# 7. SUMMARY AND HIGHLIGHTS

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The past few years have witnessed an ever increasing advances in plant tissue culture and in the application of *in vitro* techniques for breeding and propagation, disease eradication, mutants resistant to various adverse conditions, storage of germplasm etc (Ammirato, 1986). However, with few exceptions, the successful application of plant culture techniques rests ultimately upon the ability to regenerate plants from cultured cells and tissues and to control the developmental pathway once established (Ammirato, 1986). Of course there are several general recipes, rules and approaches which can be tried and are sometimes successful. However, the fact remain that the empirical approach is time consuming and not scientifically satisfying (Yeoman, 1986). Molecular knowledge of the underlying control of regeneration, including the triggers and markers of this process is mandatory for better comprehension of the process and its appropriate application. However, because of certain peculiarities of the plant developmental processes and baring few well defined experimental systems, viz. somatic embryogenesis (Nomura and Kommaine, 1986), epidermal cell layers (Tran Thanh Van, 1973) etc., detailed molecular analysis of these processes have not been achieved (Sanchez-Martinez *et al*., 1986; Tran Thanh Van and Trinh, 1986; Yeoman, 1986), because of their own intrinsic disadvantages. Through the present investigation we have attempted, to show the potential of the

epiphyllous buds of Kalanchoe mortagei that offers an excellent experimental material fulfilling the criteria of Thorpe (1979) to study molecular aspects of plant development. Leaves of K. mortagei in their notches on their either sides contain number of meristems (epiphyllous buds) which develops into complete plantlet with some trigger. This system offers following advantages for basic studies pertaining to plant development -

1) Availability of large number of experimental material.
2) Known site of bud development.
3) Rapidity of the response.
4) Ease of experimental manipulations of the explants.
5) Availability of variants for the induction of bud development.

The present study was undertaken to examine following events underlying epiphyllous bud outgrowth in K. mortagei.

1) Anatomical studies of the epiphyllous buds during their dormancy and reactivation of growth.
2) Involvement of auxin in maintenance of dormant state and of ethylene in the breaking of dormancy of the epiphyllous buds.
3) Quantitative and qualitative changes of soluble proteins during dormancy and bud growth.
4) Involvement of de novo synthesis of IAA oxidase in the reactivation of epiphyllous bud outgrowth.

The epiphyllous bud meristems are being laid down during

the ontogenesis of the leaf. Leaf maturity (when it attains a 4th nodal position in a plant), coincides with the development of bud primordia. However, subsequently the bud primordia undergoes dormancy till the onset of favourable conditions which is the detachment of the leaf from mother plant. During the course of bud outgrowth, proteins and carbohydrates from the surrounding mesophyll tissue, are channalized to the growing bud meristem - a process analogous to the germinating seeds.

Preliminary studies demonstrated that the very first epiphyllous bud to be reactivated for growth were located at the leaf tip. Gradually, the successive lower buds exhibited growth thereby showing polarity in epiphyllous bud growth in a leaf. However, using BAP application we have been able to reverse the polarity phenomenon. That means, following isolation of leaf and subsequent basal application of BAP, the first dormant buds to be reactivated were localized at the lowermost notches at the leaf base.

A cut made in midrib (and not in the mesophyll of the lamina) so as to induce stress in the system causes the reactivation of growth in the dormant epiphyllous buds in the attached leaves. Application of $CoCl_2$ - an inhibitor of ethylene biosynthesis, immediately to the cut in the midrib inhibited the reactivation process completely. This clearly implicates the role of ethylene in the induction of epiphyllous bud outgrowth. Further, it is known that ethylene stimulates an autocatalytic type chain of reactions (Ables,

1973; Evans, 1984) probably triggered by IAA oxidase (Shimokawa, 1984). So it seems that stress induced ethylene biosynthesis triggers the augmentation of IAA oxidase levels which then brings down the supra optimal IAA levels. Earlier, Van Aatr-jik et al. (1986) have also reported the involvement of ethylene in an early phase of the adventitious bud growth on the bulb scale explants of Lilium speciosum.

