

Discussion

DISCUSSION

I. General growth and development

Except a few reports (Chatterjee, 1977; Chatterjee et al., 1982; Chatterjee et al., 1985 a,b) physiological studies on growth and development of ipecac have not been frequently mentioned in literature. Though it has been under extensive commercial cultivation in India since some decades, this wonder-drug plant has not received its due recognition from the plant physiologists in India as well as abroad.

Chatterjee (1980) and Chatterjee et al. (1982) reported significantly higher occurrence of alkaloids in ipecac during reproductive stage of development. Different developmental phases in the life cycle of the plant in terms of reproductive status was recognised which revealed characteristic occurrence of phenolic and non-phenolic alkaloids during these developmental phases (Chatterjee, 1977).

Detailed analysis of different developmental phases reported in the present work could not strictly confirm the observation made earlier by Chatterjee (1977) and this study could identify more specifically three distinct reproductive and post-reproductive phases during its economic life cycle of normally 42 months; each phase being characterised by a definite proportion of occurrence of flowers and fruits. Growth behaviour and assay of alkaloids in roots of the plants growing at varying altitudes have been studied separately during different developmental stages and quantitative data on growth and formation of active principles showed that emetine content was low and cephaeline content was high in higher altitude.

One aspect of growth phenomenon of ipecac which appears interesting is its higher rate of linear growth during three reproductive phases and its lower expression during post-reproductive phases. While looking for a correlation between the growth activity and alkaloid formation, results revealed that

occurrence of alkaloid during three reproductive phases could find a distinct relationship with extension growth, more particularly during R I where the correlation coefficient values were highly significant. Rate of alkaloid synthesis became reduced in line with the decline in the rate of extension growth during these phases.

% increase (+)/decrease (-)	R I *	R II	R III
Extension growth	+ 35.30	+ 23.50	+ 12.27
Alkaloid	+ 60.59	+20.31	+ 2.92

* $r = 0.88$; $P = 0.01$.

Correlation, could also be found between chlorophyll content and leaf formation, on one hand, and alkaloid content, on the other, which was particularly significant during R I phase of development. Bharati et al. (1986) have reported similar correlation of chlorophyll content with diosgenin formation in Costus speciosus.

% increase (+)/decrease (-)	R I	R II	R III
Chlorophyll	+ 49.54 *	+ 43.53	+ 35.62
Leaf formation	+ 160.36 **	+ 66.52	+ 68.36
Alkaloid	+ 60.50	+ 20.31	+ 2.92

* $r = 0.92$; $P = 0.01$. ** $r = 0.94$; $P = 0.01$.

Such agreements between parameters like extension growth, chlorophyll content and leaf formation on one hand, and alkaloid content on the other, could not, however, be traced during post-reproductive phases; rather, negative correlation was quite evident between laminar formation and alkaloid content during these phases reminding the observation of Chatterjee et al. (1987).

% increase (+)/decrease (-)	PR I*	PR II	PR III
Laminar formation	+ 11.03	+ 24.04	+ 13.56
TA content	- 12.19	- 4.98	+ 0.40

* $r = 0.84$; $P = 0.05$.

Ghosh (1979), while analysing the growth behaviour and active principle formation in essential oil yielding plant during different developmental phases, clearly showed that maximum rates of extension growth, leaf formation and dry weight accumulation remained associated with maximum rate of essential oil formation. In studies with alkaloid yielding Catharanthus plants, Chatterjee and Nandi (1977) reported identical correlation between growth behaviour and active principle formation. In the present study, though a correlation amongst extension growth, leaf formation and alkaloid content existed during reproductive phase, parameters like dry weight accumulation could not be related with the change in alkaloid content of roots.

Numerous reports are available indicating that the formation of active principles like alkaloids, glycosides and essential oils remain most pronounced during reproductive phase of development. Mention may be made of the observations of Chatterjee (1980), Chatterjee et al. (1982) and Bharati and Chatterjee (1986) in case of alkaloids; Chatterjee (1980), Panda (1982) and Bharati et al. (1986) in case of glycosides and Ghosh (1979) in case of essential oils. Such features have been convincingly revealed in the present study where increased formation of alkaloid has been quite evident during three consecutive reproductive phases (R I, R II and R III). It would be further interesting to note that average number of floral units per plant during reproductive phases generally remained positively correlated with average TA content. Similar positive correlation has been reported between flower/fruit ratio and diosgenin in Costus speciosus (Bharati et al., 1986).

Parameters*	R I	R II	R III
Flowers/plant	6.50	24.58	24.88
% TA content	1.00	2.15	2.45

* $r = 0.98$; $P = 0.05$.

Some over-simplification has been made by Chatterjee and Nandi (1977) while correlating the patterns of occurrence of alkaloid content during reproductive growth of plants and different reproductive units prevailing during the period. Though it has been established in the present investigation that alkaloid content and flowering behaviour are positively correlated, significant correlation between these two parameters could be obtained only during peak month of flowering (i.e., May). This contention would become clear from the present investigation in which perfect positive correlation between TA and flowering during the period of April-May has been obtained.

% increase (+)/decrease (-)	R I*	R II*	R III*
Flower formation	+ 440.00	+ 2033.00	+ 2033.00
TA	+ 20.00	+ 9.00	+ 0.23

* $r = 1.00$.

Flowering phenomenon, being an outcome of complex metabolic reactions in plants, such positive effects of flowering on the formation of a secondary product like alkaloid can be fairly justified.

In the preceding pages, it has been established that certain growth parameters like extension growth, leaf formation and flowering phenomenon maintain marked relationship with patterns of TA content in roots. We may now look into the course of changes in some biochemical parameters in leaves during the life

cycle of ipecac and their relation with alkaloid changes in roots. In general, it was found that TN content decreased during vegetative and reproductive phases. As regards SN, it increased during R I, R II and R III and decreased during other developmental phases. Identical was the case with RNA, where vegetative and post-reproductive phases registered a decrease in its content; reproductive phases registering an increase. In contrast to these changes, an increase of TA content was revealed during all the developmental phases. A significant correlation existed between RNA and TA contents, particularly during reproductive phase of development.

% increase (+)/decrease (-)	R I [*]	R II ^{**}	R III ^{***}
RNA	+ 15.61	+ 37.94	+ 46.82
TA	+ 60.37	+ 22.22	+ 2.92

* $r = 0.88$; $P = 0.01$; ** $r = 0.96$; $P = 0.01$; *** $r = 0.84$; $P = 0.05$.

A close correlation between TN and SN contents on one hand and TA content on the other has been mentioned in the literature. It has been generally shown that decrease in TN and increase in SN go hand in hand with increase in alkaloid content. Chatterjee and Nandi (1977) reported increase in TA content accompanied by decrease in TN in plants like Datura; Chatterjee (1980) in Rauwolfia and Catharanthus and Panda (1982) in Costus also reported identically. Ghosh (1979) found that increase of SN and of total carbohydrate contents remained associated with increased synthesis of essential oils in leaves of Cymbopogon.

