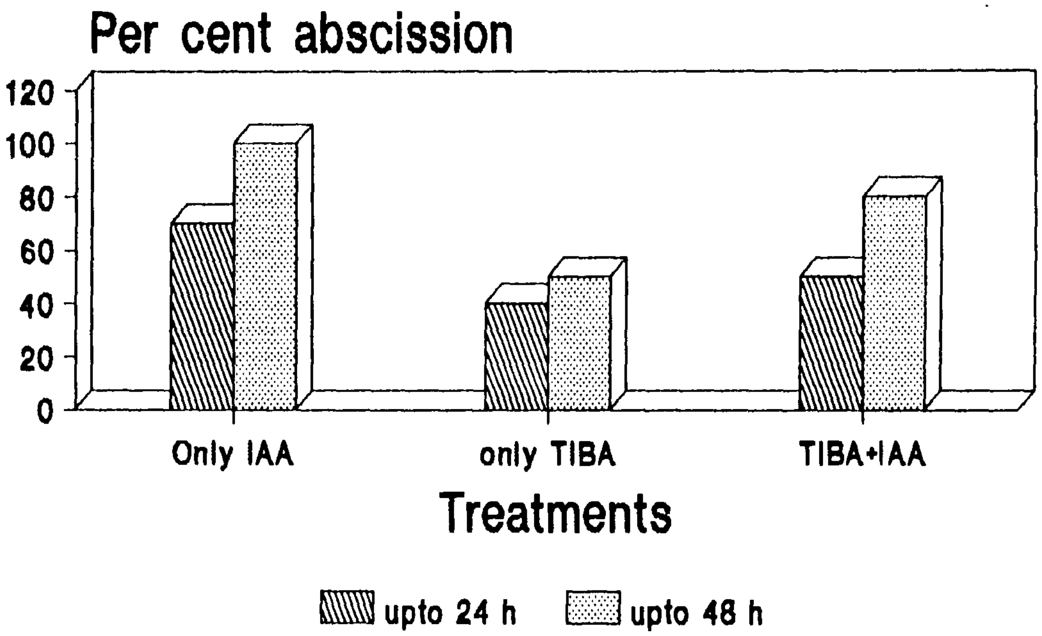


Fig 27: Effect of exogenous appln. of IAA and TIBA on lateral petiole abscission

It is evident from the results that higher concentration of IAA applied to the sub apical petioles prevents outgrowth of buds located in the axil of the petioles. With increasing IAA concentration percent bud inhibition decreased from 50% to 40% (Fig 28). This clearly suggests that a high concentration of auxin applied to the petioles inhibits bud outgrowth from the axil of the petioles.

In the same cotton explant system the extent of growth of the lateral bud as affected by IAA concentrations was studied (Fig 10). Varying concentrations of IAA were applied to both the lateral petioles and the bud growth was measured on the first day and on the final day of observation. The difference between the two gave the extent of growth of the bud over a period of time.

The results point out that at higher concentration of IAA applied, bud growth is restricted. A marginal reduction in bud growth was observed with every increment in IAA concentration (Fig 29).

Experiment II

In the debladed cotton petiole explant system with three petioles IAA of varying concentrations was applied to the terminal petiole and the number of axils of the debladed lateral and terminal petioles from which buds initiated were counted (Fig 6).

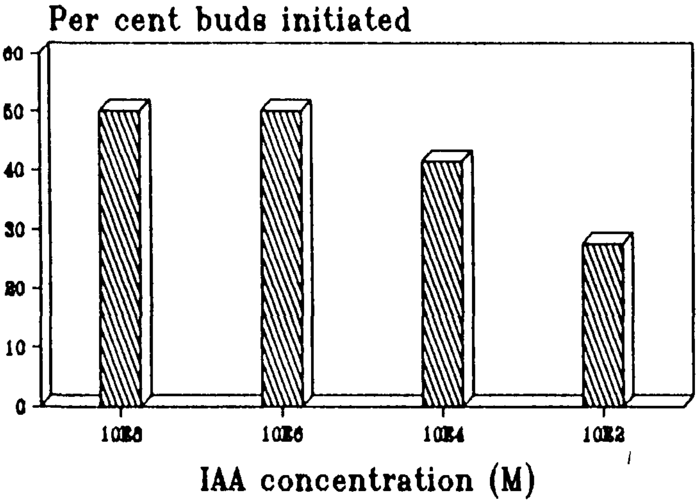


Fig 28:Effect of exogenous application of IAA on initiation of lateral buds

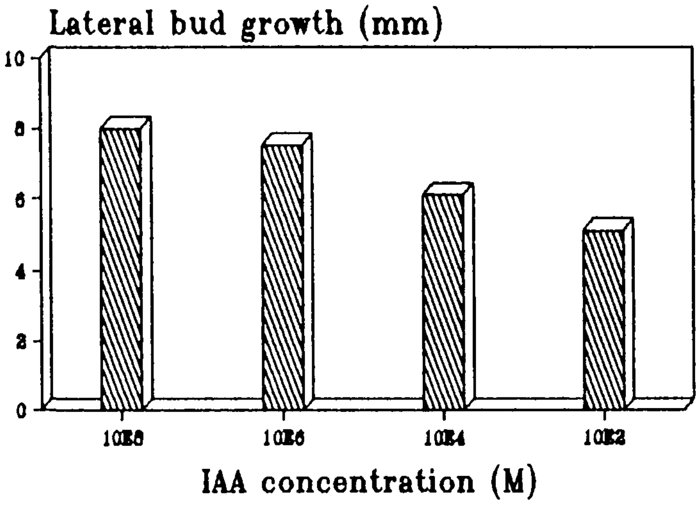


Fig 29: Effect of exogenous appln. of IAA on growth of lateral buds

It was noted that with increasing concentration of IAA applied the number of axils from which buds initiated decreased. Lateral bud initiation decreased from 70% to 25% as the concentration of applied IAA increased from 10^{-4}M to 10^{-2}M. The apical buds were also inhibited at higher concentrations of IAA. Bud initiation decreased from 70% to 40% as IAA concentration increased (Fig 30).

Experiment III

In the intact cotton seedling system the percent bud initiation was followed, when varying concentrations of IAA was applied to the leaf lamina (Fig 13). The objective was to study the need for auxin in maintenance of apical dominance. The number of lateral buds that sprouted was counted and the per cent initiation was calculated.

The results obtained indicate that at higher auxin concentration bud outgrowth was restricted. At highest concentration of IAA used the percent of buds that initiated was only 40% as compared to 70% when lowest concentration of IAA was applied (Fig 31).

These results suggest that the auxin exogenously applied is moving downward basipetally and inhibiting the growth of the axillary buds.

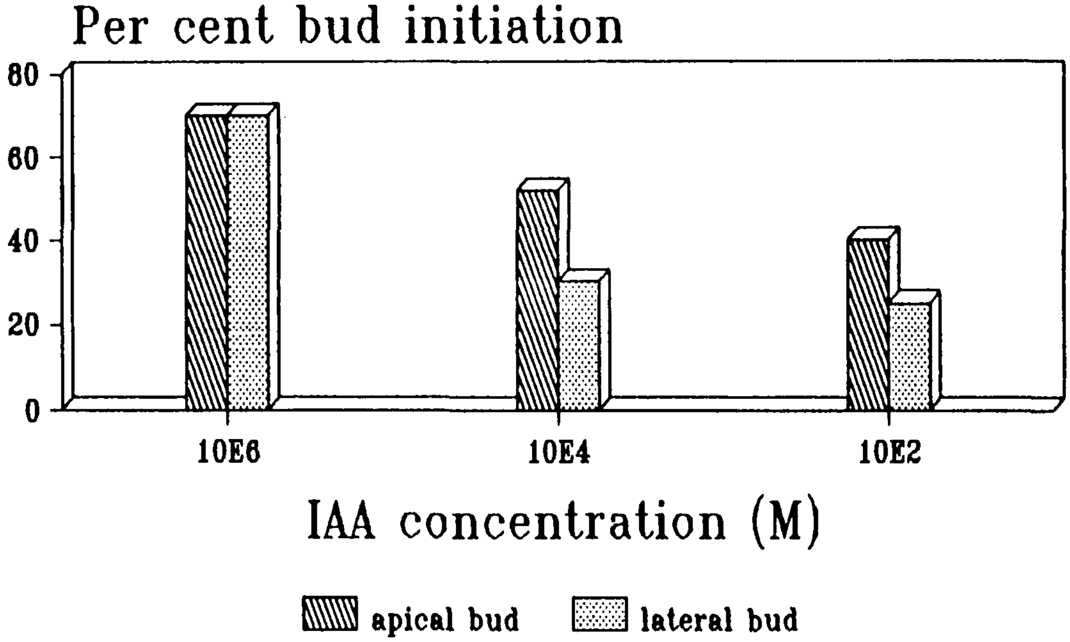


Fig 30:Effect of exogenous application of IAA to terminal petiole on apical and lateral bud initiation

In the intact system (Fig 13) the lateral bud growth from the axil of the two lateral petioles was also monitored over a period of 15 days. The increase in length of the bud from the day of initiation to the day of final observation was measured.

