4. POLARITY IN BUD OUTGROWTH

them. The surgical experiment described here indicates that the site of control for inhibition of epiphyllous bud growth is not the shoot apical or axillary meristems but located within the leaves bearing the dormant buds.

4. Polarity during epiphyllous bud outgrowth.

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POLARITY DURING EPIPHYLLOUS BUD OUTGROWTH

4.1. INTRODUCTION

Dore (1965) in his review on the physiology of regeneration concluded that most of the regeneration of plantlets from leaves is polar and occurs at the basal (proximal) end of a leaf or leaf segment. However in the present studies with Kalanchoe mortagei, it was observed that in the detached leaves, the very first buds to be released from dormancy were located in the uppermost apical notches at the leaf tip. Subsequently new buds appeared gradually on successive lower notches towards the leaf base. Thus a strong basipetal succession of bud outgrowth was observed (Fig. 4.1a and b) fulfilling the criterion of polarity (Wareing and Phillips, 1982) defined as a situation wherein two ends in a living system are different. Eventhough polarity is the first, visible morphological indication of an internal asymmetrical state within a living system and is a fundamental component of differentiation and spatial organization, it is a neglected aspect in developmental studies (Wolpert 1971; 1981; Wolpert et al., 1971). Such examples were also noted in in vitro bud formation in tobacco explant (Croes et al., 1985; Hillson and Lamotte, 1977). In tobacco, explants from the top of the plant tend to form more adventitious floral buds than the tissues from lower parts of the stem. Croes et al. (1985) suggested the role of tissue age in controlling the gradient in the morphogenetic competence.

Axial polarity has been found to be the most striking facet of the plant body, giving rise to various morphological features. Polarity in plants get established through numerous ways. Polarity becomes instituted whenever there is an unequal distribution of cellular constituents as well as hormonal levels (Coleman and Thorpe, 1985). These differences lead to polarly oriented fluxes (Sachs, 1978). Actually, such unequal distribution, which is the result of unequal division of cells in which the organelles are uniformly assorted, is required for establishing a distinct spatial relationship between daughter cells prior to induction of any developmental pathway (Hepler and Palievitz, 1974; Lang, 1974; Stebbins, 1974; Holder, 1979). The common example of unequal division is the first transverse division of zygote in Fucus (Jaffe, 1978). Before the establishment of polarity in Fucus zygote, apart from nucleus projections, mitochondria, ribosomes and fibrillar vesicles were found to be concentrated towards one half of the zygote which, thus, was more densely cytoplasmic (Quatrano, 1972; 1978). This unequal distribution, resulting in the asymmetrical division is usually accompanied by the setting up of the metabolic gradients which are the crux of polarity (Naylor, 1984). Such a phenomenon in plants can be considered as a form of positional signalling (Coleman and Thorpe, 1985). However, in the absence of any well-differentiated nervous system in plants, plant growth substances are known to accomplish the role of chemical signalling (Hillman, 1984). Thus a search was made to find out the regulatory factor(s) responsible for the polarity of

epiphyllous buds observed during their growth. Auxin, produced at the shoot tip and transported basipetally, is somehow inhibitory to the release of buds from apical dominance (Phillips, 1975) and further this effect of auxin is antagonised by cytokinin (Rubinstein and Nagao, 1976; Aung and Byrne, 1978). This indicates that cytokinin may be limiting for bud growth which requires continuous mobilisation of metabolites. In this regard a relationship between endogenous cytokinin levels and axillary bud growth has been demonstrated (Woolley and Wareing, 1972; Lee et al., 1974; Mapelli and Lombardi, 1982; Prochazka and Jacobs, 1984).

Exogenous application of hormones is one of the most common methods to study their effect or mode of action (Zeroni and Hall, 1980). The logic for such approaches is based mainly on the idea of replacement and control of the endogenous naturally occurring hormone by the exogenous hormone the level of which can be controlled and effects monitored, with the assumption that there is a relationship between the magnitude of the induced response and the concentration of the regulating substance (Firn, 1986).

4.2. MATERIALS AND METHODS

4.2A. Studies with the isolated leaves/leaf parts for understanding the polarity:

To understand the factor and its probable location, responsible for the polarity phenomenon during the epiphyllous

bud outgrowth the following surgical experiments were performed.

4.2A.1. Effect of notch position in a leaf:

In order to identify the part of the leaf exerting its effect on the polarity, the leaf was cut into two halves - upper and lower. The lower portion was kept for incubation with its petiole dipped in dist water. From the apical part (upper portion) of the leaf, the paired notches were cut serially and kept on filter paper soaked with dist water (Fig. 4.1 d). In another set the whole upper portion was kept as such for control (Fig. 4.1 d).

4.2A.2. Correlative inhibition of the epiphyllous buds:

To understand the role of correlative inhibition in controlling the polarity in bud growth, the leaves were cut into three parts (with almost equal number of notches in each) - upper, middle and basal and were incubated on the filter paper for bud outgrowth. In a second set, notches from each segments were cut into individual notches measuring 1 cm^2 and separately incubated on the filter paper for the bud outgrowth.

4.2A.3. Effect of axillary bud:

To check the probable control of the axillary bud on the outgrowth of epiphyllous buds, two sets of leaves were kept in cultures in dist water; one along with the axillary bud and other without it.

4.2B. Effect of PGR on the polarity of the bud outgrowth in the isolated leaves:

Auxins, cytokinins and to some extent gibberellins, ethylene and abscisic acid have been implicated in maintaining or releasing of the correlative inhibition (Hillman, 1984). Therefore, following experiments were done to examine if they have any role in the process of bud outgrowth.

4.2B.1. Basal application of PGR:

The PGRs tested for the polarity in the epiphyllous bud outgrowth were:

i)	Auxins	:	Indole-3-acetic acid (IAA)
ii		:	α -Naphthaleneacetic acid (NAA)
ii)	Cytokinin	:	6-Benzylaminopurine (BAP)
iii)	Gibberellin	:	Gibberellic acid - III (GA_3)
iv)	Ethylene	:	2-Chloroethylphosphonic acid (Ethrel)(ETH)
v)	Abscisin	:	Abscisic acid (ABA)
vi)	Antiauxin	:	2,3,5-triiodobenzoic acid (TIBA).

The PGRs were applied to the whole leaf through petiole. In this study, the washed leaves were kept standing, with their petiole dipped in test solutions (pH 5.8) in the concentration range of 0.01 to 100 μM (or as mentioned at respective places).

4.2B.2. Apical application of PGRs:

PGRs mentioned above were also applied to leaf tip (tip application) to study their effect on the bud growth. Thoroughly washed leaves were kept in the cultures with their petiole dipped in dist water. A small part (2-3 mm) of extreme tip of the leaves was removed surgically and immediately a cotton swab (absorbant cotton) soaked in dist water or test solutions was placed over the cut portion. At every 12 hr interval fresh solutions were added to the cotton swabs and incubation flasks.

4.2C. Quantiative analyses of *in vivo* IAA and IAA oxidase in the leaves and the epiphyllous buds during bud outgrowth:

To examine the role of endogenous auxin in controlling the polarity in epiphyllous bud outgrowth the relative levels of IAA and IAA oxidase enzyme were followed.

4.2C.1. Quantification of endogenous IAA in various parts of a leaf:

The procedure followed for the extraction (Henson and Wareing, 1977) and flourometric quantitation of IAA (Stoessl and Venis, 1970) is being described in chapter 6.

4.2C.2. Changes in IAA oxidase in different parts of a leaf:

The IAA oxidase levels were studied by the modified method of Gordon and Weber (1951). The extraction and partial purification of the enzyme and its assay is being described in chapter 6.