The present studies, in finding out a possible biochemical indexing involving nitrogenous fractions in leaves and TA contents in roots of ipecac, could not be that assertive as had been mentioned by other workers (Chatterjee, 1980; Panda, 1982 and Ghosh, 1979). While tracing out for an agreement between patterns of occurrence of nitrogenous fractions and TA contents with developmental growth from vegetative to post-reproductive phase, a generalised conclusion could not be arrived at. But restricting the data analysis to specified

periods of maximum flowering during active R I (11 - 12 months), R II (23 - 24 months) and R III (35 - 36 months) respectively, it could be shown that there existed significant correlation between decrease of TN and increase of SN fraction in leaves and increase of TA content in roots.

% increase (+)/decrease (-)	R I	R II	R III
SN	+ 5.26 [*]	+ 8.54 [*]	+ 1.19 [*]
TN	- 7.41 ^{**}	- 8.64 ^{**}	- 2.08 ^{**}
TA	+ 20.00	+ 9.01	+ 0.83

^{*} $r = + 1.00$; ^{**} $r = - 1.00$.

Statistical analysis to establish this generalisation shows that whereas correlation coefficient is positive and perfect between SN and TA ($r = + 1.00$) in R I, R II and R III; the same is negative and perfect between TN and TA ($r = -1.00$) in all the three reproductive phases.

By restricting the analysis of changes of these biochemical parameters during specified periods of maximum fruiting (13 - 14, 25 - 26 and 37 - 38 months of PR I, PR II and PR III respectively) it was also possible to establish the pattern of decrease in TA contents in roots with concomitant increase of TN in leaves.

% increase (+)/decrease (-)	PR I	PR II	PR III
TA	- 6.43	- 5.46	0.00
TN	+ 19.28 [*]	+ 26.92 [*]	+ 33.93 [*]

^{*} $r = - 1.00$

The foregoing account relating to the developmental physiology and growth of ipecac plants would suggest a regulatory role of reproductive phenomenon

on alkaloid formation. It was observed that reproductive units like flowers had certain bearing on the content of alkaloid in roots and the period of peak formation of flowers could be correlated with period of peak formation of alkaloids. Studies also showed that the physiological indices like rate of extension growth and leaf formation and biochemical indices like changes in RNA, SN and TN contents reflect the quality of roots in terms of its alkaloid content.

II. Pharmacognostic studies

Though studies on developmental physiology of ipecac have not been extensively reported, pharmacognosy of this species has received some recognition; but that too, in a rather conventional way. In the following pages, results of some pharmacognostic studies of ipecac have been discussed and the conclusions arrived at therefrom point out to some new findings of pharmacopoeial interest.

The pharmacognostic study was undertaken to ascertain macroscopic and microscopic differences of ipecac plants in relation to age and altitude. Pharmacognostic investigations were also conducted to locate the presence of alkaloids among different tissue systems of stem and root and their possible relationship with alkaloid contents in roots. The principal alkaloids of ipecac are emetine, cephaeline and psychotrine together with O-methyl psychotrine and emetamine. The medicinal properties of ipecac reside chiefly in the alkaloids emetine and cephaeline although the limitation of the British Pharmacopoeia to the non-phenolic alkaloids suggest that cephaeline is of minor importance (Bal and Dutta, 1946). In this study relative occurrence of phenolic and non-phenolic alkaloids in the roots of ipecac in relation to altitude was analysed and results proved to be interestingly specific.

Detailed morphological analyses of stem, leaf and root of ipecac plants growing in two altitudes could lead to some interesting conclusions. Low altitude ipecac plants had higher length and girth of stem; larger number and area of leaves as well as greater length, girth, volume and unit dry weight of roots. These characteristic morphological traits of ipecac plants growing in lower altitude remained linked with higher TA contents in roots.

Comparing the root system of ipecac plants growing in two altitudes, it could be ascertained that main root component accommodated higher alkaloid content as compared to other root components viz., branch roots, root tips and root hairs. No difference in this pattern of alkaloid distribution could be distinguished in plants growing in high and low altitudes.

In literature (Mitra and Chakraborty, 1948; Gupta, 1971) ipecac plants have been reported to possess two types of flowers (pin-eyed and thrum-eyed) with respect to androecium characters. In this investigation also, detailed search for flower types was made in plant populations growing in high and low altitudes and while uniform occurrence of two types of stamens could be confirmed, some special features of gynoecium could also be located which showed three types of pistil viz., long-styled, mid-styled and short-styled. However, these floral features could not be linked with any difference in total alkaloid contents of roots.

Microscopic evaluation led to some more interesting conclusions. Features like stomatal frequency and stomatal index remained linked with age of the plant as well as altitude in which the plants were growing. While, generally, both stomatal frequency and index were higher in younger plants of low altitudes; in older plants, these were more at higher altitude. On the other hand, stomata appeared to be of higher dimension in plants, growing in lower altitudes.

Features like palisade ratio, vein-islet number and vein-termination number were also analysed in population of varying altitudes and clear-cut conclusions could be arrived at. Palisade ratio always remained higher in low altitude plants whereas number of vein-islet and vein-termination number could be generally found higher in high altitude plants. Moreover, frequency of trichome distribution as well as their dimensions were lower in plants of higher altitude. So long as features of pollen grains are concerned, while their shape, morphology and contents were identical in flowers of both high and low altitude plants, the grains were of bigger dimensions in low altitude plants.

Pharmacognostic study of ipecac has not been extensively conducted excepting some pioneering work of Bal and Dutta (1946) and Banerjee (1974). But their

studies did not take into account detailed macroscopic and microscopic evaluation of ipecac plant growing at different altitudes. The present observations in this respect are of unique nature and it is more significant in the context of correlating certain specific macroscopic and microscopic feature(s) with alkaloid content of the root. Correlation of occurrence of different macroscopic features with alkaloid contents has already been mentioned in the preceding pages. Identically, certain microscopic features like higher stomatal frequency and index, larger stoma, higher palisade ratio, lower vein-islet number, bigger trichomes as well as larger pollen grains remained distinctly linked with higher alkaloid contents in roots of ipecac growing in lower altitudes. Such pharmacognostic indexing in terms of certain macroscopic and microscopic features with the quality of ipecac drug in terms of higher potency i.e alkaloids has not been reported elsewhere so far.

Our studies relating to pharmacognosy of ipecac also point out to the fact that total as well as non-phenolic alkaloid contents reach their maximum limit in plants of three years of age. It is for this reason the optimum period of harvesting of ipecac has been fixed from 3-year old plants. Pharmacopoeial requirements prescribe that ipecac roots should not contain less than 2% of the total alkaloid and not less than 55% of these should be emetine. The results of this study would point out that to attain such a pharmacopoeial quality, altitude as well as age play some important roles in the production of standard quality of the drug. Whereas ipecac population at lower altitude can satisfy pharmacopoeial requirements of the root drug at younger age of the plant, in populations placed at higher altitudes, the plants will have to complete at least two years of life cycle to come to this desired quality.