The results clearly indicate that there was a marked reduction in the bud growth with increasing concentration of IAA applied to the leaf lamina. The bud growth was 6.7mm at a concentration of 10^{-2}M as compared to 14mm at 10^{-8}M IAA (Fig. 32).

Further, in the intact system the initiation and growth of the apical bud in the axil of the first leaf was studied (Fig 13). The growth of the bud was measured both on the first day of observation and on the final day of observation. The difference of these two gives the extent of bud growth during that period.

The results observed are similar to the one obtained for lateral bud initiation and growth. Bud initiation decreased from 80% to 20% as the concentration increased from 10^{-8}M to 10^{-2}M (Fig 33). A marked reduction in the growth of the apical buds was observed. From about 7.9 mm in 10^{-8}M IAA the bud growth decreased to 3 mm at 10^{-2}M IAA (Fig. 34).

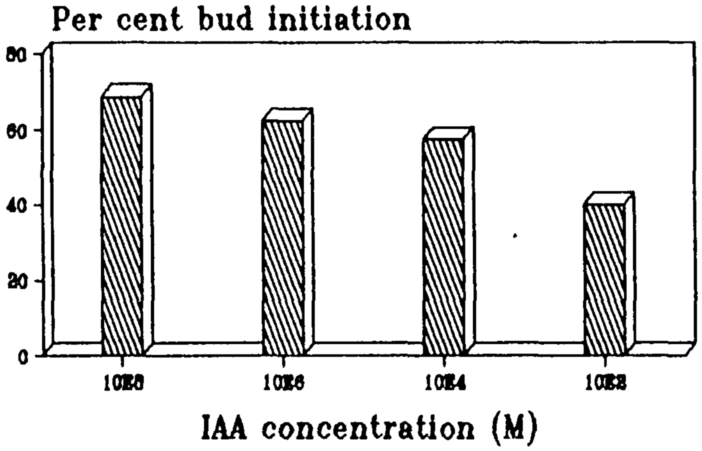


Fig 31:Effect of exogenous application of IAA to terminal leaf on lateral bud initiation

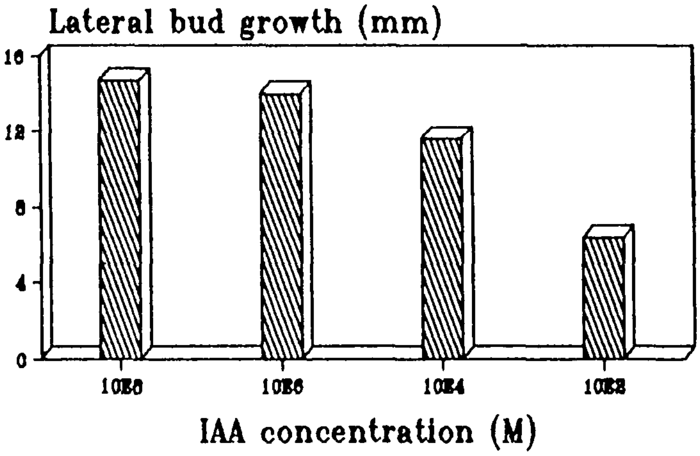


Fig 32:Effect of exogenous application of IAA to terminal leaf on growth of lateral buds

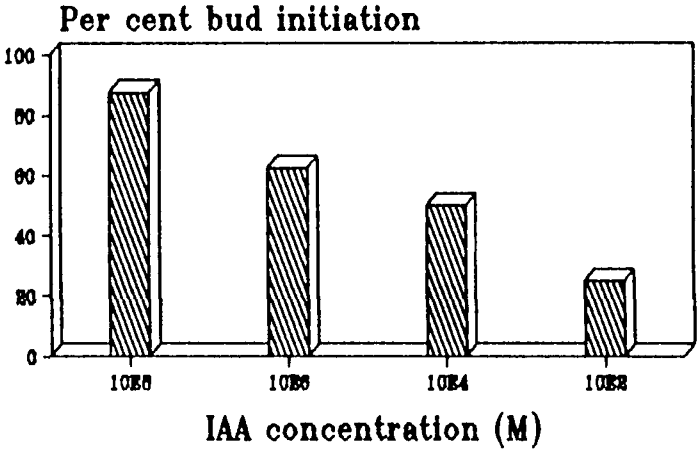


Fig 33:Effect of exogenous application of IAA to terminal leaf on apical bud initiation

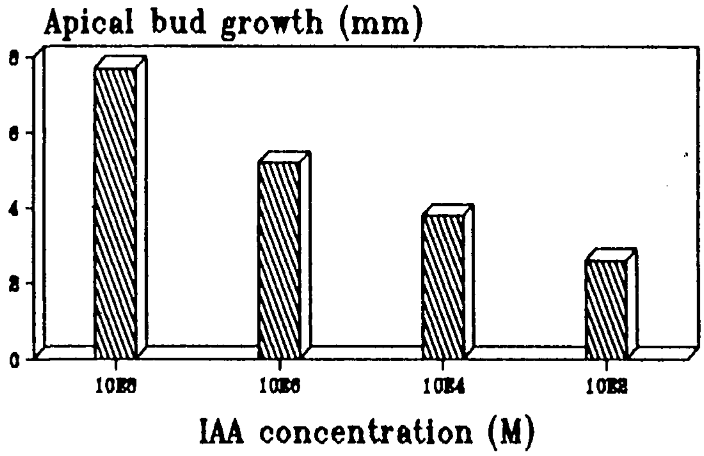


Fig 34:Effect of exogenous application of IAA to terminal leaf on growth of apical bud

In the intact system (Fig 13) the effect of IAA on the growth of the internode was also assessed. The internodal length increased from 15 mm at a concentration of 10^{-8}M to 27 mm at 10^{-2}M IAA (Fig 35).

The results indicate that IAA induces elongation of internode although it inhibits growth of buds.

Effect of application of a combination of hormones in the phenomenon of apical dominance

Using Mestha and Cowpea as test crops a pot culture experiment was conducted to study the influence of application of IAA, TIBA or IAA+BA on apical dominance. Hormone application starting from 10 days after germination was given continuously for 30 days to the apex, twice a day. Observations on plant height, leaf area and the number of lateral buds produced per plant were recorded.

The results obtained on plant height indicate that IAA application increased plant height. Plants treated with TIBA showed a marked reduction in plant height, in both Mestha and Cowpea (Fig 36,37).

The leaf area was also calculated. In Mestha it was noted that a combination of (IAA 10^{-4}M + BA 0.2ppm), increased the leaf area much more than that of either control or only IAA applied plants. In case of Cowpea the

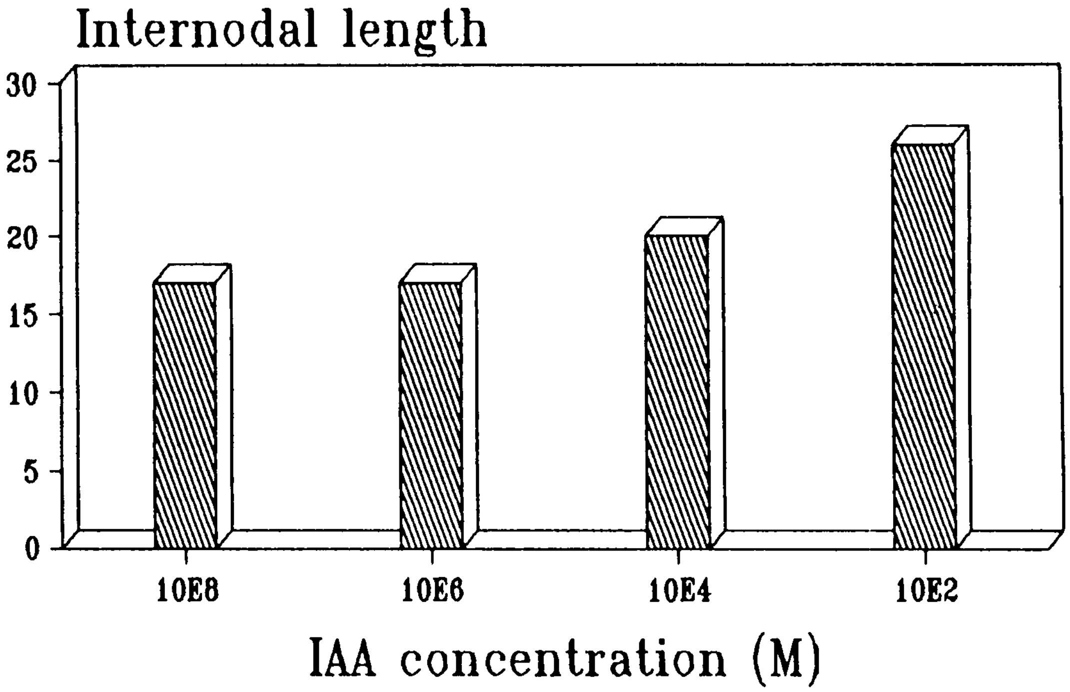


Fig 35:Effect of exogenous application of IAA to terminal leaf on internodal length

leaf area did not increase with application of IAA 10^{-4}M + BA 0.2ppm. Plants treated with only IAA showed an increase in leaf area. In both Mestha and Cowpea, TIBA treated plants showed a considerable reduction on leaf area when compared to other treatments (Fig 36a, 37a).