4.3 RESULTS

4.3A. Studies on isolated leaves/leaf parts:

To find out the site of the regulatory factor responsible for the polarity of epiphyllous bud growth (Fig.4.1a), the lamina was cut transversely at a mid point and the lower part was cultured with its petiole dipped in distilled water. The very first buds to be released from dormancy were located in the uppermost notches near the cut end (Fig. 4.1c and b). Gradually new buds appeared in subsequent lower notches. Thus even the cut lamina exhibited the same pattern of bud growth as displayed by the intact leaf in the culture (Fig. 4.1a to e).

Similarly, the upper portion of the leaf also showed the same pattern of bud outgrowth (Fig. 4.1 d). However, when separated into paired notches (transversely), the apical part demonstrated bud outgrowth from all the notches on almost the same day (Fig. 4.1 d).

Isolation of leaf into various halves and into segments containing varying number of notches also demonstrated the polarity phenomenon. Epiphyllous buds also grew equally well in the isolated notches (measuring 1 cm^2) (Fig. 4.1 e). Presence of axillary bud did not change the polarity phenomenon of the epiphyllous bud outgrowth.

To test whether the correlative inhibition is responsible for the polarity of epiphyllous bud growth, the leaf was cut

transversely into three parts - apical, middle and basal. All of them were placed on filter paper soaked with dist water.

Two sets were kept, one with intact leaf parts and second one having leaf parts separated into individual notches (1 cm^2).

In case of the intact leaf parts, the bud growth response was maximum (70 %) from the basal part of the lamina; whereas minimum (58 %) response was obtained in apical part (Fig. 4.2). Growth of buds from basal and middle parts of the lamina was observed on 7-8 day and 3-4 day of incubation respectively, while in the apical part on the day 3.

When the isolated notches were incubated in cultures, the maximum response (96 %) was found in the notches excised from middle part of the leaf (Fig. 4.2). The notches derived from the apical and basal parts registered 50 and 79 % bud growth respectively. While the bud growth response in apical notches was observed on 3-5 day after incubation, buds of the middle and basal notches displayed growth on 4th and 7th day respectively. In case of control (whole leaf) the bud growth response noted was 85, 76 and 56 respectively in apical, middle and basal notches. The appearance of buds in apical, middle and basal notches was observed on 3-4, 4-6 and 7 day after isolation and subsequent incubation.

4.3B. Effect of plant growth regulators on the polarity in epiphyllous bud outgrowth:

Various plant growth regulators were applied through the apical and basal end of the leaf (4th nodal) in order to study

their effect on the process of probable correlative inhibition (polarity) as observed in the growth of epiphyllous buds.

4.38.1. Basal application of growth regulators:

a) Auxin - NAA was used as the auxin due to its greater stability than IAA against light and enzymatic degradation. In control, the leaves showed 60 % of bud growth response (Fig. 4.3 a). Treatment with NAA in the concentration range of 0.01 to 10 µM did not change the bud growth response. At 100 µM the bud growth response was however, reduced by 40 % (Fig. 4.4 a).

Similary the basal application of IAA caused slight inhibition of bud outgrowth upto 10 µM concentration (Fig. 4.3 b), but further increase in the IAA promoted bud growth.

When, TIBA an inhibitor of auxin transport was applied to the base of the leaf, inhibition of bud growth was noticed at higher concentrations (1.0-100 µM) (Fig. 4.5b). At lower concentration the bud growth response was marginally higher than the control (Fig. 4.3 c).

In contrast to the weak inhibitory effects of NAA and TIBA, BAP - a cytokinin, exhibited strong inhibition of bud growth. At 0.01 µM concentration of BAP the bud growth response was reduced to 50 % of the control. At 10 and 100 µM, the response was drastically reduced to only 03 and 01 % respectively (Fig. 4.4 a).

The inhibition of bud outgrowth by BAP at higher concentration was however temporary; for after a lag of 16-18 days, the buds located at the lower most end of lamina exhibited growth. The entire basipetal progression of bud growth which is characteristic in control leaf, was completely reversed to acropetal succession by BAP treatment, not only to the entire leaf, but also to its part (Fig. 4.5 d and e).

When the leaves were treated basally by GA_3, the bud growth was found to be slighly higher (67 %) at 0.01 µM concentration than that in the control (59 %) (Fig. 4.4 b). As the concentration of GA_3 was raised further, the bud growth response decreased progressively. The response was found to be 38 and 21 % at 0.1 and 100 µM concentrations respectively (Fig. 4.6a). Complete inhibition of bud growth analogous to BAP treatment did not occur by GA_3 treatment.

Application of ETH through cut petiole caused increase in the bud growth response (Fig. 4.4c). The bud growth was 74 and 88 percent at 0.1 and 10 mM concentrations of ETH respectively compared to 62 % in the control leaves. Higher concentration of ethrel (100mM) was toxic to the leaf as it showed pronounced wilting and senescence (Fig. 4.6b). Therefore, the treatment was discontinued.

ABA caused weak inhibition of the bud growth. Compared to 64 % bud growth in the control, ABA displayed 55 and 46 % bud growth at 1.0 and 100 µM concentration respectively (Fig 4.4d).

4.3B.2 Apical application (i.e. tip application) of plant growth regulators:

Apical application of PGR refers to the application at the leaf tip and not at the plant apex. Apical application of NAA upto 1.0 μM concentration enhanced the bud growth response. At 0.01 μM NAA, 80 % of buds showed growth compared to 67 % in the control leaves (Fig. 4.3a). No inhibitory effect was observed even when the concentration was raised to 100 μM.

Apical tip application of IAA demonstrated the same pattern as for the basal application. While the lower concentrations (upto 10 μM) of IAA were inhibitory, 100 μM IAA was stimulatory for bud growth response (Fig. 4.3b).

Though bud growth response was slightly stimulated by 1 μM IBA application, not much inhibitory effect was found at its higher concentration (Fig. 4.3c).

Like the basal application, the apical treatment of BAP was markedly inhibitory for the bud growth. At 1.0 and 100 μM concentration the bud growth response was reduced to 56 and 06 % respectively (Fig. 4.4a). At the highest concentration (100 μM) used, the buds appeared only on the top most notches which were nearest to the point of application (Fig. 4.6c).

In case of GA_3 application through the tip of the leaf, the bud growth was inhibited; but not as strongly as that observed in BAP application. The bud growth obtained was 65 and

30 % at the minimum (0.01 μM) and maximum (100 μM) concentration used compared to 70 % in case of the control (Fig. 4.4b).

The apical application of ETH caused a slight promotion of bud growth response upto 100 μM concentration. At higher (100 μM) concentration, the response recorded was 85 % compared to 69 % in case of the control leaves (Fig. 4.4c). However, further rise in ETH concentration suppressed the bud growth to 65 % at the highest concentration tested (10 mM).

ABA treatment displayed a weak stimulatory effect. At 1.0 and 100 μM of ABA, the response registered was marginally higher (83 and 80 % respectively) compared to the control (72 %) leaves (Fig 4.4d).

Due to the polarity phenomenon during the epiphyllous bud outgrowth, the response is nonsynchronous. For biochemical analysis it is mandatory to have certain synchrony in the system. This led to explore the use of above mentioned PGRs to achieve synchronous response.

Since notches from middle part of the leaf demonstrated the maximum bud growth response, for all biochemical analysis notches only from middle leaf were used. While control (dist water) notches exhibited almost 63 % of synchronous bud growth (Table 4.1) all the PGRs caused great inhibition of bud growth. However, in comparison to all other PGRs, BAP exhibited around 68 % synchrony.

However, this response is not much significant compared to the dist water control. Therefore, in all biochemical investigation, notches only from middle part of the leaf were used without any hormonal treatment.

