3. ETHYLENE AND REACTIVATION OF DORMANT BUDS

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CORRELATIVE INHIBITION AND EPIPHYLLOUS BUD OUTGROWTH

3.1. INTRODUCTION

Various cells, tissues and organs which together constitute a multicellular plant, do not exist independently of one another but rather their activities are interrelated. Usually each shoot apex in plants somehow influences the development and positioning of lateral structures derived from the same or different apices. Specially in herbaceous plants the lack of branching is usually attributed to an inhibition of axillary bud outgrowth by the apical bud of the main stem. This phenomenon is called the apical dominance. Sometimes, this dominance of the apical region over the lateral buds is termed as the correlative inhibition (Hillman, 1984). This phenomenon is always found with varying degree in all seed plants and obviously is of profound significance as it determines the growth form of a plant.

The plants particularly from temperate and arid regions, show one more type of bud growth inhibition called the bud dormancy. This can be defined as cessation of observable growth of a bud (Berrie, 1984). It is generally accepted that the primary factor for inducing the bud dormancy is daylength (Downs and Borthwick, 1956). However, other factors such as nutrition, temperature, water status etc can also influence bud dormancy (Perry, 1971). The role of plant

growth substances, particularly that of abscisic acid in maintaining and stimulating the bud dormancy is well known (Hillman, 1984). Thus the axillary buds inhibited by the apical meristem are distinctly different from the dormant buds (Berrie, 1984).

It goes without saying that hormones are the principal factors involved in most of the developmental processes in plants. It is now well documented that in most of the cases eventhough hormones possibly cannot be determining the way in which a cell or tissue responds but their role is likely to be that of inducing certain responses. The nature of these responses are probably, determined by factors intrinsic to the cell (Wareing, 1971; Hall, 1976). Various mechanisms have been hypothesized to explain the dormancy and reactivation of growth of dormant epiphyllous buds of Bryophyllum spp. (Loeb, 1917, Heide, 1965; Henson and Wareing, 1977). At this stage, it would be imperative to go in detail on the present status of the phenomenon of correlative inhibition.

3.1A. Various theories on the factors responsible for correlative inhibition:

Several theories have been put forward, from time to time, to explain the factors responsible for the correlative inhibition -

1) Nutritive theory: Earlier investigators (Goebel, 1900; Loeb, 1915; 1918; Dostal, 1926) interpreted correlative

phenomenon in terms of competition for nutrients between the main shoot apex and lateral bud meristems.

2) Direct theory of auxin inhibition: Thimann and Skoog (1933, 1934) in their classic and pioneer studies found that the auxin from the shoot apices of Vicia faba can be detected in the agar blocks and that the auxin is produced by inhibited buds or older leaves. Further exogenous IAA could inhibit the bud outgrowth in decapitated plants. From these studies Skoog and Thimann (1934), suggested that the auxin synthesized in the shoot apex reaches the lateral buds and inhibits the localised production of IAA necessary for bud outgrowth.

3) Indirect theory of auxin inhibition: With the help of a series of experiments Snow (1940) concluded that as a result of auxin action an inhibitor is formed which moves into lateral buds and inhibits their growth.

4) Nutritional diversion theory: According to this theory, auxin creates a flow of growth factors towards the point of auxin production - the apex. Thus the lateral buds are starved in a manner similar to nutritive theory (Went, 1938, 1939).

5) Vascular connection theory: Based upon the theories of direct and nutrition diversion, Overbeek (1938) suggested that auxin or auxin induced inhibitor prevents the entry of growth factors into the lateral buds through their

suppressive effect on the establishment of vascular connection between bud and the stem.

6) Hormone balance theory: With the identifications of gibberellins, cytokinins, ethylene and abscisic acid as endogenous plant growth substances and their effects on general growth led to the conclusion that the balance of hormones controls the inhibition and stimulation of bud development (Saunders, 1978; Hillman, 1984).