Plants treated with only IAA or IAA+BA or in untreated plants there was no branching. Plants treated with TIBA showed profuse branching both in Cowpea as well as in Mestha. These results clearly indicate the role of auxin in maintaining apical dominance, by inhibiting lateral bud growth (Fig 36b,37b).

4.4 Estimation of endogenous concentration of IAA in ovaries of cowpea

The endogenous IAA concentration of ovaries at various racemes was estimated using HPLC. On the day of anthesis the ovaries were collected and after extraction and partial purification the concentration of IAA was estimated.

The results obtained indicate that the weight of the ovaries from raceme I was higher than that of those at raceme II. On an average each ovary at raceme I weighed about 10.3 mg, while those at raceme II weighed about 8.6 mg Though the weight of the ovary was different the IAA concentration did not differ. The IAA concentration of

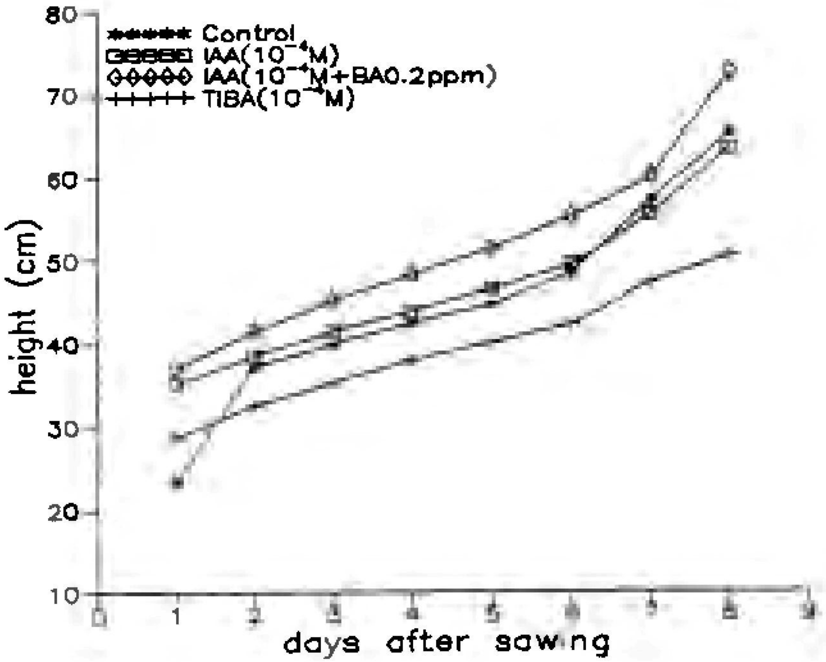


Fig 36: Effect of application af harmanes on the plant height of Mestha

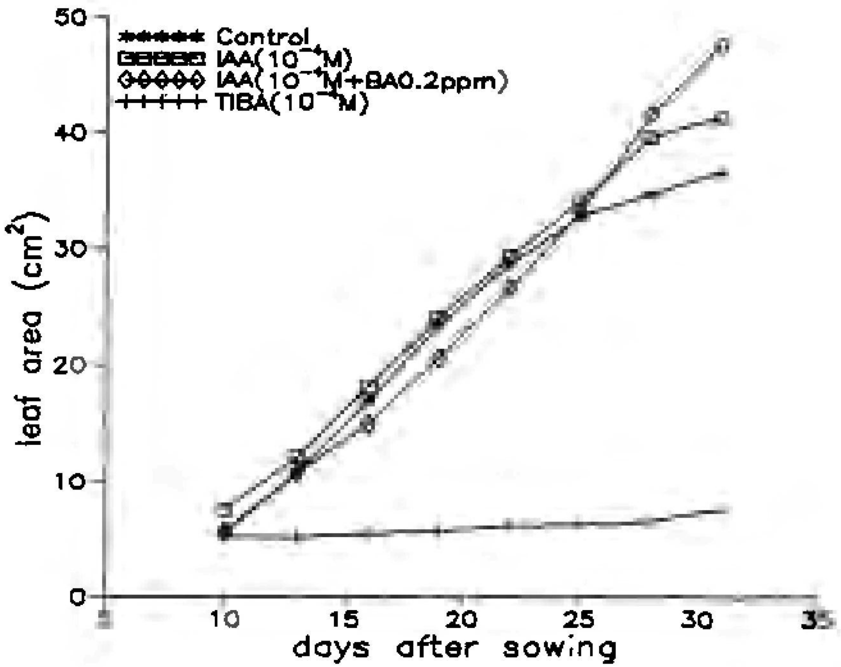


Fig 36a:Effect af application of hormones on the leaf area of Mestha

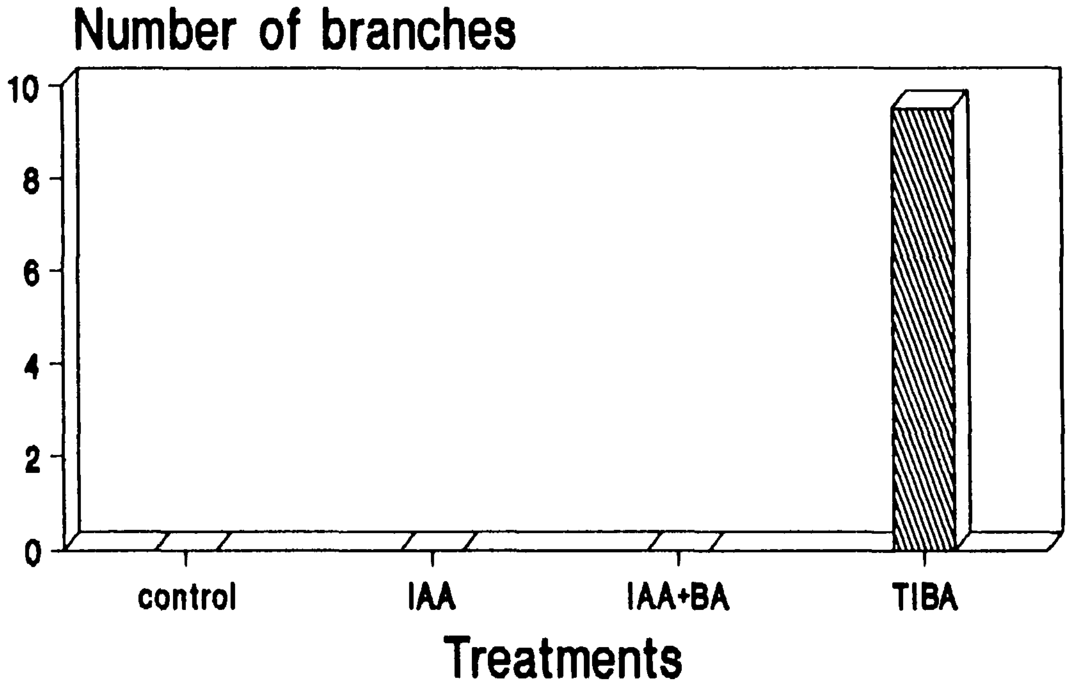


Fig 36b:Effect of exogenous application of hormones on the number of branches produced in mestha