4.3C. Changes in the endogenous IAA and IAA decarboxylating enzyme (IAA oxidase) levels:

Since high levels of auxin in the apical part of the attached Bryophyllum stem was reported to be inhibitory to the outgrowth of epiphyllous buds of leaves (Sebanek et al., 1978), the levels of IAA in various parts of the leaf of K.mortagei was examined. An inverse relationship between IAA oxidase activity and endogenous IAA levels has been demonstrated in various morphogenetic processes (Jain et al.,1969; De Greef et al.,1977; Jasdanwala et al.,1977). These results prompted to study the correlation between the level of IAA on the one hand and IAA oxidase on other in dormant epiphyllous buds.

In a leaf the highest amount of IAA was recorded in the basal part. Further, the IAA content exhibited a decreasing trend towards the leaf apex, where the content estimated was about 67 % less than that in the basal portion (Fig. 4.7). The maximum amount of IAA oxidase was observed in the basal part of the leaf (Fig. 4.7). While the uppermost apical part registered the minimum level of enzyme (2.8 units) the middle portion showed 3.6 units/mg protein almost 3.5 fold less than the maximum amount in the basal portion.

4.4. DISCUSSION

Although the complete understanding of the pattern inception in plants is still elusive (Raven and Rubery, 1982), there exist theoritical frameworks and model chemical reactions (Turing, 1952; Winfree, 1973) allowing relatively stable regularities to arise from an initially homogenous situation. While these may be the useful paradigms, the molecular organisation of cells themselves is not uniform; for example, the polarity of carrot embryoids (and other systems) first become visibly established by an unequal division of the single somatic cell (Backs-Husemann and Reinert, 1970). Also, tissues as such meristems or even callus cultures will inevitably be non-uniform due to the physical and chemical gradients in the system and due to the cells' responses at different positions in such gradients (Trewavas, 1982b). Cells communicate with one other by electrical (ionic) messages and more specifically by chemical messengers (growth regulators) (Bentrup, 1977; Jaffe, 1980; Wareing, 1977; Trewavas, 1976; 1982a) through receptors (Trewavas, 1981; 1983; Jacobson et al., 1987), calcium and inositol phospholipid turnover (Hepler and Wyne, 1985; Roux et al., 1986; Reddy et al., 1987; Poovaiah and Reddy, 1987; Poovaiah et al., 1987).

Thus, the important feature of coordinated spatial responses in plants is the gradient(s) of hormone(s) which can differentially activate the receptors and target reactions in a part relative to another (Raven and Rubery, 1982). This led

to the present investigations of effect of PGR on the polarity phenomenon in the epiphyllous bud outgrowth.

A phenomenon of polarity, similar to one observed in present studies, was also reported earlier in B. crenatum (Obhlidalova et al., 1979). These workers implicated gibberellins as the causal agent of polarity for it was associated with the decreasing levels of endogenous gibberellins towards the base of the leaf. Earlier, Nooden and Weber (1978) suggested that the dormancy in buds and seeds may be regulated by variations in the endogenous levels of gibberellins and possibly of cytokinins. If this could be the case in K. mortagei then the exogenous application of gibberellins should stimulate the growth of epiphyllous buds. However, GA_3 application did not alter the polarity phenomenon when applied apically or basally to the leaf. On the contrary, GA_3 application caused great reduction the number of buds undergoing reactivation. This is in contrast to well known effect of gibberellins in stimulating germination of dormant buds and seeds (Wareing and Phillips, 1982; Leopold and Nooden, 1984). As such gibberellins are known to exert their effects by altering the auxin status of the tissue, by increasing the endogenous auxin levels which has been demonstrated in many cases. Application of gibberellin augmented the auxin levels in rosette Hyocyamus plants (Kuraishi and Muir, 1963), bean shoots (Nitsch and Nitsch, 1959). There are evidences to show that gibberellin increases auxin levels either by the enhancement of auxin biosynthesis (Sastry and Muir, 1965;

Jindal and Hemberg, 1976) or by retardation of auxin destruction (Kogl and Elema, 1960) presumably by reducing the levels of IAA oxidase and peroxidase (Galston and McCune, 1961).

In certain systems, gibberellins are found to be inhibiting cell division (Setterfield, 1963; Kaufman et al., 1969) including cell expansion (Brain and Hemming, 1955; Wright and Aung, 1975), presumably due to the increased sensitivity of responding tissue to IAA (Katsumi and Kazama, 1974) and reduced sensitivity to ethylene (Palmer, 1972; 1975). Perhaps, since primordia of shoots are already laid in the notches during leaf ontogenesis in K. mortagei the earlier manifestation of bud outgrowth seems to be mere cell expansion. Usually the growth of inhibited buds involves two phases - an initial release from inhibition involving cell expansion (Hall and Hillman, 1975; Couot-Castelier, 1978; Yeang and Hillman, 1981b) followed by rapid establishment of the shoot through cell division. Thus the inhibitory effect of GA_3 in the present study is likely to be due to the limitation on the cell expansion phase during early stages of bud outgrowth in K. mortagei.

Each of the five major plant hormones - auxins, cytokinins, gibberellins, ethylene and abscisic acid has been implicated in the regulatory control of specific developmental processes occurring at the cellular, tissue or organ level. However, in nearly all of these specific responses have proved to be very complex (Letham, 1978). Further, the exogenous application for the influence and magnitude of the response depends upon

the efficiency of uptake and extent of degradation enroute to the active site (Firn, 1986) or likely to be through changes in the growth substance sensitivity (Trewavas, 1982a, 1983).

The application of a synthetic auxin - NAA to both apical and basal ends of the leaf, did not affect the pattern of polarity of epiphyllous bud outgrowth per se. As such leaves are known to contain auxins, specially young leaves being the sites of auxin biosynthesis (Digby and Wareing, 1966; Koukkari and Warde, 1985). However, the distribution of diffusible auxin in a maturing leaf has been found to be decreasing at the tip of the leaf first. This corresponds to the fact that in a growing leaf, growth ceases first at the tip. Further, on detachment of the leaf, auxin is found to be present in the diffusate (Burgess, 1985). It is also known that auxin transport occurs in a highly polarized manner in the basipetal direction (Jacobs, 1984), through polar diffusion in cells without any metabolic energy being used (Rubery and Sheldrake 1974; Raven, 1975; Goldsmith, 1977, Goldsmith et al., 1981) and thus accumulates at the base of an excised leaf (Elliott, 1977).

One may hypothesize that upon excision of Kalanchoe leaf, the supraoptimal auxin at the leaf tip begins its basipetal movement and accumulates at the base of the leaf. Further, low IAA oxidase levels compared to the higher content of IAA in upper part of the leaf, unlike equal amounts in the middle

part/notches implies the occurrance of a basipetal displacement of auxin in the isolated leaves (Fig. 4.7). If this could be the case, then application of auxin transport inhibitor e.g. TIBA (Kuse, 1953) should show an inhibitory effect on the bud outgrowth. Data shown (Fig. 4.5b) clearly indicates the inhibitory effect of TIBA on the induction of bud growth only if applied basaly through petiole. Tip application of TIBA had little effect presumably due to the likely diffusion of auxin through nonvascular tissue (Phillips, 1975). Localized degradation of auxin may also be having some role in promoting bud growth as the exogenous application of PCA (p-coumaric acid) - a cofactor of IAA oxidase enzyme (Gortner et al., 1958), was shown to stimulate this process (Houck and Rieseberg, 1983). Thus severing off the leaves from plant must have caused rapid degradation of auxin in the system enabling the induction of bud growth from isolated notches or in detached leaves. Exogenous application of NAA probably, strives to bring back the supraoptimal level of auxin similar to the intact leaves and thus exhibiting some inhibitory effect. Recent work with bud - bearing isolated stem sections of Phaseolus demonstrated the polarity to be a factor in the response of axillary buds to auxin. Though apical application of auxin caused inhibition, the basal treatment had no effect or caused a slight stimulation of bud growth. It was concluded that the basipetal tendency of auxin transport seems to be necessary for the correlative inhibition (Tamas, 1987).