3.1B. Validity of different theories:

A growth stimulation of lateral (axillary) inhibited buds of stem can be accomplished by either decapitation of the shoot apex or cytokinin application to the inhibited buds or increased nutrient availability (Hillman, 1984). According to Overbeek (1938), the apical dominance is due to the insufficient vascular connections between the lateral buds and the stem. However, as noted by Cutter (1972) from the work of Sorokin and Thimann (1964), it is evident that the lateral bud growth can be measured many hours before the increased vascular connections become prominent. Probably, the increased connections serve only to maintain an accelerated growth rate rather than acting as a control for the onset of bud growth (Rubinstein and Nagao, 1976). In support of the nutritive theory, there are many reports indicating the nitrogen (McIntyre, 1971; 1973; McIntyre and Larmour, 1975), phosphorus (McIntyre, 1968; Thimann et al., 1971) and potassium (Wakhloo, 1970), as limiting factors in the growth of inhibited buds on intact plants. On the other hand,

total nitrogen, phosphorous and potassium content per unit dry weight is found to be higher in the inhibited buds than the buds released by decapitation (Phillips,1968).

A role for hormone(s) seems more likely for the correlative inhibition - a phenomenon involving communication between two different portions viz apical meristem and axillary meristems (Rubinstein and Nagao,1976). Since the early work of Thimann and Skoog (1933) auxin have been implicated as an inhibitory factor in the apical dominance. Application of auxin has been found to inhibit the lateral bud outgrowth in Tradescantia (Naylor, 1958), Soybean (Ali and Fletcher, 1970) and Phaseolus (Jackson and Field, 1972) likely to be through the prevention of DNA synthesis and cell division (Nagl, 1972).

Interestingly, there are reports demonstrating that decapitation and subsequent auxin treatment to the decapitated region could direct the transport of ^{32}P to the site of hormone application (Booth et al.,1962; Davies and Wareing,1965; Husain and Linck, 1966; Seth and Wareing 1967). If nutrients are indeed controlling the growth of lateral buds, then the diversion of these substances to the apex through auxin may be related to the mechanism of indirect effect of auxin on bud growth. Under natural conditions, presumably, the apical meristem must be acting in a similar manner as that of applied auxin (Rubinstein and Nagao,1976).

At the same time, the direct theory of auxin action on apical dominance implies that auxin must be near or in the lateral bud to exert its inhibitory effects. Hillman et al.(1977) demonstrated that in Phaseolus IAA levels of lateral buds rise following the removal of shoot apex. In support of this theory, auxin which is mainly produced by the apex (Scott and Briggs, 1960) has been shown to travel down the plant part - the lateral buds (Morris and Kadir,1972; Morris et al.,1973; Goldsmith et al.,1974). Further, the basipetal polarity of correlative inhibition is in accord with the known transport characteristics of radioactivity from labelled exogenous IAA in shoots (Nonhebel,1982). This was further corroborated using inhibitors of IAA transport in various systems (Beyer,1972; Brown et al.,1972; White and Hillman 1972). These results clearly appear to be firm evidence in favour of a central role for IAA in the correlative inhibition phenomenon.

Mature leaves of various species of Kalanchoe have long been used to demonstrate vegetative reproduction occurring through buds located on the either margins (Karpoff,1982). However, these buds remain dormant for long periods of time, due to still obscure reasons.

In most of the species of Kalanchoe the dormant state of buds is found to be broken when the leaves are detached from the parent plant (Resende,1959). The involvement of photoperiodic induction for bud development has been studied extensively (Gotz, 1953; Meyer, 1953; Kroner,1955). In context with this event,

workers have demonstrated that terminal and axillary buds inhibited the epiphyllous bud outgrowth (Loeb,1915; 1917). Heide (1965) has also found similar results and indicated the role of auxin in the process of epiphyllous bud growth as also concluded by Vardar and Acarer (1957). However, Dostal and Maskova (1949) found the inhibitory effect of IAA to be rather weak, which led Dore (1965) to consider auxin as an unlikely factor in the control of bud outgrowth.

Based upon all these reasoning following sets of experiments were undertaken to reveal the factor(s) responsible for bud dormancy and their subsequent reactivation of the growth.

3.2 MATERIALS AND METHODS

A homogenous stock of Kalanchoe mortagei, Raymond Hamet and Perrier, plants was generated by producing large number of plants, asexually from a single plant growing in the Botanical Garden of the Maharaja Sayajirao University, Baroda.

The leaves were excised just before the experiment, usually in the morning time. Such isolated leaves were washed thoroughly first with tap water and followed by a rinse with double glass distilled water (dist water). These detached and washed leaves or parts thereof were used for all investigations as inocula. All test solutions including dist water were adjusted to pH 5.8 ± 0.1 with 0.1 N NaOH or HCl and sterilized.