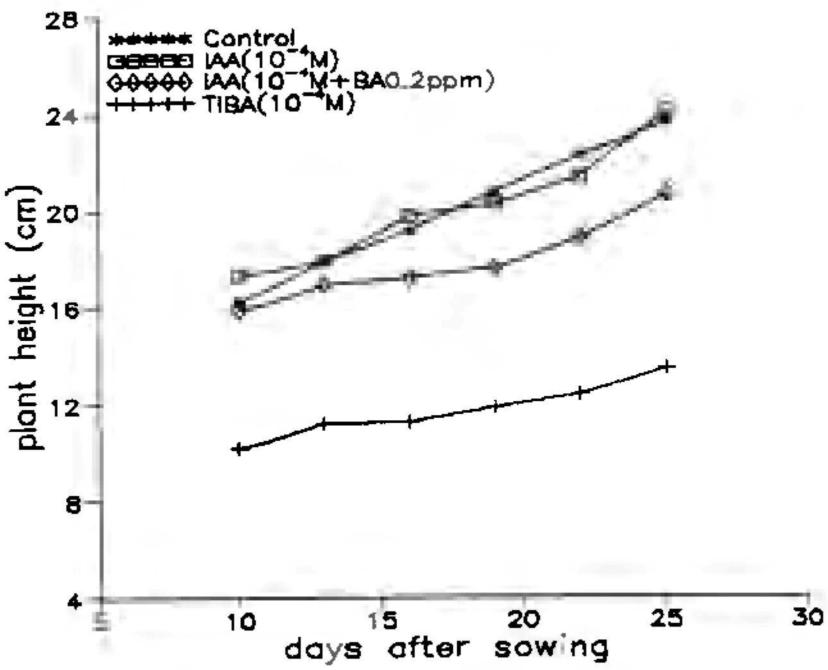


Fig 37: Effect of application of hormones on the plant height of Cowpea

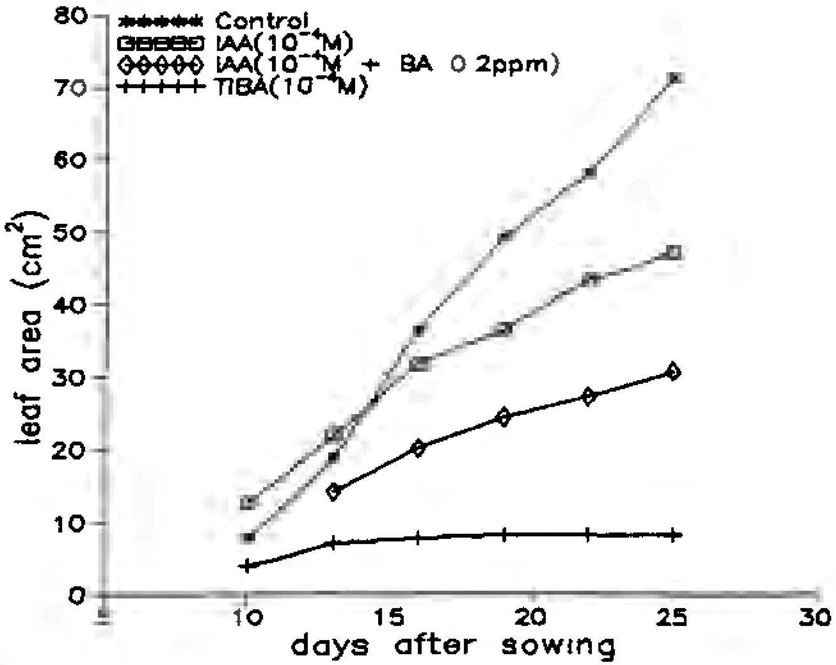


Fig 37a: Effect of application of hormones on the leaf area of Cowpea

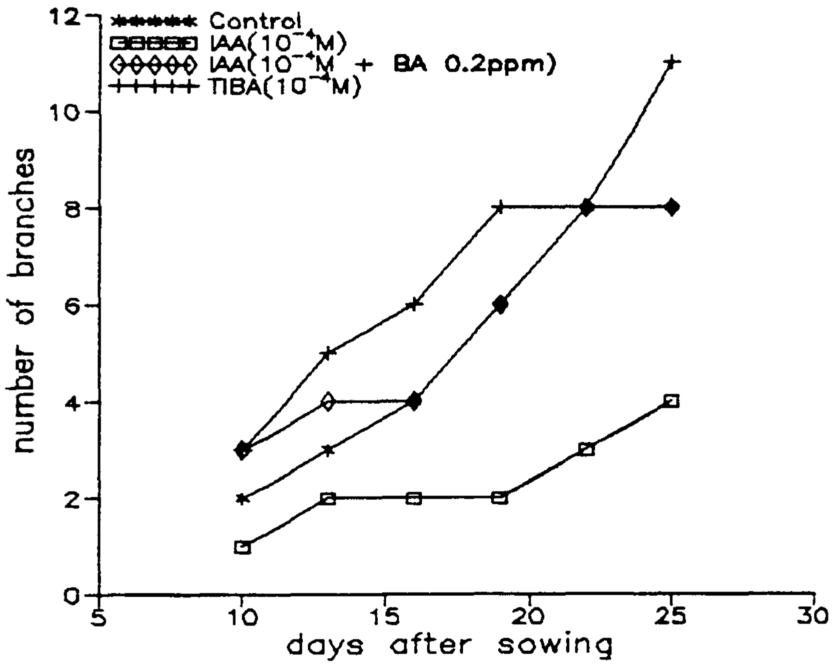


Fig 37b: Effect of application of hormones on the number of branches in Cowpea

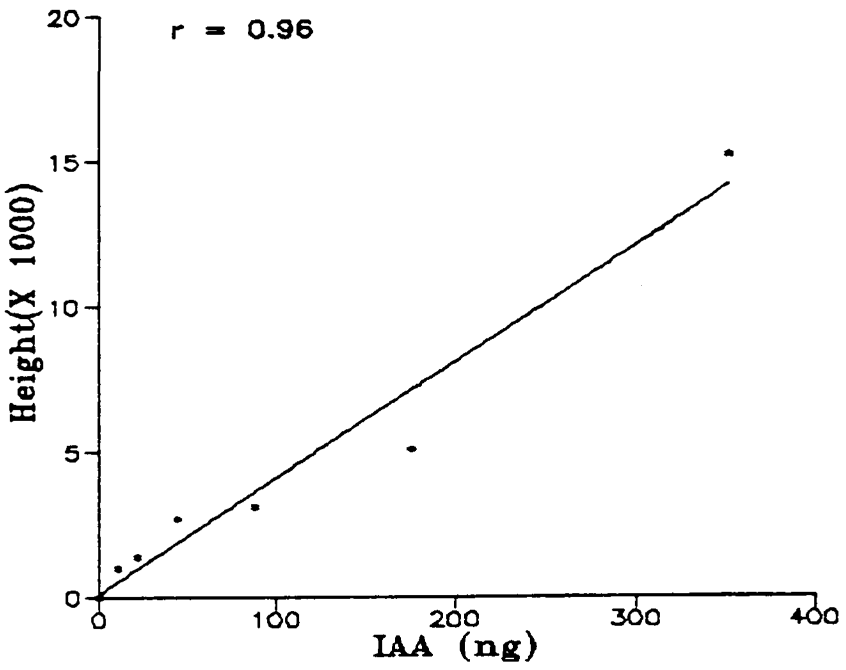


Fig 38: Standard curve for IAA

ovaries were 740 ng and 724 ng at raceme I and raceme II respectively (Table 2). Though there is a difference in weight of the ovaries this difference does not contribute to the auxin concentration.

4.5 Influence of IAA on assimilate transport and establishment of dominant sink

At early stages of development of a sink increased auxin production and transport downward may inhibit auxin transport from the competitive sinks thus establishing early dominance of the sink. The increased IAA content and transport may in turn affect assimilate transport which then may favour dominant organ in further growth and development. This hypothesis was tested in a series of experiments in the present investigation. Studies were conducted in which mobilization and accumulation of ^{14}C metabolites between competitive sinks as influenced by exogenous application of IAA to one or two of the sinks were determined.

4.5.1 Effect of IAA on the uptake of radiolabelled Sucrose

In cotton petiole explant system the influence of application of IAA to one of the two lateral petioles or to both the lateral petioles on uptake and translocation of ^{14}C -Sucrose given through the cut end of the morphological base of the explant was studied (Fig 39).

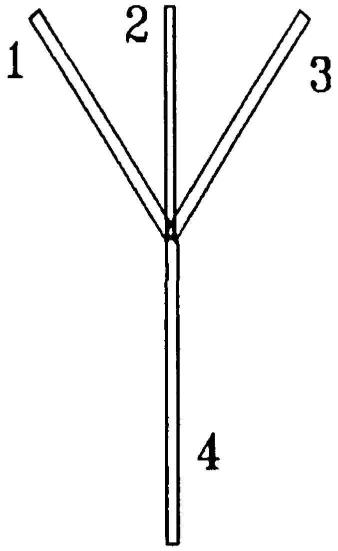
Table 2: Ovary weight and IAA concentration in ovaries collected from different racemes in inflorescences of cowpea

Nodal position	weight/ovary (mg)	IAA conc/ovary (ng)
I	10.3	740.3
II	8.6	724.6

In all the treatments large concentrations of radioactive Sucrose was retained in the basal portion of the explant. IAA application increased the total amount of radioactivity recovered in the explant. The petiole treated with IAA had more radioactivity compared to untreated petiole. At the end of 24 h of experimental period in the treatments in which only one petiole was treated with IAA, the petiole had 6.8% of total ^{14}C activity recovered in the explant compared to 1.16% by the untreated petiole. When both the petioles were treated with IAA they had 11.4% and 16.2% of total activity (Table 3). Similarly, when the stem section above the lateral petiole was treated with IAA, it had 7.2 percent of the activity and the activity that moved to the lateral petiole was less to an extent of 2.5 and 3.5%. These results indicated that application of IAA to any part of the explant increases the mobilization of Sucrose to that part.