With respect to uptake, mobility and metabolism, there are dramatic differences between the naturally occurring IAA and synthetic auxins. While NAA is quite stable, it has been found to register very little uptake and subsequent very slow mobility in the intact pea seedlings (Jacobson, 1984a), likely to be due to the lack of conjugation with auxin binding proteins (Jacobson, 1984b; Jacobson et al., 1987). In apple seedlings, (^{14}C)NAA was not mobilized at all (Hatch and Powell, 1971). This could be one of the probable reasons of partial inhibitory effect of exogenous NAA on the bud outgrowth rather than absolute. This observation was supported for application of IAA showed greater inhibitory effect upto 10 μM concentration than the synthetic one (NAA).

Cytokinins are known to induce shoot bud formation (Cornejo-Martin et al., 1979; Horgan, 1984; Matthysse and Scott, 1984). But, in the present studies BAP was found extremely inhibitory for the bud outgrowth when applied exogenously at either apical or basal end. Further, the basal treatment with higher concentration (≥ 1 μM) resulted in complete reversal of polarity in epiphyllous bud outgrowth (Fig 4.5d,e). Similarly, the apical application of BAP promoted the bud growth only at the site of treatment (Fig. 4.6c).

When kinetin was sprayed directly on the leaves, its effect was found to be quite localized. The inhibitory effect of cytokinin can be interpreted in terms of well known sink

effect of cytokinin (Mothes and Engelbrecht, 1961; Sachs and Thimann, 1964; Ross, 1986; Ross and Murfet, 1985 a and b). It seems that the cytokinin applied to the Kalanchoe leaf diverted all nutrient supply towards the site of application. Deprived of nutrients, the epiphyllous buds located distally to the site of application failed to grow. This hypothesis was supported by our observation that a small cut in the midrib below the site of cytokinin application relieved the epiphyllous buds from the inhibitory effect so observed (Fig. 4.6d). Apart from this numerous adventitious buds developed from the cut part of petiole or leaf tip by BAP treatment (Fig. 4.8a to c).

Other possibility could be the establishment of optimum auxin/cytokinin ratio (Skoog and Miller, 1957) through their effect on IAA oxidase activity. High kinetin levels has been found to be inhibitory to IAA oxidase activity (Lee, 1971; 1974). Similar results were obtained in the present studies during epiphyllous bud outgrowth (chapter VI). Another possibility of perferential transport of cytokinin to the uppermost notches in vivo to the exclusion of middle and basal notches can not be ruled out as noted by Tucker (1979) in tomato. However, there is also a likelihood of induction of bud growth first in upper notches in response to locally produced cytokinin (Tucker, 1977).

Ethylene is known to be involved in the control of a wide range of developmental responses including growth, abscission, senescence, fruit ripening, etc, (Lieberman, 1979a and b;

Sanders et al., 1986). Induction of physiological stress either by wounding, mechanical perturbation, drought or nutritional stress causes ethylene biosynthesis (Wright, 1974, 1977; Guinn, 1976a and b; Hanson and Kende, 1976; Yang and Pratt, 1978; Boyer et al., 1983; Biro and Jaffe 1984; Yang and Hoffman, 1984; Casselle and Tamma, 1986). Thus the detachment of the leaf from plant and subsequent isolation of notches in K. mortagei could result in ethylene formation whose augmented level might be triggering the epiphyllous bud outgrowth. However, the mechanism by which ethylene biosynthesis shoots up and affects physiological process is not yet fully understood (Moore, 1978; Prasad and Cline, 1985a,b; Sanders et al., 1986). According to one hypothesis, exogenous treatment with ethylene induces an autocatalytic type chain of reactions (Abeles, 1973; Evans, 1984) probably triggered by the formation of unique isozyme of perioxidase. This isozyme has been suggested to play a role in ethylene production (Ku et al., 1970). This theory was supported by the observation that ethylene formation from ACC is catalyzed by IAA oxidase in vitro. Shimokawa (1984) has demonstrated the role of IAA oxidase system in vivo in the last step of ethylene biosynthesis. This seemed to show that exogenous application of ETH might be boosting the IAA oxidase levels via its effect on its own augmented biosynthesis leading to enhanced bud outgrowth. Further, it was observed that the epiphyllous buds of the attached leaves when sprayed with ETH were released from their dormant state and initiated growth (Fig. 3.7b).

Ethylene and IAA work as balancing members of a feedback system, wherein IAA stimulates the synthesis of ethylene which in turn inhibits the synthesis as well as transport of IAA (Burg and Burg, 1966; 1967; Pratt and Goeschl, 1969; Kang et al., 1971; Kang, 1979). It has been shown that ethylene lowered the auxin release from coleoptile tip to about 65 % and auxin production to about 25 % of the controls (Van der Laan, 1934). Further, the ethylene induced inhibition of auxin transport seems to be very fast as demonstrated in etiolated Pisum epicotyles (Burg and Burg, 1966). Thus it is likely that the apical application of ethylene to the detached leaf of K. mortagei blocks the movement of auxin from the tip portion of the leaf and probably at the same time augmenting IAA oxidase levels. All these changes ultimately might be leading to the reduced levels of supraoptimal IAA. Indeed it has been shown that the capacity for germination in seeds start to develop at a time when endogenous auxin levels are very low in apple (Kopecky et al., 1975) and Acer (Nikolaeva, 1977; Tillberg and Pinfield 1981). Similarly, Wood (1983) observed a low auxin level in buds of pecan prior to bud break. It thus seems that the ethylene biosynthesis is involved in the early stages of bud development in K. mortagei. Similar conclusions were drawn by Van Aatrijk (1986) for bud formation in Lilium speciosum.

Effect of basal application of ethylene on epiphylleus bud growth of K. mortagei appears to be concentration dependent as linear increase in the number of bud outgrowth was observed with

a rise in ETH concentration (Fig. 4.4c). A rapid senescence of the leaves was observed at 0.1 M concentration of ETH. This could be due to the observation that ethylene enhancement is involved with senescing tissue (Lieberman, 1979b) and higher concentrations are often inhibitory to the growth in general (Matthysse and Scott, 1984). Similar observations were noted in the apical application of ETH, where higher concentrations ($\geq$ 1 mM) were inhibitory to bud growth.

Finally, the stimulatory effect of ABA application of outgrowth of epiphyllous buds of K. mortagei is also interesting as ABA, in fact, is known to promote and maintain the bud dormancy (Wareing and Saunders, 1971; Tucker and Mansfield, 1973; During and Bachmann, 1975; Fernandez-Muniz and Sanchez-Tames, 1982; Rodriguez and Sanchez-Tames, 1986). Perhaps this stimulatory effect of ABA may be indirect via imposition on transport (Milborrow, 1978) and degradation (Anker, 1975; Milborrow, 1966) of endogenous auxin. ABA induced growth of axillary buds has been reported when applied to the apex (Bellandi and Droffling 1974), to cut stumps (Hillman, 1970; Hartung and Fufer, 1981) or directly to the lateral bud itself (Hartung and Steigerwald, 1977).

These studies implies that isolation of leaf induces the wellknown phenomenon of basipetal transport of auxin from apical uppermost notches. This basipetal transport might also be accompanied by localized destruction of IAA for rapid achievement

of appropriate auxin/cytokinin ratio; thus, stimulating bud development first in those topmost notches, which acts as a temporary sink for nutrients and other stimulatory factors. Once meristems of the first notches become reactivated and begin to make its own auxin, their sink effect is reduced. Thereafter the successive notches act as sink and the pocess process continues on the subsequent lower notches. This could be the most plausible mechanism of the polarity observed during the outgrowth of epiphyllous buds.