The cultures were incubated in the culture-room at 25 ± 1 °C temperature and 16 hr photoperiod (10.6 Wm^{-2}). Every 24 hr interval leaves were observed for visual appearance of buds from the notches and percent bud outgrowth per leaf was calculated. All the experiments were repeated more than four times with each treatment triplicated due to the simplicity and rapidity of experimental system.

3.2A. Preliminary studies on the epiphyllous bud outgrowth:

The leaves, thoroughly washed, were kept for bud outgrowth with their petiole dipped in dist water kept in an Erlenmeyer flask (50 ml capacity).

3.2B. Effect of leaf age and plant age on bud outgrowth:

3.2B.1 Leaf age - Leaves from 1st to 5th nodes were used for this experiment.

3.2B.2. Plant age - Two different age groups of plants were used.

a. Vegetatively growing plants (6-8 month old) (Fig. 3.1a).

b. Plants reproductive phase (12 month old) (Fig. 3.1b).

The leaves (from 1st to 6th node) were excised from the plants and kept in cultures as mentioned earlier.

3.2C. Studies with intact plants (or attached leaves):

3.2C.1 Effect of the apical and exillary meristems on the epiphyllous bud growth

To study the effect of plant apex and lateral buds (axillary) on the epiphyllous bud growth in the attached leaves, the following experiments were undertaken:

a. Removal of plant apex.
b. Elimination of axillary bud of the experimental leaf. As a control, the axillary bud of the opposite leaf kept intact.
c. Discontinuity in the vasculature or lamina of the experimental leaf.
d. Removal of the young developing leaves (1-3 nodes) along with the apical meristem.

In order to localize the factor responsible for the inhibition of epiphyllous bud growth, either the apical or axillary meristem was surgically removed and observed for the growth of epiphyllous buds. In another set of experiment a cut in the vascular system of midrib or in the mesophyll region of lamina were made and their subsequent effect on the growth of epiphyllous bud was monitored.

3.2C.2 Role of plant growth regulators -

Cytokinins and ethylene are found to be involved in the process of reactivation of dormant buds (Lee et al.,1974; Rubinstein and Nagao,1976; Aung and Byrne,1978; Yeang and Hillman,1984a). With this background in mind, we attempted to ascertain the role of these two plant growth

regulators in the reactivation of dormant epiphyllous bud growth in K.mortagei. BAP was applied to the leaf by immersing the leaf tip in its solution. Ethylene was applied in form of ethrel spray.

In both the treatments controls were treated with water.

3.2D. Effect of soil contact of leaf on the epiphyllous bud outgrowth:

In natural condition, the epiphyllous buds of K.mortagei begin to grow only when the subtending leaf accidently touches the ground. In order to understand the involvement of thigmotropism in breaking the dormancy of epiphyllous buds the leaves of fourth node were made to touch the soil along the notches located at various positions on the leaf and observed for bud outgrowth.

3.2E. Role of long day photoperiods on bud growth:

To see the effect of long day conditions on the stimulation of bud growth on attached leaves, the plants were kept in culture room with 18 hr photoperiod for 15 days.

3.3 RESULTS

3.3A Preliminary studies on the epiphyllous bud outgrowth:

A fully developed leaf of Kalanchoe mortagei contains as many as 45-70 notches of its either lateral margins. Each botch

harbours a single dormant shoot meristem. These meristems are activated upon detachment of leaves from the mother plant. For this, the detached leaves were cultured in an erect position by dipping the petiole in distilled water as shown in Fig. 3.2. Under this condition the following observations were made -

1) On isolation and subsequent incubation, the shoots appeared after about 4-5 days of incubation, followed by roots.

2) The very first buds to develop are located in the uppermost apical notches (Fig. 3.1a). Gradually, the remaining buds appeared from the successive lower notches on a leaf.

3.3B. Effect of leaf age and plant age on epiphyllous bud outgrowth:

3.3B.1 Due to the opposite decussate nature of phylotaxy, leaf age in Kalanchoe mortagei could not be determined using the 'Plastochron index' criteria of Erickson and Michelini (1957). Therefore, leaf age was determined based upon their position on the node number of the main stem. The first node was considered as the one that is nearest to the apical meristem. It was found that the buds of those leaves located on 4th and subsequent higher node numbers could grow (Fig. 3.3). Epiphyllous buds of 1st, 2nd and 3rd nodeal leaves failed to show any development.