III. Temperature stress

Ipecac is a shade-loving plant and its growth is sensitive to direct exposure of light, high and low temperatures. The growth and development of this species clearly go out of gear below 15 - 16°C and above 32 - 33°C when extension growth, leaf formation, accumulation of dry matter, different biochemical

functions and total alkaloid formation become inhibited.

The present investigation has followed separately the effects of high (50°C) and low temperature (5°C) on growth performances as well as chlorophyll, RNA, soluble and total nitrogen and total alkaloid contents. The study has also included the effects of some growth promoters and inhibitors (individually or in combination with temperature) to assess the role of these growth substances in alleviating the damages caused to the ipecac plants in high and low temperature stress environments. The temperature - stress treatments were conveniently done in water medium as this was reported to be 15 times more effective compared to aerial temperature - treatment (Nyland and Gohen, 1969).

A. High temperature

In general, high temperature (50°C) treatment during pre-reproductive phase of development has inhibited growth parameters like linear expansion, leaf formation, accumulation of dry weight and biochemical parameters like chlorophyll, RNA and nitrogen fractions; inhibitory effects becoming more pronounced in higher duration of treatments. High temperature effects on growth inhibition has been amply demonstrated in the studies of Bean (1971); Onwueme and Laude (1972); Gur *et al.* (1972); Pulgar and Laude (1974) and Musaelyn (1975). Inhibition of chlorophyll synthesis due to high temperature, as reported in this study, has also been frequently reported (Onwueme and Lawanson 1973); Gur *et al.* (1972); Feirabend and Mikus (1977); Daniell *et al.* (1969) and Ghosh (1978). Similarly decrease of RNA or its denaturation by high temperature has been reported by Baker and Jung (1970).

High temperature exposure resulted in overall increase in accumulation of soluble nitrogen. Identical observation has been made by Sato and Inaba (1973) and Walgenbach *et al.* (1981) while studying the changes of different nitrogenous fractions in plants due to high temperature exposure. It is of further interest to record that high temperature treatment of lower duration augmented total alkaloid contents in roots. Stimulated production of total alkaloid by high

and alternating temperatures has also been observed by Frischnecht and Baumann (1985). Studies of Chatterjee *et al.* (1986) also confirmed higher alkaloid yield in ipecac growing under high temperature environment.

Treatments of different growth substances on ipecac revealed that while extension growth was promoted maximum by GA₃ treatments, augmentation of radial growth i.e., leaf formation, became more pronounced with KN treatments. Such effects are in conformity with observations reported by Kuraishi and Muir (1964); Jones and Lang (1968); Leopold (1971); Ray (1986) and Pressman and Negbi (1987) on GA₃ induced extension growth and by Miller (1956) on stimulation of leaf formation by KN in plants. The contents of chlorophyll, RNA as well as TN were also augmented maximum by kinetin and similar reports had been made by Roychowdhury and Sen (1965), Meidner (1967) and Rijven and Parkash (1971). GA₃ treatments also increased chlorophyll and RNA formation considerably and in this connection, mention may be made to the studies of McComb and McComb (1970), Broughton (1969) and Johry and Varner (1968); which showed increase in RNA synthesis by treatments with GA₃. It is to be noted, however, that in case of NAA treatments also radial growth of lamina of ipecac plants was augmented appreciably; an effect generally not found in the literature of plant growth.

The effect of inhibitors like MH and also KSCN are very clear in the sense that nearly all the morphological and biochemical parameters have been inhibited with the singular exception of alkaloid formation which, in every case, has been promoted. In this connection mention may be made of the observations made by Nooden (1972) on the effect of MH on shoot length and nucleic acid synthesis; Pearson and Wareing (1969); Belhanofi and Collet (1970); Mullins and Osborne (1970); Poulson and Beever (1970); Khan and Anojulu (1970); and Leshem and Schwarzj (1972) regarding effects of ABA on RNA synthesis; and Jin-Sung and Sung (1986) and Page-Degivry *et al.* (1987) on chlorophyll synthesis.

One interesting finding appears to be that of the effect of MH on augmentation of laminar growth. Though, observations of similar nature have not been reported in literature, it might be noted that in some plants, ABA has been reported

to increase overall growth (Takahashi, 1972). Regarding augmentation of alkaloid formation with inhibitor application, available reports are scanty. There are reports of increased alkaloid synthesis in plants under water stress condition (Mann et al., 1980; and Yaniv and Palevitch, 1982 and Bharati et al., 1986 and positive relation between inhibitors and water stress conditions has been mentioned by Wright and Hiron (1969). It might be a fortuitous coincidence but augmentatory mechanism of the growth inhibitors upon the increased alkaloid formation through the mediation of internal moisture stress condition can not be ruled out.

Exposure of high temperature to mature plants has shown some behavioural deviation from the normal untreated plants. High temperature exposure decreased linear growth, dry weight and some biochemical parameters like nitrogen, RNA and chlorophyll when examined during two developmental stages; R II and PR II. It is interesting to note, however, that with the advancement of age, the natural recovery-process was remarkably accomplished. Other studies (Lahiri et al., 1973) have shown that the adverse effect of high temperature could be minimised or even evaded, when an adequate nutrition was made available to the plants. Provision of nitrogen to rice varieties did protect growth and repaired metabolic injury inflicted upon by high temperature (Ghosh, 1978) and in this study the supply of some growth promoters like KN and GA₃ and inhibitors like MH to ipecac plants could produce similar effects.

While examining the high temperature effects on individual growth parameters in detail, it became clear that accumulation of dry matter in leaves had been inhibited more as compared to other growth parameters; whereas, in case of different biochemical parameters, inhibitory effects of high temperature was maximum on total RNA content. Furthermore, remarkably pronounced effect of high temperature induced inhibition was revealed during reproductive phase of development of the plant as compared to post-reproductive stage. This was particularly true in cases of leaf formation and accumulation of dry weight. Similarly, with different biochemical parameters, high temperature effect was comparatively pronounced during reproductive stage, particularly, on chlorophyll and RNA contents. In case of TA formation also, reproductive stage

of development appeared to be more sensitive. Treatments with growth substances like GA₃, KN and NAA also revealed higher manifestation of augmented leaf formation and leaf dry weight accumulation whereas in case of growth inhibitors, such differential responsiveness of two developmental phases like R II and PR II was not generally seen. Such varying sensitiveness of different developmental phases in plants has been shown by other workers engaged in researches on developmental physiology of plants. Thus mention may be made of the studies of Chatterjee and Nandi (1977) on medicinal plants like Catharanthus and Datura; Panda (1982) on Costus and Chatterjee et al. (1983) on Dioscorea.

