Abstract

ABSTRACT

Authentic reports in the area of growth and developmental physiology of ipecac (Cephaelis ipecacuanha, family Rubiaceae) are completely lacking and the present investigation dealing with detailed analysis of growth and developmental features of the plant is the first systematic approach in this direction. Nowhere in the world, except the district of Darjeeling, India, organised commercial cultivation of ipecac exists and against this background the locale of this investigation at Darjeeling Hills (home of ipecac plantations) has given added importance to this study.

The present study could identify specifically three distinct reproductive and post-reproductive phases during its economic life cycle; each phase enjoying a well-defined proportion of different reproductive units. Analysis of growth behaviour and synthesis of alkaloids conducted during different phases pointed out a characteristic feature of occurrence of total alkaloid and its constituents depending on age, developmental phase as well as topographical conditions.

The results of the present study yielded some interesting informations on correlative control of growth and formation of active principles. Studies revealed that occurrence of alkaloids during three reproductive phases possessed a distinct relationship with extension growth and alkaloid content decreased in line with decline in the rate of extension growth during these phases. Identical correlation could also be found between increase in chlorophyll content and leaf formation with increase in alkaloid content . Such agreement between the parameters like extension growth, leaf formation and chlorophyll content on one hand and alkaloid content, on the other, could not however be traced during post-reproductive phases when rather a negative correlation between leaf and alkaloid formation became evident.

The experimental results also revealed that formation of alkaloid had remained pronounced during reproductive phase of development and the average number of floral units during three reproductive phases generally showed positive correlation with the average TA content. However, significant correlation between

these two parameters could only be obtained during peak months of flowering. Flowering phenomenon being an outcome of complex metabolic reactions in plants, such positive effects of flowering on the formation of secondary products like alkaloids could be fairly justified.

Looking into the course of changes of some biochemical parameters in leaves during the life cycle of ipecac and their relationship with alkaloid changes in roots, it could be ascertained that while TN content decreased during vegetative and reproductive phases, SN content increased during reproductive phases and decreased during other developmental phases. Identically, RNA was found to decrease during vegetative and post-reproductive phases, reproductive phases registering an increase in its content. In the overall context of these changes, an increase of TA content was recorded during all the developmental phases and, particularly during reproductive phase, a significant correlation was found to exist between RNA and TA content. By further restricting the data analysis to specific period of maximum flowering, during three reproductive phases, it could also be shown that there existed significant correlation between decrease of TN and increase of SN fractions in leaves and increase of TA content in roots.

Again, by restricting the changes of these biochemical parameters during specified periods of maximum fruiting in three post-reproductive phases, it was also possible to establish the pattern of decrease of TA content in roots with concomitant increase of TN and decrease of SN contents in leaves.

Some new findings of pharmacognostic importance as reported in the present investigation would be of considerable pharmacopoeial interest. Detailed morphological analysis showed that low altitude ipecac plants had higher length and girth of stem, larger number and area of leaves as well as greater girth, volume and unit dry weight of roots. These characteristic morphological traits of ipecac plants growing in lower altitudes remained linked with higher TA content in roots. Identically, certain microscopic features like lower stomatal frequency and index, smaller stoma, lower palisade ratio, higher vein-islet number, smaller trichomes as well as smaller pollen grains remained distinctly linked with lower

alkaloid content in roots of ipecac growing at higher altitudes. Such observations of inter-relationship between some pharmacognostic indices like certain microscopic features and the quality of ipecac drug in terms of higher potency could be claimed to be unique.

The present investigation followed separately the effects of high (50°C) temperature on growth performances as well as chlorophyll, RNA, SN, TN and TA contents. The study also assessed the roles of some growth substances in alleviating the damages caused to the plants in high and low temperture stress environments.

While high temperature treatments had overall effects of inhibition on different growth parameters and biochemical balances, examination of effects in detail made clear that accumulation of dry matter in leaves and their RNA contents had been inhibited maximum as compared to other growth and biochemical parameters. Moreover, remarkably pronounced effects of high temperature-induced inhibition was revealed during reproductive phase of development of the plant. Such effects were particularly significant in case of leaf formation and accumulation of dry weight on one hand and chlorophyll and RNA contents on the other. Regarding TA formation also, reproductive phase of development appeared to be more responsive.