3.3B.2 Leaves of 1st to 6th nodes were removed from (i) 6 month old vegetative plant and (ii) 12 month old reproductive (just after bolting) plants. It was observed that the epiphyllous buds located on all the leaves (1st to 6th nodes) of reproductive plants exhibited growth (Fig. 3.3b). On the other hand, the buds of the leaves of 1st to 3rd nodes failed to grow in case of vegetative plants. Only the buds located on 4th and subsequent nodal leaves, displayed the bud outgrowth (Fig. 3.3b).

3.3C. Studies with intact (attached) leaves:

Epiphyllous buds do not develop on the leaf which is still attached to the mother plant. Thus some tissue part of the mother plant must be exerting an inhibitory control over the epiphyllous buds. In order to understand role, if any of the main apex in controlling the dormancy of epiphyllous buds, various surgical experiments were done. The observations are summarized as follows.

3.3C.1 Effect of apical and axillary meristem -

Removal of shoot apex along with the first or 1-3 pairs of leaf (Fig. 3.4a, 3.5a) did not break the dormancy of the epiphyllous buds of the leaves. However, the axillary meristems located at the first node did develope into branches (Fig. 3.5a). This suggests that the apical shoot meristem has no inhibitory control on the growth of epiphyllous buds. Removal of the axillary buds also

could not trigger the buds outgrowth (Fig. 3.4b, 3.5b).

Surgical removal of a portion of the midrib at the base of lamina, however, triggered the epiphyllous bud outgrowth of that particular leaf only (Fig. 3.6a and c). Application of $CoCl_2$ (0.1 mM) in cotton swab immediately to the cut in the midrib of the leaf inhibited the development of buds (Fig. 3.6c). On the other hand, cut made in the mesophyll region of lamina had no effect on triggering the bud outgrowth (Fig. 3.6b). Thus, it appears that it is the stress and not the injury to the leaf that breaks the dormancy of buds. Further the apical meristem had no inhibitory effect on the epiphyllous bud dormancy on the attached leaves.

3.3C.2 Role of plant growth hormones -

Since BAP treatment to the excised leaf suppressed the growth of epiphyllous buds (chapter 4), it was of interest to study its effect on the intact leaves.

Application of BAP (1.0 µM) through leaf tip, stimulated epiphyllous bud outgrowth (Fig. 3.7a). This bud growth response was found on 10th day and only on the treated leaves. Similarly, spray of ETH to the intact plant stimulated the development of the buds on all the leaves (Fig. 3.7b).

3.3D. Effect of soil contact with leaf on bud outgrowth:

No epiphyllous bud growth was found on the leaves which were made to touch the soil along the notches located on

various parts of the leaf.

3.3E The role of long day photoperiod:

The plants kept in 18 hr photoperiod also failed to display any bud growth (Fig. 3.7E).

3.4 DISCUSSION

The apical part of a shoot usually grows more vigrously than the axillary buds, despite the fact that it is apparently the least favourably situated (distance wise) with respect to nutrients from mature leaves and/or root systems (Wareing and Phillips, 1982). The apical dominance or correlative inhibition, leading ultimately to lack of branching in plants has been attributed to the inhibition of axillary bud outgrowth (Leopold and Kriedemann, 1975). Although atleast, fourteen types of treatments (Table 3.1) are known to activate growth in the inhibited buds, yet there is no specific site or basic process which can be clearly defined as locus of inhibition (Hillman, 1984). Because of the simplicity and rapidness of response, the epiphyllous buds of Kalanchoe spp. have remained a favourable experimental system with plant developmental biologists since long (Howe, 1931; Naylor, 1932; Yarbrough, 1932; Karpoff, 1982). Two questions that have been frequently attempted are —

1) control and mechanism of development of these epiphyllous buds,
2) factors responsible for their release from dormancy.

Anatomical studies have shown that these buds are formed from the meristematic cells of leaf primordia. While most of the meristematic cells differentiate into various tissues of the leaf, islands of meristematic cells remain perpetually undifferentiated on the either lateral sides in notches and subsequently forming primordia of shoot and root. When the leaf reaches maturity, cell division ceases in these regions (notches) and the bud primordia undergo dormancy (Naylor, 1932).

Release of these buds from their dormancy has been extensively studied. In case of B. calycinum it has been shown that detachment of the leaf of injury allowed the dormant buds to develop into plantlets (Loeb, 1915). In case of Kalanchoe mortagei, present experimental system, also the detachment of the leaves from the mother plant, causes the dormant epiphyllous buds to develop into complete plantlets.