In the present study, protective roles of growth substances like GA₃, KN and NAA and inhibitors like MH, ABA and KSCN against high temperature damage have been analysed. Protective effects of GA₃ and NAA against heat injury have been mentioned by Onwueme and Laude (1972); of MH by Alexandrov (1964) and Gorban (1968) and of KN and ABA by Moorie (1970). Protective effects of GA₃, KN and other growth substances against high temperature injury have also been revealed in the studies of Joshua and Heydecker (1971) and Mishra and Sahu (1969). Ovcharov (1977) has shown augmentation of reduced level of growth due to high temperature by GA₃ treatments. According to Ghosh (1978), GA₃ treatments increased RNA levels and a positive effect of GA₃ on modulating the plant system leading to increased growth and RNA metabolism was stressed upon.

As pointed out by Ghosh (1978), the protection offered by KN has been mainly mediated through increase of RNA. Heat treatments of paddy seedlings resulted in biochemical lesions to a varying degree and availability of KN to such seedlings favoured the rate of formation of metabolites like RNA. Engelbrecht and Mothes (1964) explained protective effect of kinetin due to its role in increased protein synthesis. Shininger and Polley (1977) showed that kinetin induced two to four-fold enhancement of rate of RNA synthesis.

In the present study, the protective roles of a particular chemical treatment could be assessed in terms of manifestation of different morphological and biochemical lesions which increased in intensity or decreased under different

conditions of experiment. Growth substances have been applied either before (pre-treatment) or after (post-treatment) the high temperature application and the protective roles of the chemicals have been rated. In general, KN has established itself as one of the potent protective chemicals against the high temperature induced damages of ipecac plants. In case of KN, morphological parameters like extension growth, leaf formation and leaf dry weight and biochemical parameters like chlorophyll, RNA and total nitrogen contents have been appreciably increased when applied as pre-or post-treatment. GA_3 , the protective action of which has also been markedly demonstrated in this study, has revealed promotive effects on extension growth, leaf formation and dry weight accumulation as well as on augmentation of chlorophyll, RNA and TN contents when applied as post-treatment only. Suitability of NAA as a protectant when analysed against these criteria is also noteworthy. A comparison of effects might be of interest :

Treatments	Overall % increase (+)/decrease (-)		
	KN	NAA	GA_3
Pre	+ 5.10	+ 0.65	- 3.18
Post	+ 7.00	+ 6.72	+ 6.12

It would be clear that while KN application (both as pre and post) augmented the levels of morphological and biochemical parameters appreciably, GA_3 and NAA application could do so only in post-treatments and to a comparatively lower degree.

The effectiveness of KN and GA_3 in alleviating high temperature-induced damage has been mentioned in literature. It has been shown that KN when applied before heat stress, diminishes the effect of heat on catabolic processes like increase in membrane permeability (Benzioni and Itai, 1973); changes in lipid composition (Skogqvist, 1974); rise in amylolytic activity (Benzioni and Itai, 1975) and also prevents accelerated senescence (Mothes, 1964). KN has also been shown to increase chlorophyll (Gur *et al.*, 1972) as well as RNA contents (Parthier and

Wollgiehn, 1961; Roychowdhury and Sen, 1964). KN has also been shown by Mayak and Halevy (1974) to slow the dry weight reduction and maintain an optimum turgidity of tissues subjected to stress conditions. As regards GA₃, it has been reported to be effective in restoring elongation growth (Roy, 1986; Pressman and Negbie, 1987). Protective actions of NAA against high temperature induced damage has not been frequently mentioned in literature and the results obtained with NAA in this study may open up possibilities for further investigations in the area of the amelioration of heat-stress injury in plants.

ABA has been reported to increase resistance to environmental stress (Boussiba *et al.*, 1975; and Mizrahi and Richmond, 1972) but not to heat stress. It has been mentioned that ABA might not alter resistance of the plant towards stress but rather affect the recovery from it (Itai *et al.*, 1978). In the present study, ABA could recover lesions induced by high temperature on extension growth and dry weight accumulation when application was made before temperature treatment. ABA treatment could also recover the damages on chlorophyll and RNA when applied as pre-treatments. Though ABA has been generally described as a growth inhibitor, it has also been reported to increase growth (Takahashi, 1972). The recovery from extension damage, as revealed in this study, will corroborate reports on such types of ABA action.

MH is a growth inhibitor and prevents elongation as well as radial growth of leaf (Alexandrov, 1977). In this study, pre-treatments with MH were found to relieve temperature damage on extension growth, leaf formation and accumulation of dry weight. It could also alleviate the damages of chlorophyll, RNA and TN contents when applied as pre-treatments. Thus, like ABA, MH though an inhibitor, could play as a protectant against high temperature injury. MH has been found to impart protective effects against heat injury by Alexandrov (1964) and against heat inactivation by Gorban (1968). It has also been reported by Ghosh (1978) that the pre-treatment of MH augments particularly RNA and TN, while affording protection to high temperature treated rice plants.

Though no reports on alleviation of temperature injury by KSCN are available in the literature, our study has been able to establish protective functions

of KSCN against such injury. Results have revealed that when applied as pre-treatments, KSCN is effective in recovering leaf dry weight loss and also losses in RNA and chlorophyll caused by high temperature.

A comparison of the overall effectiveness of the three growth inhibitors as protectants against high temperature injury could thus be interesting.

Treatments	Overall % increase (+)/decrease (-)		
	MH	ABA	KSCN
Pre	+ 3.93	- 2.26	- 0.56
Post	- 7.22	- 4.42	- 6.03

Retardation of plant growth by heat stress has been reported for Helianthus, Sesamum etc. (David, 1940); for wheat coleoptiles (Allan et al., 1962, Sunderman, 1964); for emerging seedlings of Phalaris, Festuca, Lolium etc. (Laude et al., 1952) and for newly emerged barley seedlings (Onwueme et al., 1971). Mechanism of high temperature retardation has been variously reported as inhibition of cell elongation (Allan et al., 1962); inactivation of endogenous growth hormones (David, 1940); enzymes (Langridge, 1963) or by rendering various metabolic deficiencies (Bonner, 1957; Ketellaper, 1963). In light of these observations and from the results obtained in the present study, it might be said that growth inhibitors provide protections to heat injury through inhibition of growth processes, thus exposing the tissues to lesser risk of damage.

The analysis of effects of high temperature, in conjunction with different growth substances or inhibitors and consideration of patterns of modulation of different morphological and biochemical parameters in ipecac plants, reveal interesting features in protective actions of different chemical treatments. Comparing the effects on extension growth, it could be ascertained that GA₃ treatments ensured highest protection followed by KN, NAA, MH and ABA whereas no protection could be seen in KSCN treatments. Contrary to this observation, GA₃ treatments were reported to afford no protection against heat induced

reduction in growth (Onwueme and Laude, 1972). Benzioni and Itai (1972) reported failure of KN to reverse the effect of heat treatments though KN application on leaf blades was shown to increase heat resistance of plants (Engelbrecht and Mothes, 1964). It was reported that KN treatment was effective in increasing the ability of plants to survive harmful consequences of heat, even if the treatment followed the exposure to high temperature.