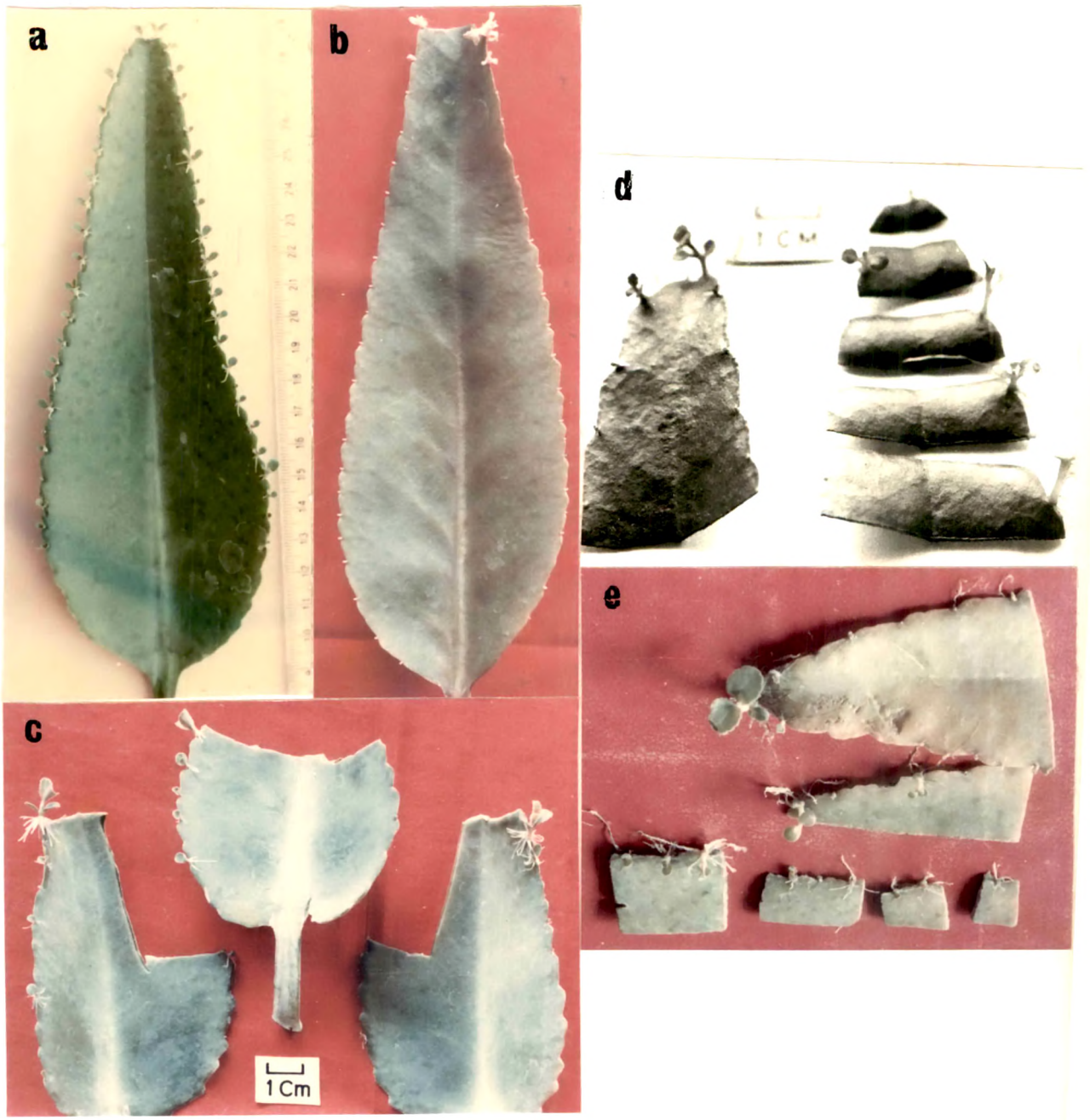
Thus following observations and forgoing discussion clearly implicate the likelyhood of a presence of phenomena similar to correlative inhibition (Hillman, 1984) in the epiphyllous bud outgrowth in K. mortagei.

1) The ability of the uppermost notches to demonstrate very first bud outgrowth compared to other substending notches.
2) Removal of upper notches (extreme or all from the upper part of the leaf) causes the subsequent physiologically upper notches to behave as the topmost one and show first bud outgrowth.
3) Separation of leaf into individual notches (measuring 1 cm^2) giving equally good and synchronous bud outgrowth response.

Further, the highest auxin levels in the uppermost part of leaf compared to the middle part and several fold increase in IAA oxidase activity in the early stages of bud outgrowth implicates the role of IAA in correlative inhibition. It was

demonstrated that the bud growth inhibition can be relieved when a ring of lanolin containing TIBA was placed around the stem between the apex and the bud (Tucker, 1978). Furthermore, TIBA induced inhibition of epiphylleus bud outgrowth in present studies, supports the involvement of IAA transport in polarity. When branching (lacking apical dominance) and non-branching lines of tomato were tested for their ability to export radio-labeled IAA from the shoot apex, only the latter was able to do so (Salerno and Brenner, 1983), indicating that branching character is due to the failure of shoot apex to export IAA. It may be concluded from these points that the release of IAA from the apex and its subsequent transport is an essential component responsible for correlative inhibition (Tamas, 1987). A similar conclusion was reported about the possible role of IAA in reproductive dominance (developing fruits and seeds) over axillary bud growth in Phaseolus and Glycine (Tamas et al., 1981; 1985). In this context developing fruits and seeds have been recognized to be rich in IAA (Bandurski and Schulze, 1977).

Fig. 4.1. Polarity in epiphyllous bud outgrowth in detached leaf (a).
Note that (1) the polarity in bud outgrowth reestablishes upon the removal of uppermost apical notches (b) or uneven upper part of the leaf (c).
(2) the isolation of leaf into individual notches relieves the polarity phenomenon (d and e).