In case of B.diagremontianum and B.tubiflorum, the activation of the foliar meristem is under photoperiodic control. In these species bud formation takes place on attached leaves under long day conditions (Heide, 1965). However, bud formation is possible also in short days under high temperature regimes in B. tubiflorum, whereas in B. diagremontianum budding on attached leaves is strictly bound to long day conditions (Resende, 1959). The photoperiodic requirement for bud outgrowth in these plants has been extensively studied by Gotz (1953), Meyer (1953) and Kroner (1955).

Goebel (1902) and Loeb (1915; 1917) demonstrated that terminal and axillary buds inhibited the epiphyllous bud outgrowth in Bryophyllum. This inhibiting substance was assumed to be a hormone (Loeb 1917). However in ageing leaves and in leaves which are in contact with the moist soil or immersed in water for a prolonged time, buds may be formed while the leaves are still attached to the plant. Reed (1923) reported that external conditions such as high humidity or absence of light may initiate physical or chemical changes within the leaves that stimulate epiphyllous buds to be released from dormancy. These changes included increased levels of catalase, carbohydrases, reducing sugars and glucosides, but decreased levels of starch and total carbohydrates (Mehrlich, 1931). Such observations introduced some controversy into the literature on this subject with regard to the inhibitory effects of buds - apical and axillary.

Our results indicate that since K. mortagei is a day neutral plant with respect to the epiphyllous bud formation, any photoperiodic control is unlikely. Further, contact of attached leaves with moist surface or soil also failed to reactivate these dormant buds, eliminating the possibility of any role played by either thigmotropism or humidity in the reactivation process.

In case of B. digremontianum, the removal of plant apex resulted in the induction of epiphyllous bud growth. This led Heide (1965) and subsequently Henson and Wareing (1977) to

conclude that epiphyllous bud formation is under the control of correlative inhibition similar to apical dominance. However, decapitation of main shoot apex or removal of axillary buds also failed to reactivate the epiphyllous buds in this study, thus ruling out any regulatory role of the main shoot apex or axillary buds on epiphyllous budding in K.mortagei.

Among the many explanations offered for the underlying mechanisms of correlative inhibition, one implies that competition for nutrients between apical - including young developing leaves (Hillman, 1984) and axillary meristems plays a crucial role, and that this process is mediated by auxin (Panigrahi and Audus,1966; Little,1970; White and Hillman,1972). Considering this theory, the young developing leaves (1-3 nodes) must be acting as a sink for continuous supply of nutrients, thus depriving the epiphyllous buds to develop on mature leaves. Then , it is likely that removal of this sink should bring about the epiphyllous bud formation on attached leaves. However in present studies, removal of young leaves along with apical meristem and axillary bud could not stimulate the epiphyllous bud outgrowth, refuting nutritional diversion hypothesis (Phillips, 1975; Patrick, 1982) as a cause of dormancy of the epiphyllous buds of K.mortagei.

The question still remains as to the nature of control (process or compound) that maintains dormant state of epiphyllous buds on intact leaves in K.mortagei. The surgical experiments

indicate that a cut on midrib or petiole but not on mesophyll tissue could trigger the growth of dormant buds. If injury alone would have been the cause of reactivation of growth, then the injury on mesophyll tissue would have sufficed to break the dormancy. But, the injury could trigger bud growth only when it is site specific i.e. on the midrib. Clearly, intruption of vascular supply would have generated sufficient stress which is known to trigger ethylene biosynthesis in wide variety of plants (Abeles and Abeles 1972; McMichael et al.,1972; Ben-Yehoshua and Aloni,1974; Yang and Pratt,1978). There is also a possibility that the severing of the vasulature might block an inhibitor entering the leaf and inhibiting bud growth. The failure of the residual inhibitor to prevent bud growth may be due to its suboptimal level. The results obtained with intact plants (removel of plant apex/young developing leaves) clearly nullifies the possibility of inhibitor from apex or young leaves. Earlier, Sebanek and Slaby (1982) suggested the correlative effects of root and basal part of stem upon the bud development in leaves of B.crenatum. However, their results with incision in the stem just below the leaf, clearly indicates the possibility of stress induced bud formation.

It is worth noting here that the application of $CoCl_2$ (0.1 mM) immediately to the cut in the midrib, inhibited the bud growth which was observed in control (Fig. 3.6c). This seems to confirm the role of ethylene in triggering bud formation. Earlier Boyer et al. (1986) and Crouzillat et al. (1985) have reported inhibition of ethylene induced responses by $CoCl_2$ in Bryonia and Bidens respectively.