Treatments with different growth substances on ipecac revealed that while extension growth had been promoted maximum by GA_3 treatments, augmentation of leaf formation became most pronounced with KN treatments. The contents of chlorophyll, RNA as well as TN were also augmented maximum by KN. In this connection mention may be made to the effect of NAA treatments which revealed a marked augmentation of radial growth — an effect generally not found in literature. As regards the effects of different growth inhibitors (viz., MH, ABA and KSCN), nearly all the morphological and biochemical parameters had been inhibited with the singular exception of alkaloid formation which, in all the cases had been promoted. All these effects showed differential responsiveness towards reproductive and post-reproductive phases and, in general, reproductive phase of development appeared to be more sensitive.

In the present study, the protective roles of growth promoters or inhibitors could be assessed in terms of manifestation of different morphological and biochemical lesions which increased in intensity or decreased under different conditions of experiments. Growth substances were applied either before (pre-treatment) or after (post-treatment) the high temperature application and the protective roles of the chemicals had been assessed. In general, KN had established itself as one of the potent protective agents against high temperature-induced damages in ipecac plants. In case of KN, morphological features like extension growth, leaf formation and leaf dry weight and biochemical parameters like chlorophyll, RNA and TN contents had been appreciably increased when applied as pre- or post-treatments. The protective action of GA_3 was clear in its promotive effects on extension growth, leaf formation and leaf dry weight as well as on augmentation of chlorophyll, RNA and TN contents when applied as post-treatments only. Role of NAA as a protectant was also noteworthy particularly when its augmentative effect on radial growth of leaf was viewed.

As regards protective roles of inhibitors, ABA could recover lesions induced by high temperature on extension growth, dry weight accumulation, chlorophyll and RNA contents. MH was found to relieve the damage on extension growth, leaf formation, dry weight accumulation, chlorophyll, RNA and TN contents. Results with KSCN showed that it was effective in recovering leaf dry weight loss and also damage in chlorophyll and RNA caused by high temperature. The interesting observation that came out of the present study was the mode of action of inhibitors which were proved to be effective protectants when applied as pre-treatments.

In this investigation, effects of cold temperature treatment on manifestation of growth and development of ipecac have been studied and physiological parameters like extension growth, leaf growth and dry weight accumulation and biochemical parameters like chlorophyll, RNA, SN and TA content as affected by low temperature alone or combined with protective measures were analysed. In general, $5^{\circ}C$ 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.

In contrast to high temperature, low temperature exposure ($5^{o}C$) to ipecac plants inhibited TA contents in roots.

Damaging effects of low temperature could be characteristically modulated by application of different growth promoters or inhibitors. KN treatments (both before and after low temperature exposures) 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, SN, TN, and TA. KN effects had been more or less uniformly demonstrated both in pre- and post-treatments. The effects of GA_3 and NAA in relieving low temperature injury were generally comparable. GA_3 recovered damages on extension growth more convincingly as compared to NAA. Unlike KN, treatments with GA_3 and NAA could not repair loss in dry weight accumulation caused by low temperature. Repairing of damages to biochemical parameters was done more competently by NAA than GA_3. As regards inhibitors, MH treatment had shown to be more protective against damages caused by low temperature to extension growth, leaf formation and dry weight accumulation. Reduction in contents of different biochemical parameters due to low temperature had also been recovered to some extent by MH. ABA and KSCN proved to be less effective as compared to MH, though their protective action against low temperature damage became clear which recovered losses against dry weight, chlorophyll, RNA and TN.

The results of the present study, thus, could clearly distinguish between mechanism of action of growth promoters and inhibitors as protectants against high and low temperature damages. While growth promoters could alleviate injuries of high and low temperature when applied after temperature treatments, growth inhibitors exhibited most of the protective roles when applied before temperature treatments. Promoters and inhibitors are known to act antagonistically in many processes but in the present study they exhibited comparable action of protection against temperature injuries. But the types of responses these growth substances would show had been more indicative of the fact that while growth promoters could help in recovering the damages, the inhibitors would make the plants more resistant.