4.5.2 Influence of IAA on mobilisation of metabolites

Cotton seedlings were exposed to $^{14}CO_2$ for half an hour. Twenty four hours after feeding $^{14}CO_2$ cotton petiole explants were prepared from the seedlings (Fig 39). These explants will now have ^{14}C metabolites almost evenly distributed in both the petioles. Then for one or both the



T1: IAA applied to petiole 1

T2: IAA applied to petioles 1 & 3

T3: IAA applied to petiole 2

T4: control

Fig 39: Cotton explant showing treatment details

Table 3 : Effect of IAA application on uptake and distribution of ^{14}C Sucrose

Treatment Sample	T_1	T_2	T_3	T_4
1	618.4 *(6.8)*	603.9 *(11.4)*	201.5 *(2.5)*	285.6 *(3.3)*
2	237.0 *(2.6)*	218.3 *(4.1)*	572.0 *(7.2)*	409.2 *(4.7)*
3	105.4 *(1.16)*	856.1 *(16.2)*	279.5 *(3.5)*	275.8 *(3.2)*
4	8085.3 *(89.3)*	3582.6 *(68.1)*	6860.6 *(86.6)*	7713.2 *(88.8)*
Total	9046.1	5260.9	7913.6	8683.8

Note: Italised values in parenthesis represent per cent radioactivity of the total.

petioles IAA was applied and 24h after application of IAA the distribution of ^{14}C activity in different parts of the explant were assessed.

Application of IAA to only one of the petiole increased the total amount of radioactivity recovered in that petiole compared to the untreated petiole. The percent radioactivity recovered in IAA treated and untreated petioles were 18.7 and 11.6% respectively. Application of IAA to both the lateral petioles mobilized ^{14}C activity from the basal portion of the explant and the percent activity recovered was 16.9 and 18.4 in the two petioles. IAA applied to the cut end of the stem above the two lateral petioles also increased the ^{14}C activity recovered in that part (Table 4).

In untreated explants major amount of ^{14}C activity was retained in the basal portion of the explant which contributes to 62.6% of the total activity recovered from the explant. In all the other treatments the basal portion of the petiole explant had relatively less ^{14}C activity indicating that application of IAA mobilizes the metabolites to the part where its concentration is maintained high.

Table 4 : Effect of IAA application distribution on ^{14}C activity in different petioles in cotton explants

Treatment Sample	T_1	T_2	T_3	T_4
1	353.95 *(18.7)*	334.90 *(17.7)*	215.40 *(11.8)*	261.9 *(12.6)*
2	234.10 *(12.3)*	295.70 *(15.7)*	490.44 *(26.8)*	388.2 *(18.7)*
3	219.60 *(11.6)*	364.72 *(19.3)*	212.28 *(11.6)*	241.24 *(11.6)*
4	1084.12 *(57.3)*	888.02 *(47.1)*	909.51 *(49.7)*	1179.8 *(56.9)*
Total	1891.8	1883.3	1827.6	2071.1

Note: Italised values in parenthesis indicate the per cent radioactivity of the total

4.5.3 Influence of IAA on mobilization of radiolabelled Sucrose to different sinks in a inflorescence of cowpea

In a inflorescence of cowpea only the first flowers produced in the first raceme develop into mature pods. All the other reproductive units abscise as young pods, flowers or buds. The young developing pods may produce and transport high concentration of auxin thus diverting nutrients for their development. In this experiment, the influence of an auxin transport inhibitor on translocation of Sucrose to the developing sinks at different racemes in a inflorescence was studied.

The cut end of the peduncle of an excised inflorescence was kept in ^{14}C Sucrose solution for 24 h and the ^{14}C activity of the sinks borne at different racemes in a inflorescence was assessed (Fig 40).

At the end of 24h experimental period the amount of ^{14}C activity recovered was maximum in the inflorescence in which the developing pods were treated with IAA. Relatively high activity was observed in the pods treated with TIBA. The amount of radioactivity recovered in the reproductive units in all the racemes was 644.6 in untreated inflorescences compared to 1342.7 in TIBA treated and 730.1 in IAA treatment (Table 5).

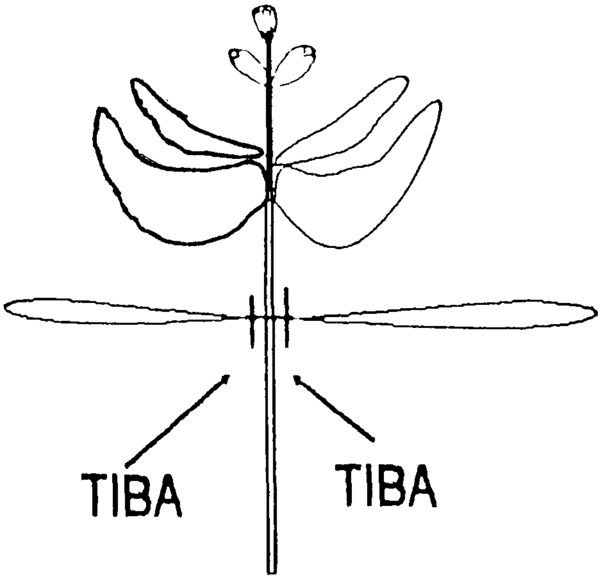


Table 5: Effect of application of IAA and TIBA on uptake of 14 C Sucrose by different racemes within an inflorescence of cowpea

Treatments	**Ist raceme**	**2nd/3rd onwards + apex**	**Peduncle**	**Total**
Control	160.8 *(5.4)*	483.8 *(16.3)*	2316.5 *(78.2)*	**2961.1**
TIBA	184.4 *(5.4)*	1158.4 *(34.4)*	2026.1 *(60.1)*	**3368.8**
IAA + BA	198.1 *(5.5)*	532.05 *(14.8)*	2842.7 *(79.5)*	**3572.8**

Note: Italised values in parenthesis represent the percent radioactivity of the total

Application of TIBA to the pedicel of the developing pods with an objective to reduce the IAA transport from the developing pods increased the amount of radioactivity recovered in the reproductive units above raceme I. The increase in radioactivity in the reproductive units was primarily due to significant reduction in the activity in the peduncle of the inflorescence.

The percent distribution of total radioactivity recovered at different parts of the inflorescence indicates that the racemes above the first received only 16.3% of ^{14}C Sucrose in untreated inflorescences compared to 34.4% in the treatments where TIBA was applied to the pedicel of pods and 14.8% in the treatment in which IAA was applied to the developing pods.

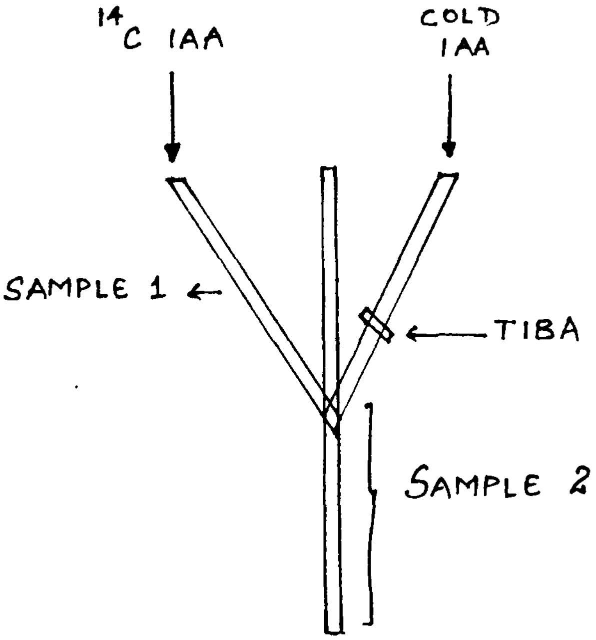
These results reveal that inhibition of IAA transport out of the developing pods in the first raceme results in increased translocation of assimilate to the young developing reproductive units in the upper racemes.