Interestingly, in absence of such stress, ETH (which generates ethylene) spray to the intact leaves, indeed triggered the growth of dormant buds in the present studies. Levy et al. (1973) reported CEPA (2-chloroethylphosphonic acid - ethylene releasing substance) spray to leaf induced bulbing in noninductive conditions in onion. So it can be concluded that the stress mediated ethylene biosynthesis is probably responsible for the reactivation of the growth of dormant epiphyllous buds. In this context, green leaves are known to contain more ACC (1-amino-cyclopropane-1-carboxylic acid - the immediate precursor of ethylene) along with the enzyme (ACC synthetase) which is responsible for ethylene biosynthesis from ACC (Woodrow and Grodzinski, 1987). Further, Hume and Lovell (1983) demonstrated that ACC might be the true transport signal and its availability in the different plant parts could be the limiting and determining factor for response. Role of ethylene in the reactivation of dormant buds have been extensively studied by Hall et al., 1957; De Wilde, 1971; Burg, 1973; Catchpole and Hillman, 1976). Recently Van Aatrijk et al.(1986) have reported the role of ethylene in the adventious bud formation in Lolium. They found direct correlation between the number of plantlets formation per explant and the enthylene production. Further, it has been shown that ethylene is essential in maintaining the growth of released buds (Yeang and Hillman, 1982). They found IAA induced transient increase in bud growth on intact Phaseolus plants. Application of an ethylene biosynthesis inhibitor AVG (aminoethoxyvinyl glycine) to the enhanced bud growth completely inhibited it

Table - 3.1. Treatments which promote growth of lateral buds held under correlative inhibition (Hillman, 1984).

1. Excision of apical portion of stem (decapitation).
2. Removal of young developing leaves.
3. Physical restriction of apical growth.
4. Isolation of the apical position of the stem by disease, bark-ringing, or steam-girdling.
5. Infection by pathogens causing witches-broom (Hexenbesen).
6. Elevated carbon dioxide levels.
7. Quantity and spectral quality of light.
8. Humidity.
9. Water and nutrient supply to root system.
10. Gravimorphic treatments.
11. Induction of the reproductive condition.
12. Application of auxin-transport inhibitors, abscisic acid, ethephon, ethylene or May & Baker 25-105 to tissues above bud.
13. Application of chemical pruning agents to shoot.
14. Application of indole-3-acetic acid or cytokinins to bud.

Fig. 3.1. A 6 month old vegetative (a) and 12 month old reproductive plant (b) used for studying the effect of plant age on bud outgrowth response in isolated leaves.


a


b

Fig. 3.2. Detached leaf kept in upright position in a flask - an experimental set up used for various studies.

Fig. 3.3. Effect of leaf age (a) and plant age (b) on epiphyllous bud outgrowth from detached leaves.


a

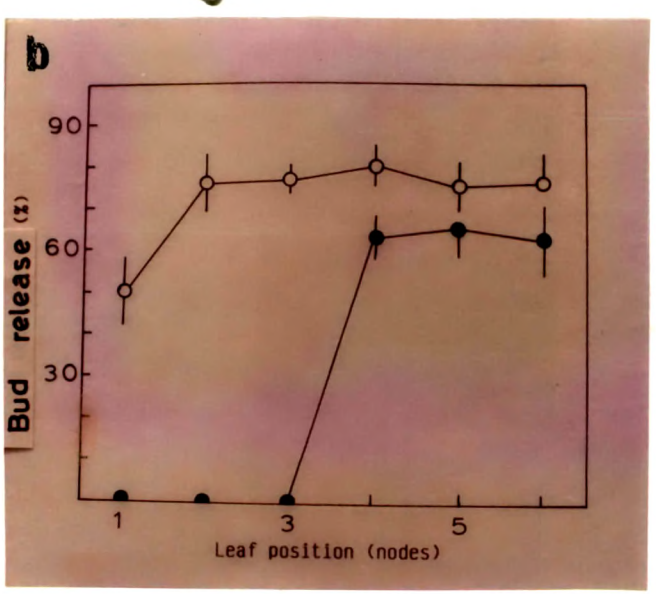
b
90
60
30
Bud release (%)
1
3
5
Leaf position (nodes)

Fig. 3.4. Effect of removal of plant apex alone (a) or axillary bud of the leaves (b) on bud outgrowth on attached leaves.


a
b
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Fig. 3.5. Removal of plant apex alongwith developing leaves, with intact axillary buds (a) and axillary bud removed (b), could not induce bud outgrowth on attached leaves.