a
b
c
1 Cm
d
1 CM
e

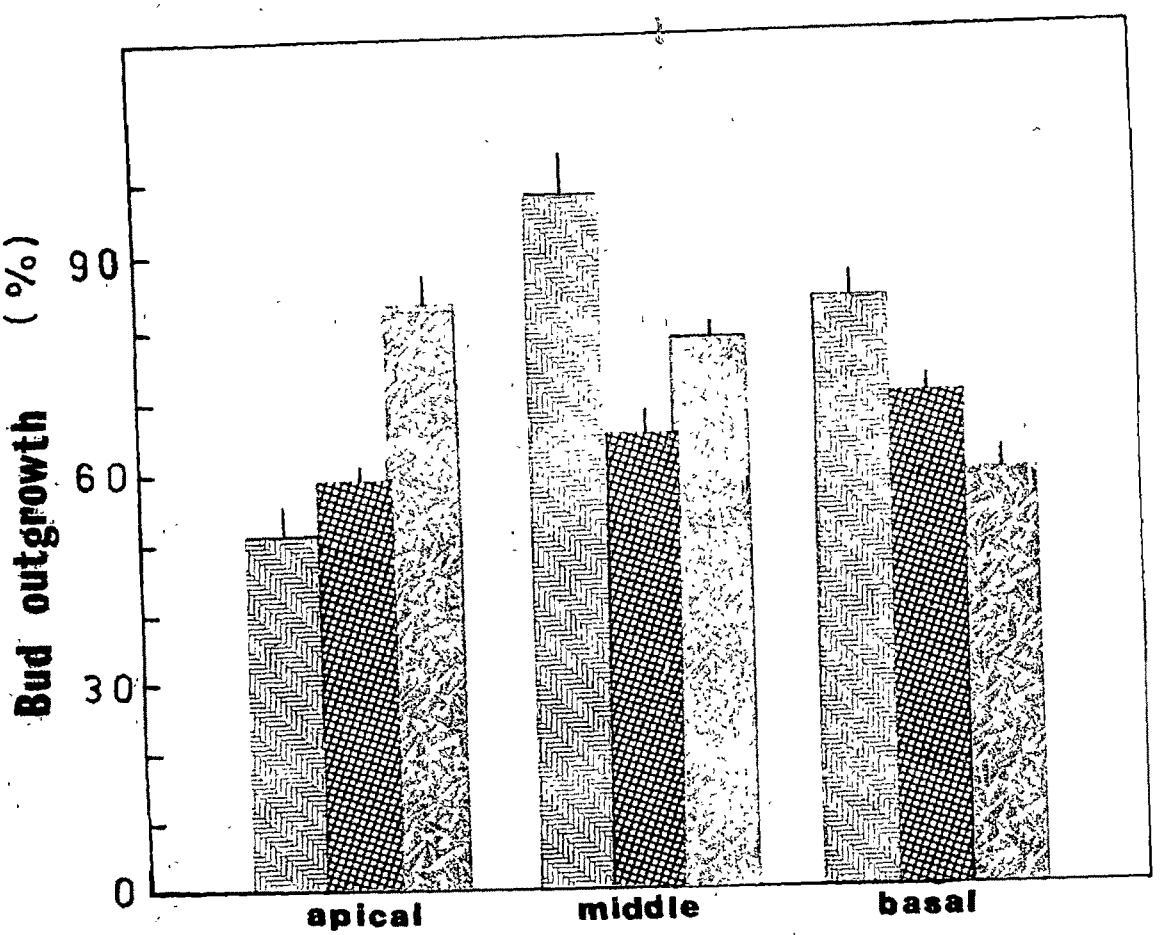


Fig. 4.2. Effect of separation of leaf into various parts (apical, middle and basal) and their individual notches on epiphyllous bud outgrowth.
(▨) Intact leaf, (▩) Intact leaf parts and
(▧) Isolated notches from various leaf parts.

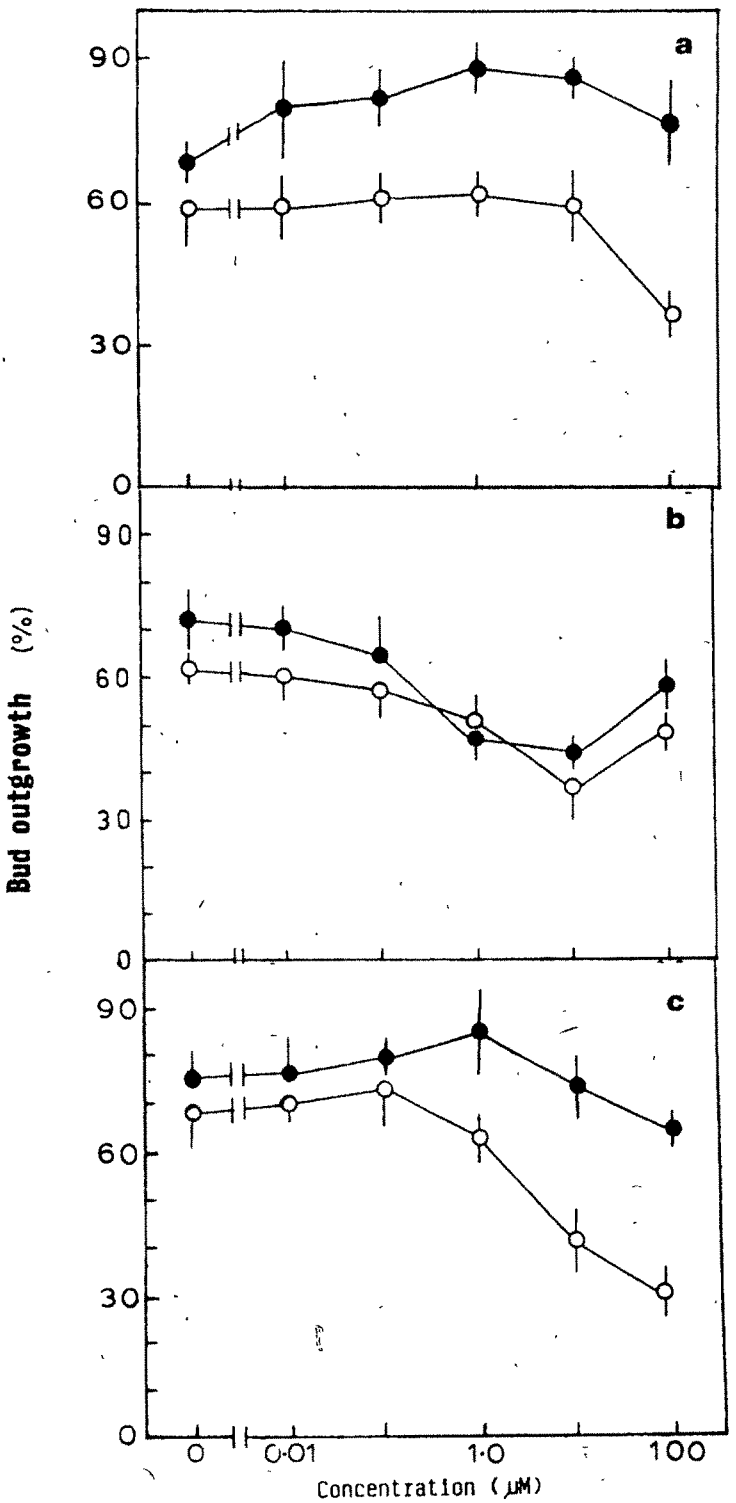


Fig. 4.3. Impact of NAA (a), IAA (b) and TIBA (c) application on bud outgrowth response. (●) apical application, (○) basal application.

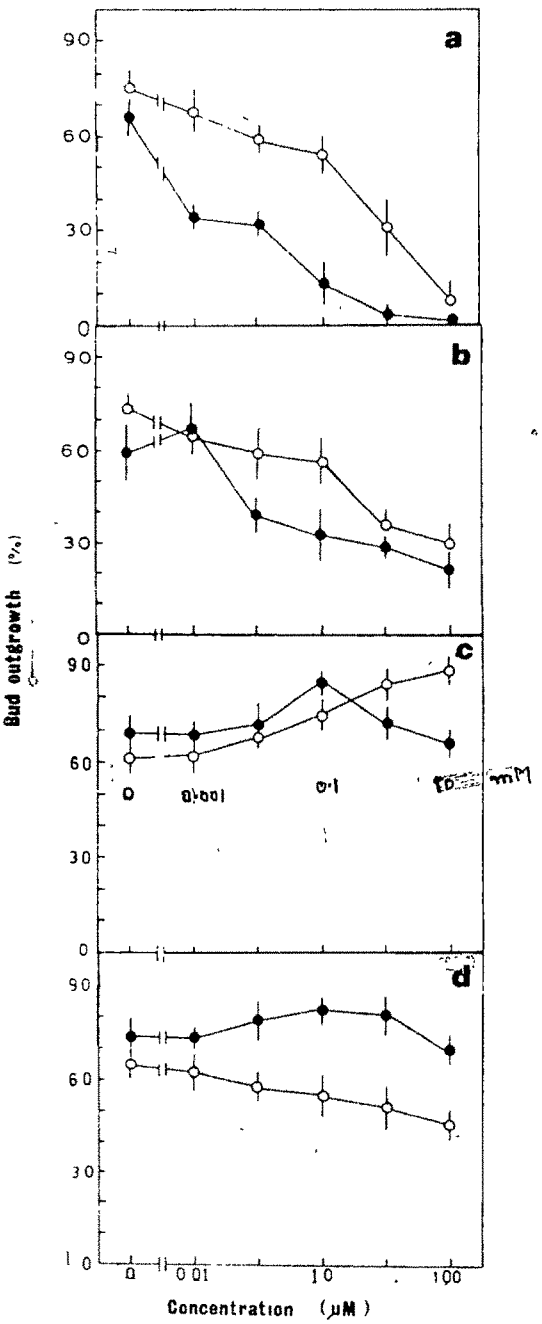


Fig. 4.4. Changes in bud outgrowth in response to basal/apical tip application of BAP (a), GA_3 (b), ETH (c) and ABA (d). For (a) and (b): (○) apical and (●) basal application. For (c) and (d): (●) apical and (○) basal application.