Results of our study might interestingly be analysed to explore whether promotive effects of high temperature on alkaloid formation could be correlated with the patterns of manifestation of growth processes. It could be shown that *inhibition of laminar formation and augmentation of stem growth due to high* temperature remained concomitantly associated with increased alkaloid formation. The results were further corroborated in our experimemtal findings where growth promoters like KN, GA_3 and NAA were used in both pre- and post-treatments and inhibitors like MH, ABA and KSCN were used as post-treatments. While attempting a biochemical indexing of alkaloid contents in the roots, one worth mentioning conclusion could be derived. In almost all the cases of pre- and post-treatments with different growth substances, particularly with inhibitors, there appeared a close correlation between decrease in RNA contents and dry weight of leaves and increase of alkaloid contents in roots. During post-reproductive phase also identical negative correlation between alkaloid contents in roots and RNA and dry weight of leaves could be established.

The impact of these observations would be far-reaching to botanists and phytochemists because, for the first time, it has been reported that the contents of total alkaloids in the root of a commercial medicinal crop like ipecac could, 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 would 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.

No information could be found in literature on P^{32} uptake and its translocation in relation to high and low temperature stress conditions applied to economically important crops. The present investigation with an important medicinal plant like ipecac can be claimed to be unique in this respect.

In the present study, effects of both high and low temperature stress on P^{32} uptake by ipecac plants were analysed alongwith the effects of some growth promoters or inhibitors with an aim to elucidate how far the protective nature of these chemical factors could be related to stress effects of temperature.

Exposure to high and low temperature depressed P^{32} uptake capacity of ipecac plants, the effects being more pronounced in low temperature.

On 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.

Studies established promotive effects of growth regulators like GA_3, KN, NAA and MH on P^{32} uptake by ipecac seedings. In general, the stimulatory effects followed decreasing pattern of KN→GA_3→MH→NAA, the maximum augmentation being in KN and the minimum, in NAA; ABA and KSCN could not augment the uptake behaviour; rather inhibited the process. Such effect of augmentation or inhibition of P^{32} uptake by different growth regulators could find parallelism in the enhancing effects of KN on RNA, SN and TN; GA_3 on chlorophyll, RNA and SN; MH on TA and leaf formation; NAA on leaf dry weight, leaf formation and chlorophyll and with the inhibitory effects of KSCN on chlorophyll, RNA and SN and of ABA on chlorophyll, RNA, SN and TN formations.

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 treatments with KN, GA_3, MH and NAA whereas ABA and KSCN aggravated the inhibitory effects of abnormal temperatures.

An analysis of the effects of chemical pre-treatments individually would reveal that the scope of effectiveness of KN and GA_3 in offering greater protection to growth and biochemical lesions due to high and low temperatures had been further broadened accomodating a more efficient uptake, distribution and incorporation of P^{32} in the system of elevated as well as lowered temperature environment; in other words, the damage inflicted upon the uptake mechanism of P^{32} was elegantly and effectively repaired by KN and GA_3; such effects being comparatively more pronounced under low temperature stress conditions. Though not as effective as KN and GA_3, 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 P^{32}. One would thus demarcate pioneering roles of KN and GA_3 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 ipecac plants subjected to stress conditions.

It might prove at this stage to be a rewarding exercise to compare the uptake and incorporation behaviour of P^{32} in respect of KN and GA_3 vis-a-vis RNA contents. In general, induction of uptake as well as incorporation were higher in KN treatment under high and low temperature conditions. Such increased uptake by KN (as compared with GA_3) was particularly interesting; if one would 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. On the background of such convincing results (implicating an important metabolite like RNA and a fascinating tool like P^{32} for assessing efficiency of ion uptake) it would be argued that temperature-stressed plants acquired 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.

High and low temperature treatments rendered the plants to incorporate more P^{32} particularly 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° and 5°C, extent of metabolic participation in these plant organs did not differ markedly.

Thus, 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 the composition

of protein, plants would remember a temperature experience and adapt their physiological system accordingly by favourably modifying their uptake and translocation behaviour.

In conclusion, it would be pertinent to put into record that the phenomenon of protection, as offered by different growth substances and inhibitors against thermal stress, appeared to be of highly specific in operation and optimisation of a particular treatment would depend on the time of application of such chemicals and their nature, stage of development of plant experiencing stress environments, range of temperature and its durations and also the type 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 investigation in the metabolic implications of thermal stress injury in a highly valuable medicinal plant like ipecac might be considered a major exercise in the area of temperature stress physiology in higher plants.