It will also be interesting to examine the effects of different growth substances/inhibitors on alleviation of injuries of leaf formation and accumulation of dry matter due to high temperature. KN both as pre- and post-treatments, proved to be a very effective protectant. Such effects of KN have not been reported in literature. The pronounced effectiveness of NAA in augmenting radial growth of leaf when applied after the high temperature treatments have also been very clear in this study and in this connection mention may be made of the work of Gorban (1968) and Onwueme and Laude (1972) on heat protective action of auxins.

Another interesting observation that came out from the present study was the mode of actions of inhibitors like MH, ABA and also KSCN when applied as pre-treatments. Application of such inhibitors before high temperature treatment could increase dry weight, chlorophyll and RNA contents in leaves. It is possible that inhibitor treatments, while inhibiting the growth as well as different metabolic processes, have rendered the plants more resistant to high temperature damage. That growth inhibition at high temperature may, in fact, be a protective device preventing more serious high temperature injury has been mentioned by Levitt (1980). Observations of Ghosh (1978) on the mechanism of MH action on high temperature can also be cited. MH, though a growth inhibitor, does not affect growth itself, but rather augments it; side by side, favours leaf dry weight, chlorophyll, RNA and TN contents. It is to be noted, however, that these effects were obtained when MH was applied as pre-treatments and not as post-treatments.

The results of the present study, thus, could clearly distinguish mechanisms of action of some growth promoters/inhibitors as 'protectants'. While growth,

promoters, in most cases, could alleviate injuries of high temperature when applied after the temperature treatments, growth inhibitors exhibited more of its protective role when applied before the high temperature. Promoters and inhibitors are known to act antagonistically in many processes but in the present study they exhibit comparable effects of protection against high temperature induced injury. But the type of responses they show had been more indicative of the fact that while the growth promoters could help in recovering the damages inflicted by high temperature, inhibitors would make the plants more resistant.

B. Low temperature

Lyons (1973) has described many symptoms of cold injury. Amongst various effects, membrane damage (Cacas *et al.*, 1965), solute leakage (Nordin, 1977), inhibition of starch translocation (Crawford and Huxter, 1977), respiratory upset (Stewart and Guinn, 1971), accumulation of toxins (Lyons, 1973) and protein breakdown (Razmaev, 1965) are some of the end-effects caused due to low temperature. In this investigation, effects of cold temperature treatment on manifestation of growth and developmental processes of ipecac have been studied and physiological parameters like extension growth, leaf growth and dry weight accumulation and biochemical parameters like chlorophyll, RNA, SN, TN and TA contents as affected by low temperature treatments, alone or followed by protective measures, have been analysed.

In general, 5°C exposure inhibited extension growth and leaf formation and also lowered the contents of chlorophyll, RNA, TN, TA and to some extent SN. One singular exception was dry weight accumulation which increased. Hilliard and West (1970) reported reduction of growth by low temperature; reasons attributed being failure of translocation of photosynthates. Pomeroy and Andrews (1975) demonstrated increase in dry matter in cold environment. Garber (1977) while studying effects of light and chilling temperatures showed decrease in chlorophyll content in plants exposed to 4°C in light. Studies of Romanova (1967) and Ostaplyuk (1967) revealed increase of amino acid nitrogen

in plants exposed to low temperature. Studies of Sakai (1957, 1962) showed increase in soluble protein in low temperature exposure whereas Li and Weiser (1967) and Devay and Paldi (1977) found increase in RNA contents in low temperature condition. Effects of low temperature on alkaloid formation in plants have not been mentioned in the literature. The present investigation, however, clearly revealed that 5°C exposure to ipecac plants inhibited TA content in roots.

Modulation of low temperature effects due to application of different growth substances and inhibitors has become clear in the results of present investigation. KN treatments (both before and after low temperature exposure) convincingly established itself as a potent protector against low temperature injury. KN promoted leaf formation, leaf dry weight accumulation, extension growth and also increased contents of RNA, TN, TA and SN. KN effects had been more or less uniformly demonstrated both in pre and post-treatments and in general, the augmenting responses were equally pronounced. In the stress literature, KN treatments have been found to be markedly effective in recovering high temperature damages in plants but its role in recovering low temperature induced injury has not been frequently mentioned. In this respect, the results of the present studies with KN have proved to be interesting.

The effects of GA₃ and NAA in modulating the injuries caused by low temperature are generally comparable. Both GA₃ and NAA could recover the damages on extension growth; the former rather more convincingly. Leaf formation was also augmented by low temperature application when combined with GA₃ or NAA. The effects on leaf formation could be found to be more revealing in post-treatments.

As regards recovery of losses of different biochemical parameters by GA₃ and NAA, results speak of higher effectiveness of NAA. Protective action of GA₃ or NAA against low temperature damage have not been frequently reported. Tumanov and Trunova (1950) found that auxin reduced low temperature hardening whereas Kacperska-Palacz et al. (1965) found no effect of IAA against

low temperature injury. Marlangeon (1969) reported application of GA_3 to be effective against cold injury. The summary data shown in the following table will justify the role of KN as a good protectant against low temperature injury in comparison to NAA and GA_3 .

Treatments	Overall % increase (+)/decrease (-)		
	KN	NAA	GA_3
Pre	+ 8.11	- 1.26	- 2.53
Post	+ 7.42	+ 2.13	+ 3.28

One of the remarkable features regarding protective effects of MH is its effectiveness in pre-treatments only. These effects are reminiscent of the protective effects of MH against high temperature treatments described earlier. MH treatments have been shown to be effective in recovering the damages on extension growth and leaf formation caused by low temperature. Reduction in contents of different biochemical parameters due to low temperature has also been recovered to some extent by MH.

Though inhibitory effects of MH on growth have been reported in literature (Zukel, 1957; Sachs and Lang, 1963); such stimulatory effects of MH in relation to low temperature have not been mentioned elsewhere. It may be of interest to speculate on the mechanism of action of MH in recovering cold injury. It might be possible that inhibition of growth activity by MH was the determining cause in lessening the quantum of damage on other parameters and such a phenomenon could explain why MH treatments became effective in pre-treatments only. Post-treatments with MH could not, however, reverse the damages inflicted upon by low temperature; which stood clear in the effects of growth promoters like NAA and KN.

ABA is not as effective as MH; but in pre-treatments, its protective action against low temperature inhibition was also noteworthy. ABA has been reported

to protect against chilling stress by Rikin and Richmond (1976) and by Walton (1980). Like MH and ABA, pre-treatments with KSCN also could offer protection against cold injury, more particularly against chlorophyll, RNA and TN losses. Such effects of KSCN have not been reported; except one finding of Ghosh and Chatterjee (1975) which showed that damages caused by low temperature could be repaired by treatments with KSCN. The summary data in the following table might help in grading the protective action of the three inhibitors.