4.5.4 Diffusable IAA and dominance phenomena in cotton petiole explants

The relationship between the concentration of auxin diffusing from one of the petioles on the diffusion from the other was studied using ^{14}C labelled IAA. ^{14}C labelled IAA with 10^{-7}M cold IAA was applied to the cut end of one of the two lateral petioles. Six hours prior to the application high concentration of cold IAA (10^{-3}M and 10^{-5}M) was applied to the opposite petiole. The amount of radioactivity diffusing out of the petiole was determined (Fig 41).

The amount of ^{14}C -IAA that diffused out of the applied petiole was relatively low to an extent of only 5 to 10 percent. Maximum diffusion of radioactivity was observed in the set up in which the opposite was treated with water. Application of high concentration of cold IAA to the petiole reduced the diffusion of ^{14}C IAA out of the opposite petiole. With increase in concentration of IAA applied to the petiole the amount of radioactive IAA moving out of the opposite petiole was inhibited to a greater extent. In another set up, TIBA an inhibitor of auxin transport was applied to the petiole with an objective to inhibit the basipetal transport of applied cold IAA and the transport of ^{14}C IAA from the opposite petiole was determined. Application of TIBA resulted in movement of more ^{14}C IAA from the opposite petiole (Table 6).

This indicated that high concentration of IAA coming from one competitive dominant sink inhibits the basipetal transport of IAA from the other non dominant sink.



TREATMENTS

T_1 : CONTROL

T_2 : 10^{-5} M IAA

T_3 : 10^{-3} M IAA

T_4 : 10^{-5} M IAA
+ 10^{-4} M TIBA

FIG 41: COTTON PETIOLE EXPLANT USED FOR RADIOACTIVE IAA TRANSLOCATION STUDIES

Table 6: Translocation of radiolabelled IAA in the presence / absence of dominant sink

Treatment	T_1	T_2	T_3	T_4
Sample				
1	2990.6 *(90.9)*	3005.2 *(95.06)*	3014.5 *(95.8)*	2953.1 *(91.8)*
2	299.39 *(9.1)*	156 *(4.94)*	129 *(4.2)*	262.2 *(8.2)*
Total	**3290**	**3161.2**	**3143.5**	**3215.1**

Index: Values in italics represent the per cent values

Inhibition of transport of IAA from the competitive dominant sink induces high diffusion of IAA from the non dominant sink.

DISCUSSION

V. DISCUSSION

Hormones are the chemical messengers which aid in intercellular communication. Hormones bring about specific formative effects in a plant.

Auxin, the first hormone to be discovered is known to regulate various plant processes. Auxin plays a role in initiation of rooting from cuttings, maintenance of apical dominance, cell elongation, leaf expansion, regulation of abscission and fruit growth.

The influence of auxin in these aspects has been studied extensively and there are a large number of reports giving evidences for the role of auxin in the phenomenon of abscission and apical dominance.

Auxin gradient between the proximal and distal regions is the probable cause for abscission (Addicott and Lyon). Since, auxin is important for the growth of an organ large concentrations of auxin have to be produced. This auxin that is synthesised has to move out of the organ for development of the organ.

The recently proposed hypothesis of "Primigenic Dominance" looks to auxin induced abscission in a new direction. The major emphasis is that auxin synthesised by the dominant organ moves basipetally, accumulating at a junction, the node from which the dominant sink develops. The high auxin concentration at the junction is supra optimal and hence the movement of auxin from the subordinate organ is restricted, ultimately leading to its abscission (Bangerth, 1989).

To test this hypothesis, and to further look into the role of auxin in the phenomenon of abscission and apical dominance the present investigation was conducted with the following objectives:

1. To study the role of auxin in apical dominance and abscission.
2. To study the influence of translocation of auxin from dominant sinks on the abscission/growth of subordinate sinks.

Pattern of flower opening and pod set in an inflorescence of cowpea

In cowpea, each fruiting point is subtended by a trifoliate leaf. The inflorescence is a raceme. Two flowers at the first raceme open first, followed by two more at the next raceme. In many cases all the reproductive units produced in the nodes above the third raceme abscise as buds.

All the flowers produced in the inflorescence do not set pods but abscise at various developmental stages. A maximum of two to three pods set in an inflorescence.

The opening of flowers and their abscission follow a regular pattern. Previous reports have shown that a relation exists between flower opening and pod set in an inflorescence (Adedipe and Omrod).

The duration required for the first flowers to open in the second raceme in various fruiting points was spread over a period of 120h (5 days) after anthesis at the first raceme. All flowers opening at the second raceme do not set into pods. Some of them abscise as flowers or as young pods. Only a small percent (1-5%) of them develop into fully mature pods.

As the time for opening of flowers between first and second raceme increases abscission of flowers at the upper racemes increases. This could be due to the presence of developing pods at the proximal position in the raceme. The developing pods in the first raceme exhibit a dominance effect on the flowers produced later in the upper raceme inducing its abscission. The abscission of buds,flowers and young fruits on the upper racemes occurs during early development stage of the fruits in the basal most raceme, implying mobilisation of metabolites by the developing fruits, and/or the production of inhibitors by such dominant fruits suppresses the development and abscission of young fruits and flowers (Adedipe and omrod, 1975; Prasad, 1986; Van Steivenick, 1971).

Ovaries are the sites of synthesis of auxin. Ovaries at the first raceme develop first and the auxin synthesised moves basipetally. This auxin moving downward probably accumulates at a junction. The concentration of auxin in the junction increases and this increased IAA inhibits basipetal movement of auxin from reproductive units developing at the upper racemes. This is because the pods at the first raceme exhibits a higher degree of dominance on these flowers which open very late at the second raceme. Hence, these flowers abscise early.

Since auxin can move only basipetally and not acropetally this theory of inhibition/abscission of flowers at the upper racemes is most probable.

Influence of source-sink manipulation on abscission of reproductive units and pod set

Early produced dominant sinks within an inflorescence always have an inhibitory effect over the later developing sinks by way of decreasing the assimilates going to the subordinate sinks, thus leading to their restricted development.

The effect of these dominant sinks can be manipulated. If the dominant sinks are removed there is more scope for development of the subordinate sinks. With this objective the first formed flowers in the first and/or second raceme is removed and its effect on flower production, abscission and fruit set was determined. Removal of first formed flowers in an inflorescence induced pod set in the upper racemes.

Removal of early formed flowers from the basal racemes reduced the dominance effect of the first formed flowers and competetion. As a result more number of flowers are produced in the upper racemes and fruit set occurred.

In another experiment a change in the source sink ratio was done by reducing the source size. For development of sinks, presence of source is essential. It is from the source that photosynthates are translocated to the sink. When the source size is reduced, translocation of photosynthates is less, leading to restricted development of the sink.

It was noticed that when the source was reduced by 33% or 66%, the number of flowers produced was less and the number of pod set was also less. In cowpea, each fruiting point is subtended by a trifoliate leaf. This leaf supplies photosynthates to that particular inflorescence. When the photosynthate supply is reduced there is less metabolite for the sink to develop. Hence the reproductive structures abscise leading to reduced pod set. However, again pod set occurs only in the basal racemes that too from the first flowers produced in the basal most retained raceme.

On the other hand, when the source is maintained and the sink is reduced there is more scope for better development of the sinks. Maintaining either one fruiting point or two fruiting points per plant increased the number of pods set in the fruiting. However, the pod set pattern was similar. Only few pods were set from the early formed flowers at the first and second raceme.

Three conclusions concerning the regulation of abscission of reproductive units can be derived from these simple experiments. First, fruit set at the basal raceme depresses flower production and fruit set in the upper racemes in an inflorescence. Secondly, alteration of sink to source ratio causes marginal effect on pod set and again the pod set occurs only in the lower most of the retained racemes.

Thirdly, it seems that an upper limit for fruit production within an inflorescence is set by availability of resources (source size and activity)

Effect of TIBA on the pattern of abscission of reproductive structures

The physiological causes leading to abscission of flowers and young fruits are not clear. The hypothesis put forth to explain the reasons range from simple competetion among developing sinks for resources through the production of hormones that regulate source-sink interactions and effectively divert resources away from later sinks to the production of hormones by the developing fruits that actively inhibit later developed young reproductive structures.

In this experiment the effect of inhibiting auxin transport from the developing young fruits on the basal racemes on flower development and fruit set on upper racemes was investigated by using TIBA.

Tri-iodo benzoic acid (TIBA) is a potent inhibitor of auxin transport. The role of TIBA in preventing basipetal auxin transport and the effect of this on pod set in an inflorescence was studied.

In a racemose inflorescence the proximal flowers set into pods while the more distal flowers would abscise. This is attributed to the basipetal movement of auxin from these pods which inhibits the flowers at the distal raceme from developing. If the auxin transport from the developing pods at the first raceme could be inhibited we can expect more reproductive units to be produced in the upper racemes and to develop into pods.

Application of TIBA to pedicels of young developing pods induced development of more number of reproductive units in the upper racemes. The flowers produced set into pods and the pod set in the second raceme was higher in TIBA treated plants.