Fig. 4.5. No effect of basal treatment of NAA (a) or TIBA (b) as compared to control (c) on polarity phenomenon in bud outgrowth. Note complete reversal of polarity by basal application of BAP to leaf (d) and leaf part(e).

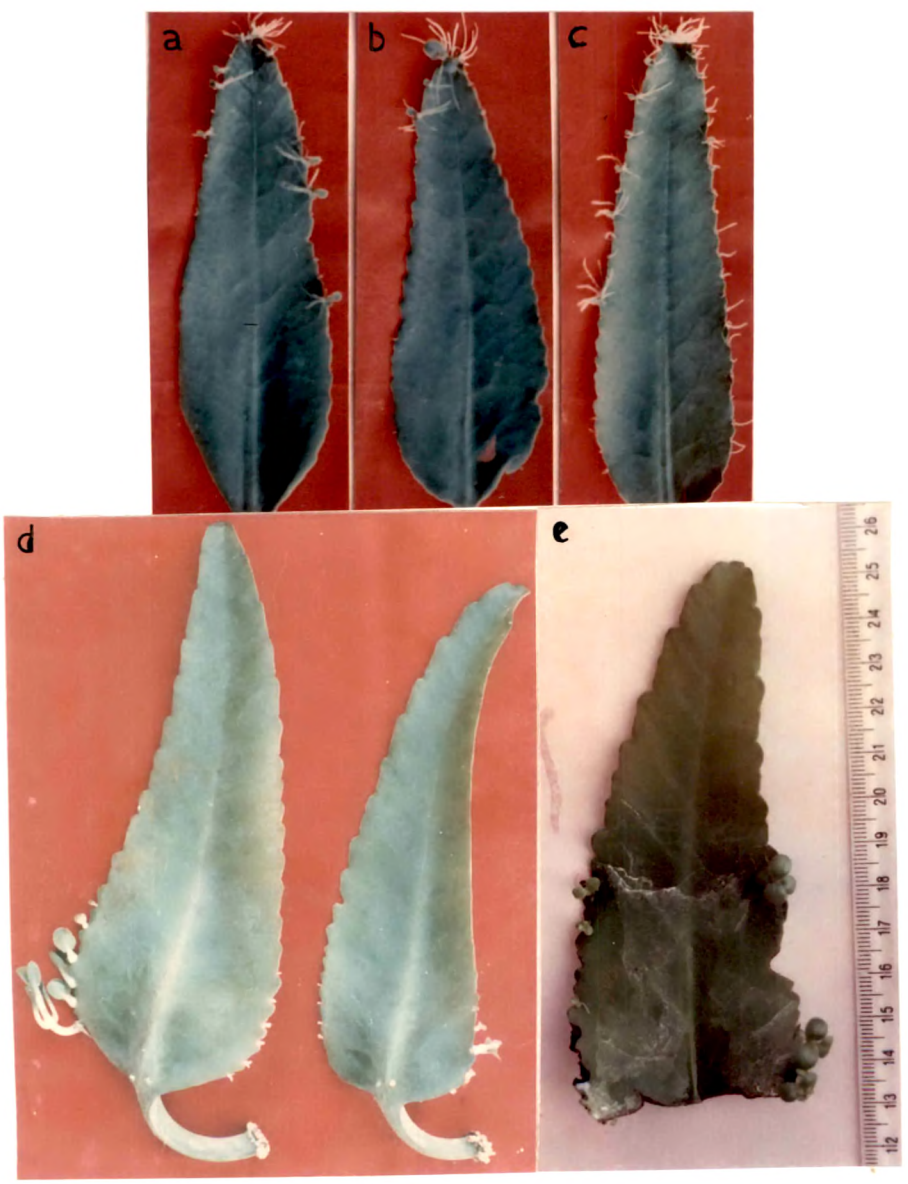
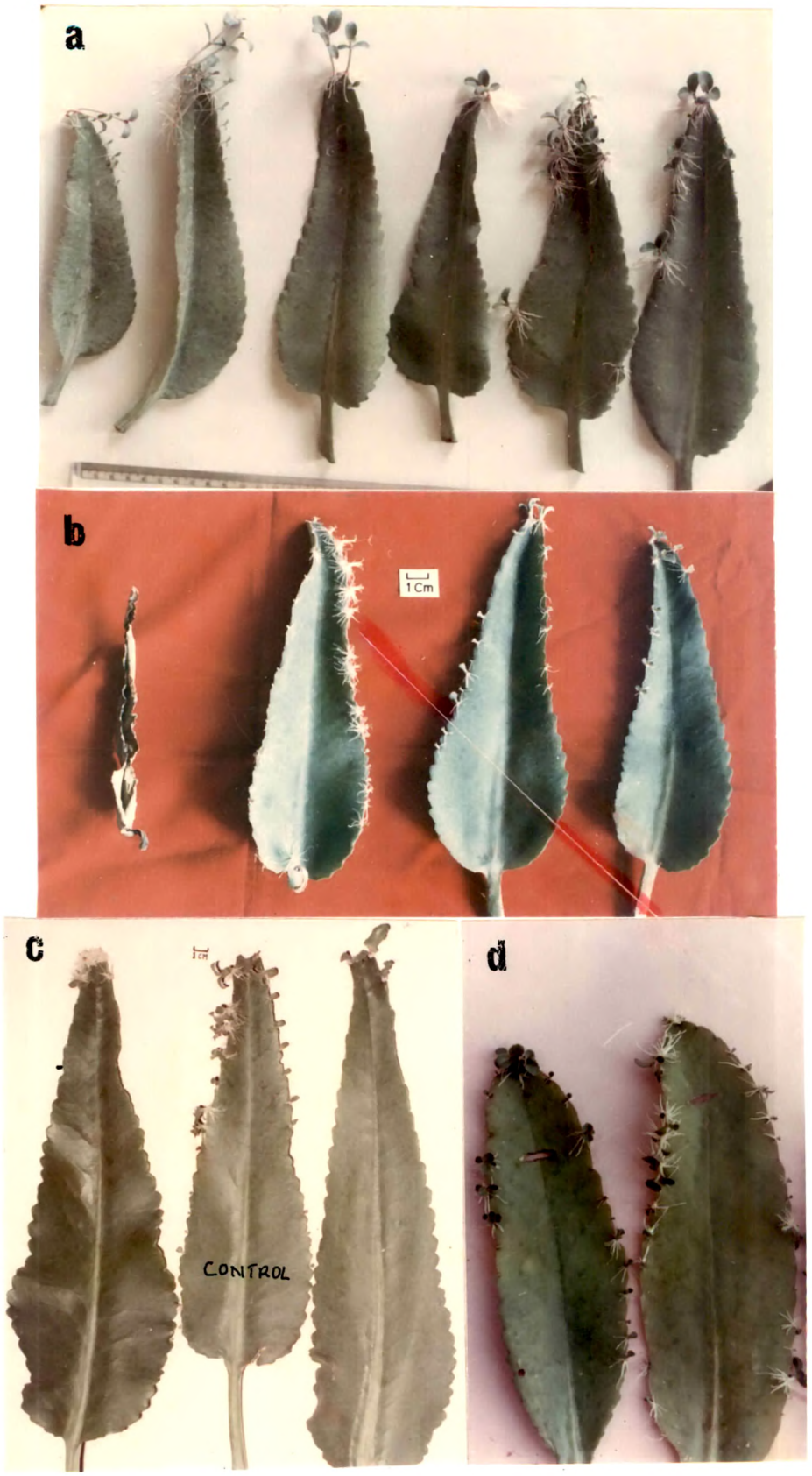

a
b
c
d
e

Fig. 4.6. Effect of basal treatment of GA_3 (a) and ETH (b) and apical application of BAP (c) on bud outgrowth. Note that the buds develop only at the site of cytokinin application, but a cut in midrib (just below the point of BAP application) stimulates the successive subtending buds' growth (d).
For (a) and (b): the concentration increases from right to left.