Treatments	Overall % increase (+)/decrease (-)		
	MH	ABA	KSCN
Pre	+ 7.45	+ 2.71	+ 1.92
Post	- 3.17	- 6.71	- 4.42

The study of the pattern of modulation of different physiological and biochemical parameters in ipecac plants due to low temperature application in conjunction with different growth promoters/inhibitors has revealed some interesting results. Looking into the effects on extension growth, GA_3 treatments have been found to be comparatively more effective. NAA applications could allow perceptible protection only in post-treatments; whereas inhibitors were found to be effective against restoring growth functions affected by cold in pre-treatments only.

Results of the experiments with different growth substances/inhibitors could demonstrate that protection against cold injury, at least partially, was achieved by altering the growth rate. The following summary-effects might be of interest to examine.

Treatments	% growth promotion (+)/inhibition (-)					
	GA_3	NAA	KN	MH	ABA	KSCN
Pre	+ 10.00	+ 4.75	+ 4.00	+ 4.25	+ 2.75	+ 0.02
Post	+ 13.00	+ 8.75	- 0.52	- 2.25	- 5.00	- 2.50

Our results could also show that the low temperature-induced inhibition of leaf formation and dry weight increase could be alleviated by different promoter/inhibitor treatments; the effect remaining most pronounced with KN (both pre- and post-treatments) and also with different inhibitors (as pre-treatments).

Treatments	KN	GA ₃	NAA	MH	ABA	KSCN
% increase (+)/decrease (-) Leaf formation						
Pre	+ 9.25	- 6.25	- 2.02	+ 7.00	- 9.25	- 6.00
Post	+13.25	+ 9.75	+ 2.75	- 5.50	-10.50	- 9.25
Dry weight accumulation						
Pre	+ 5.00	- 4.60	- 4.75	+ 4.00	+ 5.00	+ 2.92
Post	+ 6.00	- 2.75	- 2.50	- 3.75	- 11.25	-10.00

Effectiveness of growth promoters when applied as post-treatments and of inhibitors when applied as pre-treatments would thus appear clear. Kuraishi *et al.* (1966) reported prevention of chilling injury by cytokinins. Bramlage (1982) reported that only a limited benefit could be gained from the use of growth regulators in overcoming chilling injury. Rikin *et al.* (1981) and Sasson and Bramlage (1981) mentioned protection against chilling injury by ABA pre-treatments. ABA was also reported to increase plant resistance to subzero temperatures (Irving and Lanphear, 1968; Rikin *et al.*, 1975). Studies of Ghosh (1978) revealed protective roles of KN and MH against chilling damage on rice.

Effects of low temperature on biochemical changes in plants have been narrated by Levitt (1980). Ipecac plants when exposed to 5°C, showed considerable decline in the contents of chlorophyll, RNA, TN, SN and TA. Studies of Millerd and McWilliam (1968), Mckersie and Thompson (1978) and Thompson *et al.* (1978) had shown decline in chlorophyll and TN contents under conditions of low tem-

perature. SN content was, however, found to increase (Johansson *et al.*, 1955; Smith, 1968); whereas TN and PN decreased (Le Saint, 1966). Levitt (1980) mentioned that cold exposure markedly increased soluble protein which was also reported by Kacperska-Palacz *et al.* (1975). Li and Weiser (1967) mentioned increase in RNA with cold hardening and studies of Devay and Paldi (1977) revealed that chilling temperature doubled the RNA contents. Identical observation was also made by Chen and Li (1977). In the present study TN content decreased when ipecac plants were exposed to 5°C temperature. Decrease of SN contents somewhat depended on stage of development of plants when the treatments were made. Contrary to other reports, RNA contents, however, decreased as a result of low temperature treatment; the observation conforming Rochat and Therrien (1976) and Guinn (1971) who showed that chilling treatments resulted in decrease in RNA and protein contents.

When protective roles of growth regulators like KN, GA₃, NAA, MH, ABA and also of KSCN against biochemical damages were followed in the present investigation, some interesting observations could be made. Loss of chlorophyll as well as of RNA due to low temperature treatments could be arrested by KN and inhibitors like MH, ABA and KSCN. Similarly, recovery of TN and SN contents by KN as well as by different inhibitor-application could also be achieved. Effects of GA₃ and NAA in such recovery had rather been negligible. One interesting generalisation could, however, be derived from the results of this study. While recovery from damages by growth promoters are mostly effective in post-treatments, with growth inhibitors protection could only be achieved when applied as pre-treatments.

Treatments	KN	GA ₃	NAA	MH	ABA	KSCN
% increase (+)/decrease (-)						
Chl						
Pre	+ 10.00	- 5.25	- 1.40	+ 6.75	+ 5.50	+ 5.25
Post	+ 9.00	- 3.75	+ 2.00	- 4.75	- 2.30	+ 0.48
RNA						
Pre	+ 16.00	- 5.50	- 1.50	+ 8.75	+ 7.00	+ 8.25
Post	+ 12.00	- 0.75	- 1.50	- 2.25	- 7.25	- 3.00
TN						
Pre	+ 10.00	- 7.25	- 0.19	- 6.75	+ 7.70	+ 7.00
Post	+ 8.00	- 6.25	+ 2.19	- 0.07	- 8.00	- 2.25

Some investigators believed that growth regulators were important in relieving cold injury (Sulakadge and Rapava, 1973). Wolk and Herner (1982) mentioned that there might be promise in using certain growth regulators or hormones to provide at least partial protection against chilling injury. Our studies also confirm such findings.

While comparing the range of protection given by different growth promoters or inhibitors against high and low temperature injuries, it would be revealed that GA_3 , in both the temperatures, afforded maximum protection to extension growth followed by NAA; whereas in leaf formation damage, KN treatments revealed maximum protective effects followed by NAA. In case of chlorophyll and RNA, protection was maximum with KN treatments and minimum with KSCN. Earlier in the present studies, ABA and KSCN had been found to be effective in protecting against high temperature injury on SN and TA contents. MH, applied as pre-treatments, acted effectively protecting extension growth, leaf formation, dry weight accumulation and RNA synthesis in both high and low temperatures.

When over-all protective effect (OPE) was defined as the sum-total of percentage increase or decrease of all the parameters, some interesting conclusions were derived. Firstly, amongst the growth promoters, KN stood as the most effective in the protection process in both high and low temperature-caused damages. Secondly, amongst growth inhibitors, MH appeared as the most effective protectant against high and low temperature injuries. OPE values of KN in high and low temperature were + 82 and + 131, followed by NAA and GA_3 , the values were - 5 and + 20 followed by ABA and KSCN (- 35 and - 24; - 46 and - 9).