The role of auxin in abscission of flowers developing in the upper racemes is clearly indicated. This gives evidence saying that auxin produced by the developing pods at the first raceme inhibits the flowers in the upper racemes from developing. Inhibition of auxin transport from this raceme helps in better development of flowers at the upper racemes. Hence auxin that is moving basipetally from the developing pods seems to be the dominant trigger that inhibits development of flowers at the upper racemes and this is the signal for abscission.

TIBA an inhibitor of basipetal transport of IAA reduces the auxin concentration in the transport path of photosynthates. Reducing IAA concentration in the transport path may be helping in more IAA transport from the non dominant reproductive units which may start to develop. It is also possible that continuous synthesis of IAA and its transport is essential for a sink to develop. Inhibition of IAA transport from the dominant sink by TIBA might be inhibiting faster development of these sinks resulting in the transport of photosynthates to other competent sinks developing at the upper racemes.

The role of IAA in abscission phenomena

The pattern of flower opening, abscission of flowers and fruit set follows a particular sequence in all the terminal racemose inflorescences where in, flowers open in acropetal succession. In these species the first formed flowers enjoy not only a temporal but also a spatial advantage since they are most favourably located in terms of access to the resource path. During pre-anthesis developmental stages itself the dominant effect might have set in to influence fruit set. To avoid this dominance effect, further experiments were conducted using a well defined test system-cotton petiole explants in which the two petioles which are opposite to one another do not have much dominant advantage over the other. In this test system, the influence of auxin on abscission as well as apical dominance phenomena was studied.

Effect of application of auxin on abscission of petioles in explant system

In cotton petiole explants IAA of different concentrations were applied to both the petioles or only to one of the lateral petioles arising from a single node and the time taken for abscission of the petioles was observed.

Application of IAA to both the petioles delayed abscission. With increase in the concentration of IAA applied, the abscission percent reduced and at high concentration of IAA used there was no abscission upto 72h (Fig 3).

Consequently in the next series of experiments we examined the effect of application of IAA to only one of the petioles and the extent of abscission of the untreated petiole was studied. In all the three experiments, with increasing concentration of IAA applied to one of the petiole the percent abscission of the untreated petiole increased.

From these data we can conclude that when equal concentration of IAA is applied to both the petioles IAA defers abscission process. However, when only one of the petioles was treated with IAA the other petiole abscises early and with increasing concentration of IAA there was acceleration of abscission.

In a modified cotton petiole explant system with three petioles -one apical and two lateral opposite petioles (Fig 6), the influence of application of IAA to different petioles on the abscission of untreated petioles was studied. Application of IAA to both the lateral petioles did not induce abscission of the untreated apical petiole or IAA treated petioles. However, application of IAA to only the apical petiole induced abscission of untreated lateral petioles. Here also with increase in concentration of IAA applied to the apical petiole the percent abscission of the untreated lateral petiole increased. In another experiment in three petiole explant system the apical leaf was retained and different concentrations of IAA was applied to the leaf lamina. This also induced abscission of two lateral petioles (Fig 8).

This indicates that IAA applied to the apical petiole or to the leaf moves down through the stem and may be inducing abscission of the untreated lateral petioles.

In the next series of experiments the need for transport of IAA from a treated petiole to induce abscission of untreated petioles was studied using an auxin transport inhibitor TIBA. Application of TIBA to the base of the petiole 6h prior to application of IAA to the distal end of the petiole did not induce abscission of untreated petiole. These data clearly indicate the need for transport of applied auxin from treated petiole to induce abscission of untreated petiole.

Many previous reports have shown a reduction in abscission of flowers and young fruits in IAA treated plants (Osborne, 1968; Beyer and Morgan, 1971). Many other reports indicate that application of IAA accelerates abscission (Leopold and Rubinstein, 1963; Carns, 1966; Sharma and Paliwal, 1989; Morris, 1993) particularly when IAA is applied to only few of the flowers or young pods.

The main objection to implicate the role of IAA in inducing abscission is its inability to move in acropetal direction to induce abscission of flower and fruit produced in the upper nodes in a racemose inflorescence. So ABA or ethylene were proposed to transmit the effect of the primary signal into the organ to be inhibited (Tucker, 1977; Blake et al, 1983; Hillman et al, 1985). In fruits correlatively inhibited, the fruits destined to abscise were shown to contain high concentrations of ABA (Tamas et al, 1979; Petzold et al, 1992). However, the involvement of ABA in inducing premature abscission of fruits is questioned by Bohner and Bangerth, (1988). Similarly, ABA concentrations had no relationship with pod shedding (Porter, 1977; Huff and Dybing, 1980).

Exogenous application of ethylene is shown to induce abscission of leaves, flowers and young fruits (Morgan, et al., 1977). However, no information is available to suggest a role for ethylene in transmission of dominance signal to induce abscission of young developing pods (Bangerth, 1989).

The role of IAA in apical dominance

Hormonal control of apical dominance is well established. Auxin and cytokinin were shown to play opposite role on regulation of apical dominance. Indole acetic acid synthesised in the growing apical bud was implicated in influencing lateral bud growth (Snow, 1925; Laibach, 1933; Thimann and Skoog, 1933, Sachs, 1991).

In this investigation we examined the role of IAA and its transport in control of lateral bud growth both in intact and explant system. Application of high concentration of IAA through the distal cut end of the petiole or through the decapitated end of the main stem inhibited initiation of growth of axillary buds. Although, at low concentrations of auxin the bud started elongating the extent of elongation in a given time was very low. With increase in concentration of IAA applied the extent of inhibition of bud sprout and elongation growth of the bud reduced. In intact cotton petiole system also, application of IAA to the cut end of the lateral or apical petiole or to the apical leaf, inhibited lateral bud growth even though the plants were decapitated. Similarly in cowpea and Mestha continuous application of IAA inhibited the axillary bud growth. However, when IAA was applied to the apical bud of Cowpea or Mestha seedlings, the next internode elongated more.

From these data we can conclude that:

1. High concentration of IAA moving from the dominant sinks inhibits lateral/axillary bud growth.
2. Auxin moving from a dominant sink increases the growth rate of the sink from which it is moving and also the internode below the dominant sink.
3. Inhibiting IAA transport by TIBA induces growth of lateral/axillary buds and also growth rate of these buds.

The role of auxin in dominance phenomena and in inducing abscission of developing reproductive units and apical dominance

Abscission pattern of young developing reproductive units were monitored in an inflorescence of cowpea and also in cotton petiole explant. Auxin transport from the dominant sink seems to play an important role in acceleration of abscission. Moreover, the transport of IAA from dominant sinks also seems to maintain apical dominance resulting in inhibition of axillary/lateral bud growth. Restricting auxin transport reduced the dominance effect of the dominant organ. High auxin concentration moving form the apical bud in basipetal direction was said to be supra optimal for bud growth resulting in inhibition of growth . However, the relationship between auxin concentration and inhibition of axillary/lateral bud growth is questioned. Although removal of apical bud induced growth of lateral/ axillary buds the concentration of IAA in the lateral bud did not decrease. However, in few cases an increase in auxin concentration of axillary buds were reported after decapitation (Hillman *et al*., 1977; Gocal *et al*., 1991).

The movement of IAA from the apical bud in basipetal direction to the node and from there to lateral/axillary bud in acropetal direction is also questioned (Bangerth, 1989).

Bangerth (1989) opines that IAA from dominant organs can inhibit other organs without being translocated to them and without the mediating effect of secondary messengers.

Application of auxin transport inhibitors induced growth of lateral buds in the experiments indicating the primary role of auxin in correlative control of development.

We examined the need for transport of IAA from dominant to subordinate sinks in inhibition of growth of lateral/axillary buds. In cotton petiole explant system application of TIBA induced growth of lateral buds. Similarly in Mestha and Cowpea seedlings continuously treated with TIBA,it resulted in the production of a number of lateral branches but reduced the internode elongation.

From this brief examination of the data it is possible to conclude that polar IAA transport from the dominant sinks acts as a signal to induce abscission or to restrict bud growth.

Bangerth (1989) in his primigenic dominance hypothesis proposed that IAA from dominant organ can inhibit subordinate organ without being translocated into them and without the mediating effect of secondary messengers. A number of other earlier works also corroborate our finding that endogenous concentration and the rate of diffusable IAA concentration is more in dominant fruits (Sastry and Muir, 1963) Prasad, 1983, Bangerth, 1989). Manipulation of the dominance relationship was shown to alter the rate of IAA diffusion (Bangerth, 1989). When the dominant organ - the shoot tip of apple or grapes or beans is removed the nearby fruits show higher fruit set (Quinlan and Preston, 1971; Prasad, 1983; Diethelm, 1988). Removal of dominant sinks seems to greatly increase the IAA export out of the remaining fruits and presumably prevents or reduces their abscission (Chapman _et al_, 1979).