The quantification of endogenous IAA and effect of various plant growth regulators implied the involvement of auxin as the controlling factor for dormant stage of the buds on attached leaves. The surgical experiments in K. mortagei refuted the likelyhood of correlative inhibition from apical/axillary meristems as the causal tissues for the epiphyllous bud dormancy. However, studies with isolated leaves and parts thereof, demonstrated the probable occurrance of correlative inhibition imposed by uppermost apical epiphyllous buds on the growth of subtending successive lower buds located within the leaf.

Experiments involving metabolic inhibitors indicated that reactivation and subsequent growth of dorment epiphyllous buds requires synthesis of new protein(s). Further using time course analysis, it was noted that new proteins are synthesized within first 12 hr of leaf isolation and subsequent incubation.

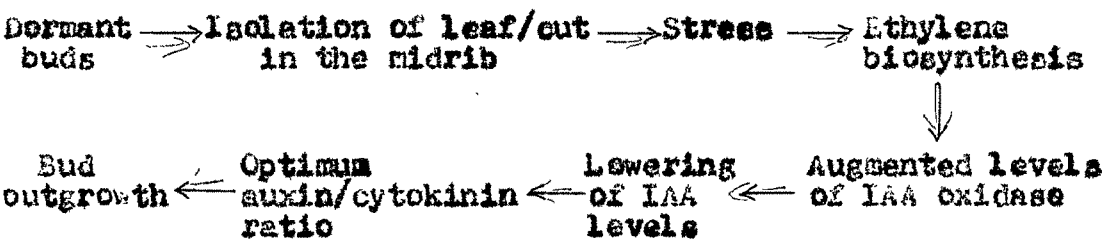
Qualitative analysis of soluble proteins by isoelectric focusing showed tremendous changes in protein pattern at various stages of epiphyllous bud outgrowth. A total of six proteins were identified as notch specific proteins.

A direct negative correlation was found between IAA levels and IAA oxidase activity during leaf development. Following leaf isolation and subsequent incubation of isolated notches, there was a six fold augmentation of IAA oxidase activity. This enhancement in the IAA decarboxylating enzyme activity coincided with the finding that new proteins are synthesized within first 12 hr of isolation. However with further progress in bud development process, the IAA oxidase activity demonstrated a declining trend. This clearly indicates the differential requirement of IAA for the reactivation of dormant bud growth and its subsequent development.

By in vivo labeling with ($^{14}$C) serine, it was found that of total incorporation in protein 13 % was found in IAA oxidase alone substantiating the earlier results.

To confirm, whether the increase in IAA oxidase activity is the cause/consequence of the bud growth process, three controls were performed. It was assumed that the function of enhanced levels of IAA oxidase is to lower down the endogenous inhibitory level of IAA to an optimum auxin/cytokinin ratio favourable for bud outgrowth. This was confirmed when the buds were incubated in the presence of BAP and pyrocatechol, where no increase in IAA oxidase level was noted. Similarly, Cycloheximide which inhibited the bud growth also repressed the IAA oxidase activity. These results clearly shows that the epiphyllous buds achieve the competence for growth when there is a decrease in supraoptimal IAA levels, through the action of IAA decarboxylating enzyme.

From these studies we present a following sequence of events for the reactivation of growth of dormant epiphyllous buds -



## HIGHLIGHTS

1) In *Kalanchoe mortagei* the dormant stage of epiphyllous buds is due to the supraoptimal levels of auxin.
2) Reactivation of the dormant buds occurs by detachment of the leaf from the plant.
3) The first step in the reactivation process is the biosynthesis of ethylene due to stress.
4) Ethylene induce *de novo* synthesis of IAA oxidase which lowers the concentration of auxin. This brings about an optimum auxin/cytokinin ratio favourable for bud outgrowth.
5) Some proteins are found specific to the notch region of the leaf.
6) Tremendous changes occur in protein pattern in the dormant buds, during their reactivation and subsequent growth.
7) There is a polarity phenomenon in the outgrowth of epiphyllous buds. This polarity can be completely reversed by cytokinin application.

Thus we present here a model system to study gene expression during plant development with a molecular marker.