Another interesting aspect of protection, offered by different promoters/inhibitors, was an agreement between the timings of their treatments when the protective effects became maximum. It became clear that in case of growth promoters like KN, GA_3 , and NAA, maximum protection against high and low temperature injury could be assured when applied as post-treatments; thus assigning these promoters to play a recovery role. On the contrary, in case with different

inhibitors (viz., MH, ABA and KSCN), the protective effects were made in pre-treatments which made the plants more resistant against temperature-induced damages.

C. Temperature and alkaloid content

Observations made in the foregoing pages have established the fact that ipecac plants are sensitive to both high and low temperature treatments and such temperature treatments affect the growth behaviour, alkaloid formation and other metabolic processes in a characteristic way. As ipecac is known for its alkaloid emetine which is used for medicinal purposes, it will be of interest to examine how different treatments mentioned in the study would affect the alkaloid contents in plants. Results indicated that ipecac plants under transitory high temperature stress conditions led to an increased synthesis of alkaloids *in vivo*; but such effects were time-dependent. Thus while a very limited and shorter duration of high temperature stress was conducive to alkaloid formation, stress of longer duration acted antagonistically. It is to be noted, however, that low temperature stress could not augment TA contents in ipecac.

Mode of action of high temperature stress on alkaloid increase can be interestingly speculated. It could be argued that increase of secondary metabolites under restricted high temperature stress occurred at the expense of growth activity as well as due to internal biochemical changes caused by such treatments. In longer durations of high temperature (as well as in low temperature), biochemical imbalances were produced irreversibly which were inhibitory to the alkaloid formation in roots. Increased level of alkaloid formation under temperature stress has not been frequently mentioned in the literature. Studies of Winters *et al.* (1947) showed increased alkaloid content in Cinchona by high temperature. Alternating temperature conditions increasing TA in Coffea arabica has been reported by Frischknecht and Baumann (1985). Palevitch's data (1985) revealed the effects of internal water stress conditions favouring alkaloid synthesis in medicinal plants. A 50°C - temperature treatment has been shown to induce increase in TA by 4, 9 and 10 to 39% after 33, 60 and 120 days respectively (Chatterjee *et al.* 1985; 1986).

Results of the report made by Chatterjee *et al.* (1986) analysed to explore whether promotive effects of high temperature on alkaloid formation could be correlated with the patterns of manifestations of growth processes. It could be shown that inhibition of laminar formation and augmentation of stem growth due to high temperature remained closely associated with increased alkaloid formation.

Treatments °C/Min.	Observation (days)	% increase (+) / decrease (-) Stem growth	Laminar formation	Total alkaloid
10	60	+ 17.32	- 20.20	+ 8.88
	120	+ 25.00	- 44.90	+ 38.50
50	30	+ 23.00	- 33.40	+ 9.14
	120	+ 42.00	- 32.50	+ 10.20

The correlation between inhibition of laminar formation and promotion of alkaloid content could also be corroborated in the present experimental findings where growth promoters like KN, GA₃ and NAA were used as both pre and post-treatments and also inhibitors like MH, ABA and KSCN were used as post-treatments.

While attempting a biochemical indexing of alkaloid contents in the roots of ipecac, experimental results for reproductive and post-reproductive phases of development were analysed separately and one conclusion could be derived. In almost all the cases of pre- and post-treatment with different growth substances, particularly with inhibitors, there appeared a close correlation between decrease in RNA contents and dry weight of leaves with increase of alkaloid contents in roots. In this context, the data obtained with inhibitors like MH, ABA and KSCN during reproductive phase of development would be interesting to examine :

% increase (+) or decrease (-)	MH + HT	ABA + HT	KSCN + HT
		Pre-treatment	
Alkaloid	- 3.25	- 7.08	- 3.67
RNA	+ 18.44	+ 11.19	+ 11.39
Leaf dry weight	+ 15.77	+ 13.65	+ 9.83
		Post-treatment	
Alkaloid	+ 3.09	+ 17.00	+ 12.58
RNA	- 1.37	- 0.83	- 1.56
Leaf dry weight	- 9.15	- 9.30	- 9.44

During post-reproductive phase also, identical negative correlation between alkaloid contents in roots and RNA and dry weight of leaves could be established.

Similar observations were made by Ghosh (1979) in studies with essential oil yielding plants where biochemical indexing involving nitrogenous fractions of leaves and essential oil content was reported.

The impact of these observations will be far-reaching to botanists and phytochemists because, for the first time, it has been reported that the contents of total alkaloid in roots of commercial medicinal plant like ipecac can, assertively, be predicted on the basis of some well-defined morphological as well as biochemical indices prevailing elsewhere in the plant body. Such experimental directions will certainly open up new horizons of applied research involving areas of developmental physiology of a medicinal crop and its inherent capacity to synthesize secondary metabolites like alkaloids.

IV. P³² - uptake

It has been reported in the literature that ion uptake by roots is a function of temperature of the medium and increase of temperature within limits enhances

uptake phenomenon (Lingle and Davis, 1959; Rickels and Lingle, 1966). Inhibition of uptake at high temperature has been reported by Webb (1967). Ghosh (1978) has studied P^{32} -uptake pattern in rice and reported that high temperature treatment increased P^{32} -uptake in lower range and depressed the same in higher range.

No information could be found in literature of P^{32} -uptake and its translocation in relation to high and low temperature stress conditions applied to economically important crops excepting the study of Ghosh (1978) in rice. The present investigation with an important medicinal plant like ipecac, thus, can be claimed to be a unique exercise in this respect.

In this study, effects of both high and low temperature stress on P^{32} - uptake by ipecac plants were analysed along with the effects of some growth promoters/inhibitors with an aim to elucidate how far the protective nature of these chemical factors could be related to the recovery of stress effects of temperature. Exposure to high and low temperature stress depressed P^{32} -uptake capacity of ipecac plants, the effects being more pronounced in low temperature.

Temperature (°C)	% decrease in P^{32} -uptake			
	Whole plant	Leaves	Stem	Root
5	- 50	- 48	- 52	- 56
50	- 30	- 34	- 45	- 8

The results obtained were at variance with the observations made by Ghosh (1978) who demonstrated increased P^{32} -uptake in high temperature which, however, got inhibited with further rise in temperature.

While analysing different growth and biochemical functions in high and low temperature systems, ipecac plants appeared to be temperature-sensitive in nature. Such sensitiveness might demand a specific uptake and distribution pattern of different ions and our studies with radioactive phosphorus in temperature-treated plants would confirm this. Not only the temperature-treated

seedlings of ipecac revealed inhibited P^{32} -uptake uniformly; incorporation of P^{32} in different tissue system also decreased.