a
b
1Cm
c
CONTROL
d

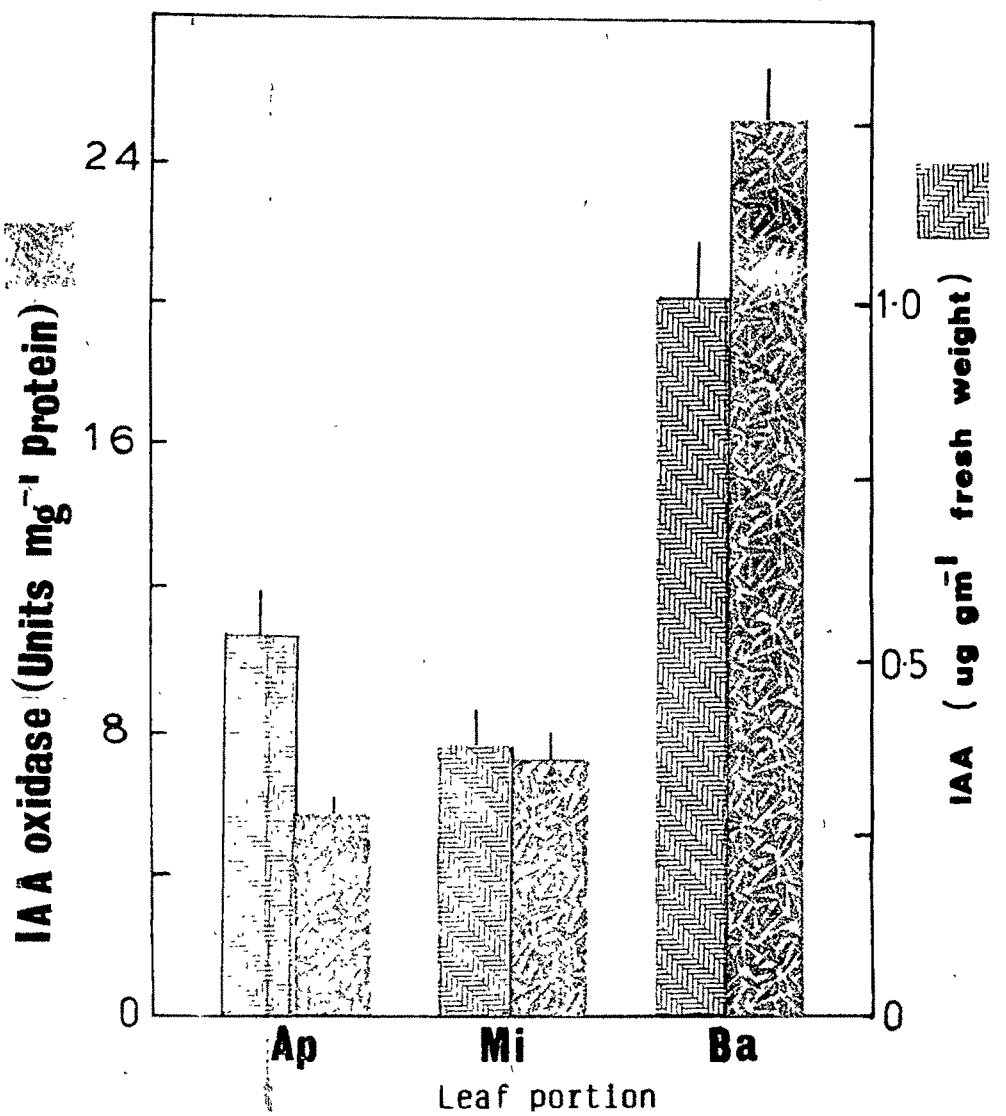


Fig. 4.7. Levels of endogenous IAA (▨) and IAA oxidase enzyme (▩) in various parts of the leaf. ap - apical, mi - middle, ba - basal.

Fig. 4.3. BAP induced adventitious bud formation from petiole (a and b) and leaf tip (c).

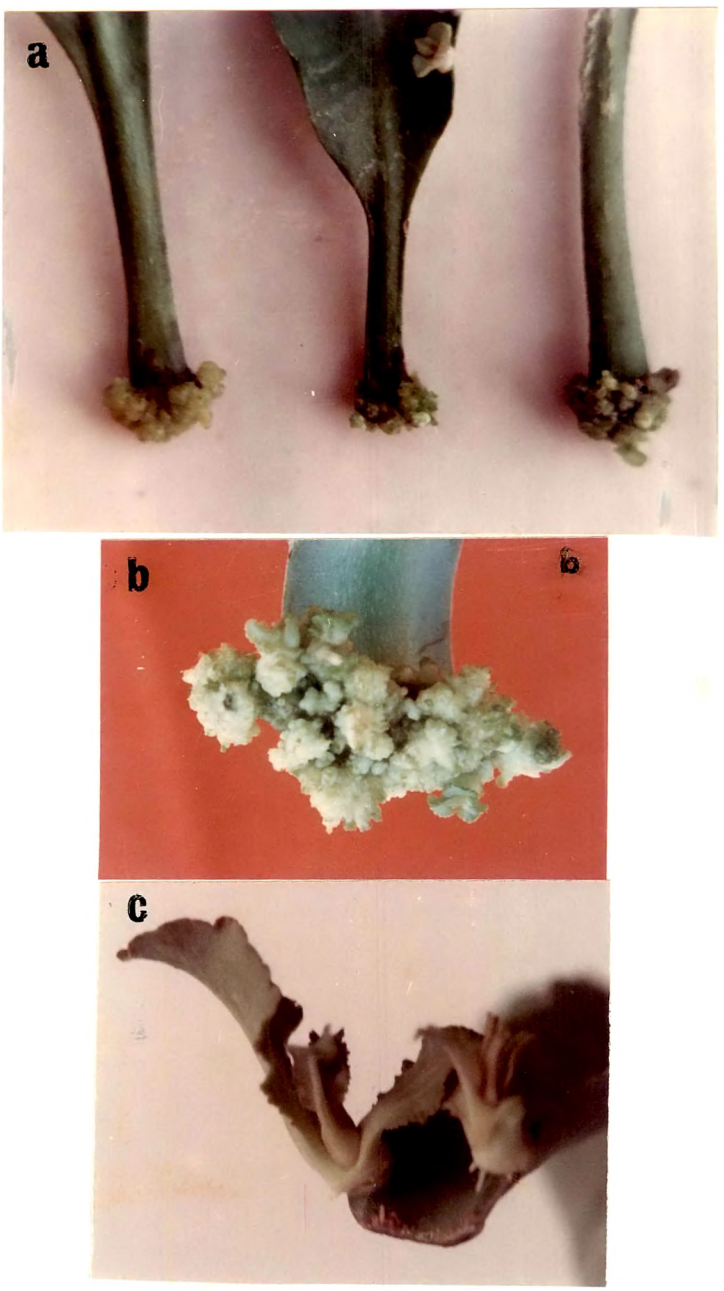

a
b
b
c

Table -4.1. Effect of various plant growth regulators on epiphyllous bud outgrowth in vitro in *Kalanchoe mortagei*

Treatment	% of buds showing synchronous outgrowth
Control (dist water)	62.8 ± 2.02
NAA	49.4 ± 3.61
IAA	43.6 ± 4.21
GA_3	40.2 ± 4.83
BAP	68.6 ± 2.62
TIBA	58.8 ± 3.47
ABA	39.1 ± 4.16
ETH	57.2 ± 3.92