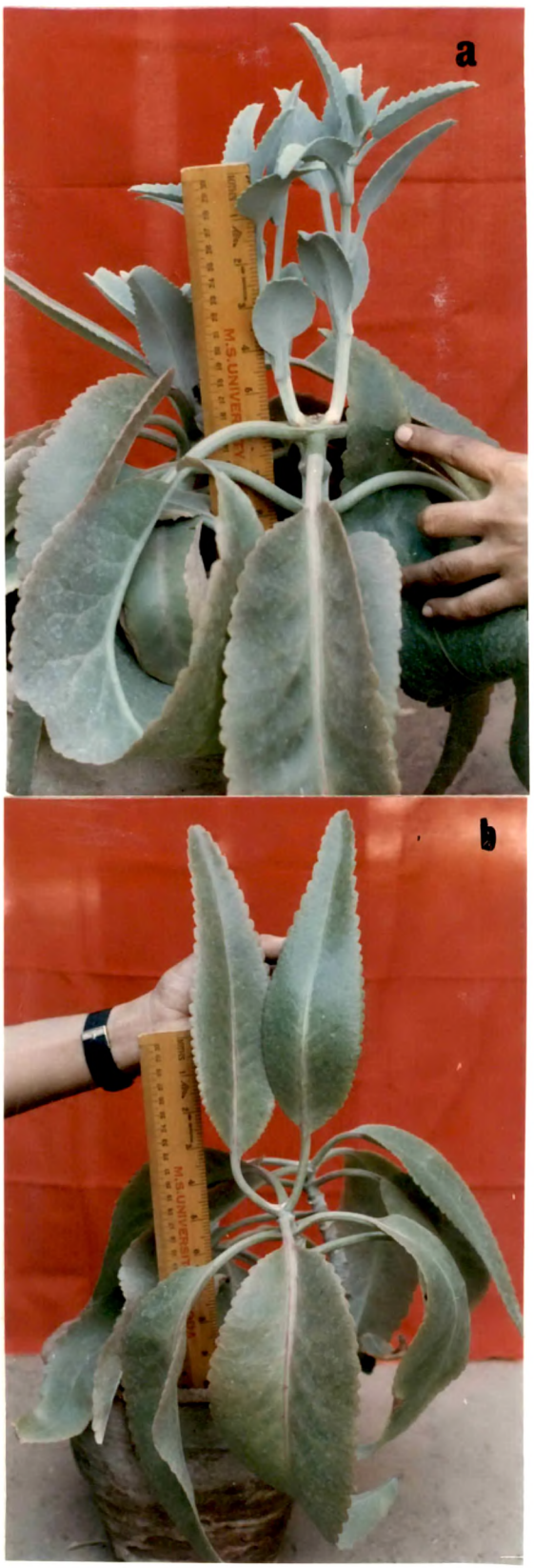

a
b

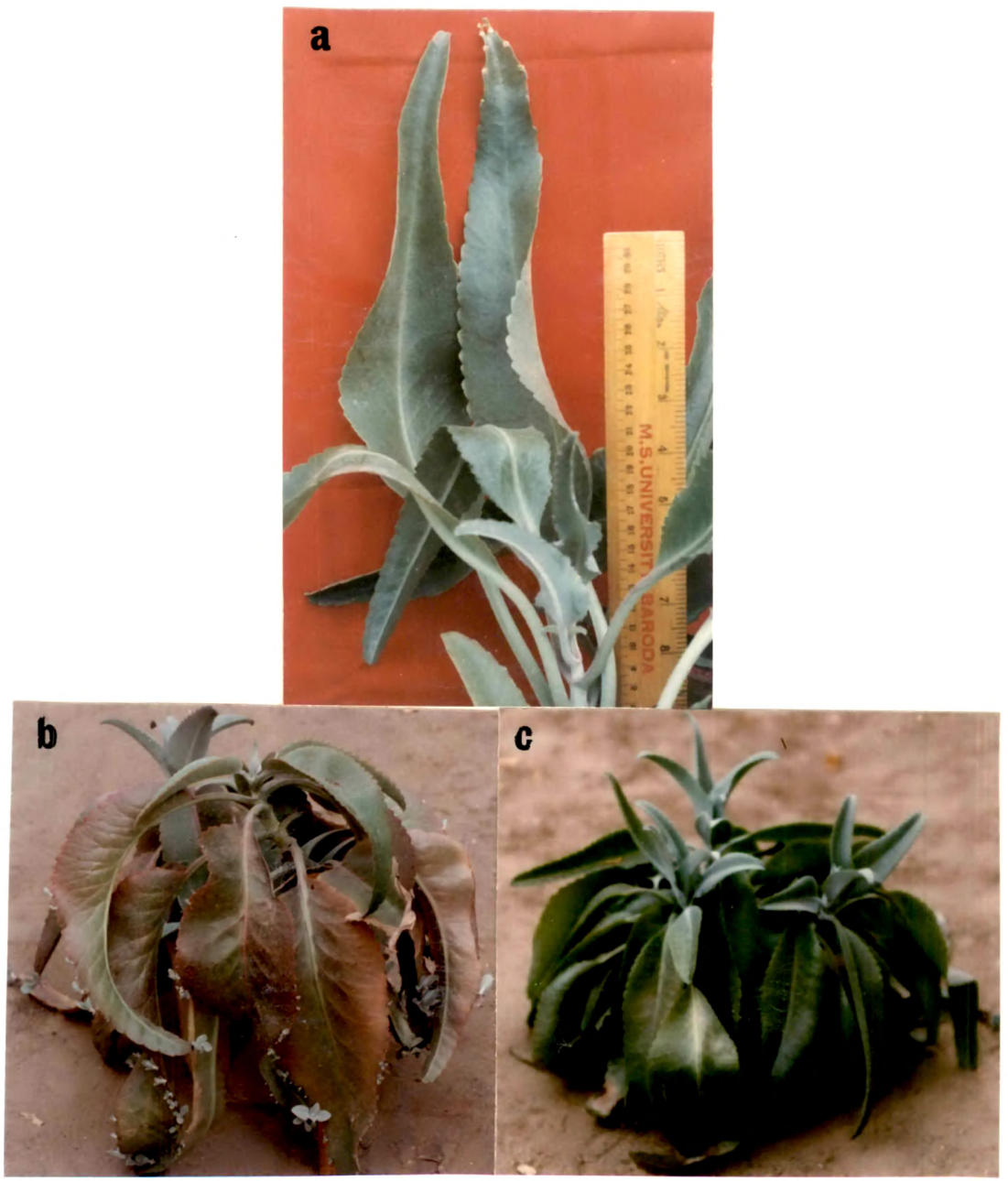
Fig. 3.6. Effect of a cut in midrib (a) or lamina (b) of a leaf on bud growth in attached leaves.
Note that the cut in midrib induces development of dormant buds and application of $CoCl_2$ inhibits the bud outgrowth (c).


a


b


c

Fig. 3.7. Effect of BAP application to the leaf tip (a), ETH spray (b) to the attached leaves and long day photoperiod (c) on growth of dormant buds.
Note the outgrowth of buds by BAP and ETH application.


a
b
c

indicating the role of ethylene in bud growth induction. In this regard, Ku et al. (1970) had reported that depending upon its free concentration IAA induces the formation of a short lived RNA required for the synthesis of highly labile protein which controls the rate of ethylene production in vegetative tissue of etiolated pea shoots.

From these studies it seems that induction of bud outgrowth follows the following sequence -

Stress → Ethylene synthesis → Induction of IAA oxidase →
→ Destruction of IAA → Optimum auxin/cytokinin ratio → Bud outgrowth

Therefore, exogenous BAP application induced bud formation in attached treated leaves in present studies could be due to the fact that exogenous cytokinin treatment bypasses all these initial steps and brings down the optimum ratio of auxin/cytokinin and thus inducing bud development. However, other likelyhood could be the cytokinin incited ethylene production (Yoshi and Imaseki, 1981) as reported in wheat leaves (Loveys and Wareing, 1971), pea stems (Fuchs and Lieberman, 1968), hypocotyl of mung bean (Imaseki et al., 1975 and Ban and Yang, 1976) and sunflower shoots (Wample and Reid, 1979). Further, the hypothesis of ethylene involvement in bud outgrowth is supported by the observation that in water stress plants, the buds were found developing even on attached leaves.

In absence of such stress, the epiphyllous buds remain dormant on the intact leaves of K. mortagei. The dormant state is maintained by the supraoptimal level of auxin within/around