P^{32} -incorporation would indicate metabolic efficiency (Ray 1977; White 1973) and decreased incorporation of P^{32} caused by both high and low temperature will generally indicate a metabolically deteriorating system in ipecac plants under stress conditions which will further justify the unstability of these functions in this plant species in environments of elevated or lowered temperatures. However, a note of caution should be there because incorporation of P^{32} increased in the leaf tissue system under low temperature stress. This phenomenon remains in agreement with increased accumulation of dry matter in leaves under condition of low temperature. The low temperature shock facilitating incorporation as well as dry matter accumulation in leaves finds parallelism in the observations relating to promotive effects of temperature shock on other growth phenomena in plants (Levitt, 1980).

Studies have established promotive effects of growth substances, like GA_3 , KN and NAA and of inhibitors like MH on P^{32} -uptake capacity of ipecac seedlings. In general, the stimulating effects on phosphorous uptake could be found to lay in the successive order of $KN \rightarrow GA_3 \rightarrow MH \rightarrow NAA$. Other two inhibitors, (ABA and KSCN) could not augment the uptake behaviour; rather inhibited the same. Such effects of augmentation or inhibition of P^{32} - uptake by different growth substances/inhibitors could find parallelism in the enhancing effects of KN on RNA, SN and TN; of GA_3 on chlorophyll, RNA and SN; of MH on TA and leaf formation; of NAA on leaf formation dry weight accumulation and chlorophyll and with the inhibitory effects of KSCN on chlorophyll, RNA and SN and of ABA on chlorophyll, RNA, SN and TN formation.

Contrary to the observation of Ghosh (1978) on the augmenting effects of high temperature on P^{32} -uptake and incorporation, ipecac plants in the present study distinctly revealed inhibition of P^{32} -uptake under high and low temperature conditions. Such inhibitions could, however, be erased by chemical treatments and side by side, excepting ABA and KSCN, pre-treatments with KN, GA_3 , MH and NAA completely alleviated inhibition of P^{32} -uptake and promoted it.

An analysis of effects of chemical pre-treatments individually will reveal that scope of effectiveness of KN and GA₃ on offering greater protection to growth and biochemical lesions due to high and low temperature has been further broadened; accommodating a more efficient uptake, distribution and incorporation of P³² in a system of elevated as well as lowered temperature environments. In other words, the damage inflicted upon the uptake mechanism of P³² was elegantly and effectively repaired by KN and GA₃; such effects being comparatively pronounced under low temperature stress conditions. Though not as effective as KN and GA₃, it would be interesting to note that auxin like NAA and inhibitor like MH also offered protection to growth and biochemical damages and induced considerable efficiency on uptake and distribution mechanism of P³². One could thus note remarkable roles of KN and GA₃ and, to some extent, NAA and MH in the overall repairing mechanism of injury by way of improving the functions of some physiological parameters as well as increasing biosynthesis of essential metabolites supplemented by more efficient modulation of uptake of ions like phosphorus in ipsecac plants subjected to stress conditions.

Ghosh (1976), while working with rice plants, could find out a relationship between the increased uptake of P³² by inhibitors like MH with enhanced synthesis of RNA. In the context of such a report, it might prove to be a rewarding exercise to compare the uptake and incorporation behaviour of P³² in respect of KN and GA₃, as reported in the present study. In general, induction of uptake as well as incorporation were higher in KN treatments under high and low temperature conditions. Such increased uptake by KN (as compared with GA₃) is particularly interesting; if we recall its augmenting effects on RNA synthesis in leaves of temperature-treated plants. Such an observation would find support even in the graded effects produced by NAA and MH on uptake of P³² vis-a-vis synthesis of RNA. On the background of such convincing results (involving an important metabolite like RNA and a fascinating tool for assessing efficiency of ion uptake by using P³²), it can be argued that temperature-stressed plants do acquire an elevated and fortified system of nucleic acid synthesis (particularly RNA) as well as an improved translocating mechanism to enable the plants to tide over the stress environments of abnormal temperature.

By examining the pattern of preferential distribution and percentage of total uptake within the ipecac plant itself, it would become apparent that apical pair of leaves incorporated a comparatively higher P^{32} followed by stem tissues. Such enhanced uptake and distribution of P^{32} might be a pointer to the fact of higher metabolic participation in these organs. This characteristic feature of relatively higher P^{32} -incorporation by apical leaves and stem remained unchanged even when different growth substances/inhibitors were applied to the plants pointing to the fact that higher metabolic efficiency of these organs persisted even under widely divergent experimental conditions.

High and low temperature treatments, rendered the plants to incorporate more of P^{32} in the apical pair of leaves. Such a phenomenon would again be suggestive of the fact that plants which have experienced temperature shock had their metabolic manifestations clearly restricted to the apical region. Further interesting aspect which came out from the observations was that both under 50°C and 5°C , extent of metabolic participation in these plant organs did not differ markedly.

We could, thus, conclude that while responses of ipecac plants to temperature stress would embody some instantaneous effects on extension and radial growth, chlorophyll synthesis, dry weight accumulation, nucleic acid synthesis and changes in composition of protein, plants do remember a temperature experience and adopt their physiological system accordingly by favourably modifying their uptake and translocation behaviour.

In the preceding pages, some basic functions of ipecac plant have been employed as indicators of thermal injury. These functions include capacities for extension growth, leaf formation, accumulation of dry matter, rate of chlorophyll, RNA, SN and TN formation, rate of TA formation and P^{32} -uptake/incorporation. Considering the performances of these indicators under conditions of various experimental diversions and analysing each of these functions against different protective treatments we might recognise this species as "low temperature loving" plant. This is particularly because our results have amply demonstrated patterns of lesser injuries in exposures to low temperature as compared to

high temperature. The following summary data may justify this :

Treat- ment (°C)	Extension growth	Leaf no.	% increase (+)/decrease (-) over control					p ³² - incor- poration (Leaves)
			DW	Chl	RNA	TN		
5	- 3.79	- 11.50	+ 1.20	- 0.43	- 0.53	- 1.63	+ 6.00	
50	- 3.83	- 14.12	- 4.55	- 0.42	- 1.13	- 1.97	0.00	

In this connection it may be mentioned from the published literature (Chatterjee *et al.*, 1980; 1985) on agrotechnology of ipecac growing in Darjeeling Hills that this species favours the low atmospheric temperature within altitudes between 300 to 500 m.

In conclusion it will be pertinent to put into record that the phenomenon of protection, as offered by different growth substances/inhibitors against thermal stress, appears to be of higher specificity in operation and optimisation of a particular treatment will depend on time of application of chemicals and their nature, stage of development of plant experiencing stress environment, range of temperature and its durations, and also the types of damages (whether biochemical or physiological) being investigated into.

When a thorough stock-taking of the literature in the area of high and low temperature stress was made, a very low percentage of the total could be ascribed to temperature injury (including recovery) in economically important medicinal plants. In the context of this, the present exercise on the metabolic implications of thermal stress injury in a highly valuable medicinal plant like ipecac might be considered a significant endeavour in the area of temperature stress physiology in higher plants.