Bangerth (1989) while explaining the role of IAA opines that higher IAA export from the first induced or dominant organ inhibits IAA export from the later induced or inhibited organs. We verified this in cotton petiole explant system.

The results revealed that application of high concentration of IAA inhibits basipetal transport of applied ^{14}C IAA from the other petiole, (Table 6) indicating that the IAA induced dominance effect and also high concentration of IAA moving downward from the dominant sinks may be inhibiting IAA transport from the less dominant or subordinate sinks. Since continuous basipetal IAA movement is necessary for the organ to develop, further restriction of polar movement of IAA may be inhibiting growth of axillary/lateral buds or inducing abscission of non dominant reproductive units. This data corroborates the findings of Morris (1977) and that of Bangerth (1989).

Transport of metabolites to dominant sinks in the dominance phenomenon

In premature abscission of reproductive units produced late in an inflorescence, the competition for assimilates is often argued as one of the main reasons for dominance. However, this can also be argued as a dominance effect since abscission of buds, flowers and young fruits occurs during very early stages of ontogeny in reproductive growth during which the actual demand for assimilates is meagre. In many cases removal of early formed dominant sinks results in diversion of assimilates towards later formed sinks, consequently reducing abscission.

Therefore, assimilate transport to the early produced reproductive sinks or apical buds seams to be influencing dominance phenomenon.

In this study, we have measured the uptake of ^{14}C assimilates by reproductive units. To examine the importance of competition for assimilates in determining the dominance effect, we also examined the role of IAA transport out of a dominant pod in influencing uptake of assimilates to the different racemes within an inflorescence.

In a developing fruiting point of cowpea, the influence of application of IAA or inhibition of transport of applied IAA by using TIBA, on mobilization of ^{14}C Sucrose into different racemes was studied. IAA was applied to the developing pods in raceme I (Fig 40), and in another treatment TIBA was applied to the peduncle of developing pods with an objective to decrease basipetal transport of IAA produced in the pods.

Application of both IAA and TIBA increases the ^{14}C Sucrose movement into applied pods. In IAA treated pods the total radioactivity recovered in the peduncle was 79% compared to control. However, the percent of radioactivity retained in different parts of the raceme remained almost similar as that of control. Application of TIBA to the peduncles of developing pods increased the amount as well as the percent of radioactivity moved into the upper racemes.

This clearly indicates that inhibiting IAA transport from the dominant organ results in higher amount of metabolites moving to the subordinate organs.

Experiments conducted with cotton petiole explant system indicated that, in the first experiment in which ^{14}C Sucrose was given through the basal cut end of the explant, ^{14}C Sucrose moved preferentially to the IAA applied region.

In the next experiment cotton seedlings were fed with $^{14}CO_2$ and petiole explants were prepared and IAA was applied to both the lateral petioles, or to one of the lateral petioles or to the stem section above the node of lateral petioles (Fig 39). High ^{14}C activity was recovered in the region which received IAA.

The results of these three experiments clearly indicate the involvement of auxin content and transport in the dominance phenomenon. Particularly, in the experiment in which transport of endogenous IAA was inhibited by TIBA, more amount of ^{14}C Sucrose that moved from the basal peduncle region to the upper racemes is interesting. These data tend to conclude that the reproductive units in the upper racemes are also capable of mobilizing metabolites but, in the presence of dominant sinks they are inhibited in mobilising metabolites. Probably, the movement of high concentration of IAA from the dominant developing pods in raceme I is inhibiting metabolite auxin transport to the upper racemes.

In cowpea inflorescence application of TIBA to the pedicelof the dominant developing pod from basal raceme increased the amount of ^{14}C Sucrose translocated to upper raceme. Two conclusions can be drawn from these results;

Firstly, developing dominant sinks releases high concentration of auxin and increases the sink capacity that is, high amount of photosynthates are translocated to the sink. Secondly, high concentration of auxin transported from dominant sink inhibits transport of auxin from subordinate sinks inhibiting further growth. Once auxin transport is inhibited from the dominant sink by TIBA, the auxin transport from the subordinate sinks are reestablished inducing further growth and transport of photosynthates to the sinks. The results of the field experiment also indicate that application of TIBA to the pedicel of growing pod at the first raceme increases pod set in the upper raceme, but in many inflorescences the pods treated with TIBA abscised within 4-5 days after application (Data not given). This further emphasizes that auxin transport from the dominant sink is essential for its survival and growth.

This study further substantiates that distal fruits have a lower potential sink strength than the proximal fruits (Ho, 1980). In cowpea, since the ovary weight of flowers from the upper racemes on the day of anthesis itself is less the potential capacity of the developing distal sinks is low even prior to flowering. The inferiority of distal reproductive sinks seems not entirely due to the late induction.

The later induced reproductive units in the distal part normally imported less assimilates, because of mobilization of major amount of assimilates by the proximal dominant sinks. The high concentration of auxin produced and translocated by the proximal dominant sink helps in increased growth of dominant sink and also helps in transport of assimilates towards them. In addition to this, the high auxin concentration coming from the dominant sink may inhibit auxin transport from the distal sink of the upper racemes inhibiting further growth of these sinks.

The reasons for high sink capacity of proximal flowers which develop into mature pods is not known. The weight of the ovaries on the day of anthesis was relatively more in proximal early produced flowers (Adedipe and Omrod, 1976).

Ho *et al*, (1983) reported that proximal fruits had relatively high IAA concentration than the distal fruits in a tomato truss. Bohner (1982), also reported that proximal fruits of tomato contained large concentration of diffusable auxin compared to distal fruits. This indicates that higher sinks capacity in terms of high growth rate is by the advantage of having high auxin concentration which helps in mobilization of metabolites. However, how this dominance effect restricts the flow of metabolites to the subordinate sinks is difficult to explain. This issue is more complicated since the abscission of reproductive units occurs at very early stages of reproductive growth, when the demand for photosynthates are relatively less.

Well developed ovaries possibly with more number of cells per ovary together with higher auxin concentration attracts assimilates. The proximal fruit may have the potential to attract large amount of assimilates resulting in abscission of all the flowers produced later at the distal racemes. As Bangerth and Ho (1984), hypothesised in a tomato truss there are possibly two ways by which early produced proximal flowers can inhibit growth of later produced distal flowers within an inflorescence:

(a) By limiting the supply of assimilates to the later induced flowers. It has been shown that flowers produced early in the proximal node act as major sink and monopolize the mobile assimilates enroute.

(b) The inhibition of late produced distal flowers may be caused by production and release of high concentration of IAA from early produced proximal flowers which in turn may inhibit the transport of IAA from distal flowers.

SUMMARY

VI. SUMMARY

The present investigation was conducted to study the role of auxin and its transport on abscission and apical dominance phenomenon.

1. The pattern of abscission and pod set was studied in a fruiting point of cowpea. Only the two basal flowers formed into pods. All the other flowers produced later abscised.

2. The weight of the ovary from the flowers at the first raceme was higher than that compared to those at the second raceme, but there was no significant difference in their auxin concentrations.

3. By altering the source - sink ratio it was noticed that reduction in source caused a reduction in the number of reproductive units produced per inflorescence. Reduction in the sink increased the number of reproductive units and it was also noticed that distal flowers produced pods in a raceme when the proximal flowers were removed.

4. Application of TIBA to the pedicel of young developing pods increased the number of reproductive units produced in the upper racemes and induced pod set at distal racemes.

5. In cotton petiole explant systems, application of auxin to one of the lateral petioles induced abscission of the untreated petiole and with increasing concentration of auxin applied, abscission percentage increased. Application of TIBA to the base of the petioles reduced abscission of untreated petioles.

6. Application of auxin to the cut end of the stem or leaf lamina, inhibited growth of the lateral/axillary buds, indicating the role of auxin in apical dominance.

7. Continuous application of TIBA to the apical bud increased the number of lateral branches produced.

8. Experiments conducted on the role of IAA in mobilisation of metabolites indicate that more amount of metabolites are mobilised to the region to which higher concentration of IAA is applied. It was also observed that basipetal movement of IAA from the dominant sink inhibited basipetal translocation of IAA from the subordinate sink. With application of TIBA to the dominant sink, basipetal translocation of IAA from the subordinate sink increased.

These results indicated that abscission of reproductive units produced in the distal racemes of an inflorescence is due to dominance effect of early formed fruits in the proximal racemes. High auxin concentration produced and translocated from the dominant sink seems to be the trigger.

The dominance effect of apical node on initiation of growth of the axillary/lateral bud also seems to be mediated by the signal IAA. High concentration of IAA translocated from the apical bud inhibits growth of the lateral buds.

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