Review of literature and scope of the present study

REVIEW OF LITERATURE AND SCOPE OF THE PRESENT STUDY

Review of work done on ipecac

Successful cultivation of ipecac is rather limited to a restricted area of the world and scientific reports on this plant are meagre. Available literature includes mainly studies on germination, cultivation, pharmacognosy and phytochemistry.

Germination studies were undertaken by Rajkhowa(1964) in Assam who stated that the seeds took 93-125 days for germination to start. Depending on cultural conditions, 20-50 percent germination was reported by Chatterjee et al.(1985). According to them, highest rate of germination could be attained by shading and mulching the seed-bed at low altitudes. Chatterjee et al. (1982) obtained improved germination by treating the seeds with lime water for 40 hours and with hydrogen peroxide for 96 hours.

Studies on the cultivation of ipecac were reported by Biswas and Sampatkumaran (1948), Chopra et al.(1948), Higbee and Kelly(1950), Roychoudhury(1951), Datta and Mukherjee(1951), Gupta(1952), Chakravarti(1962), Goswami(1963), Saha(1966), Rajkhowa(1969) and Gupta(1971). Extensive studies on different aspects of cultivation of ipecac were undertaken by Chatterjee(1977, 1980).Transplantation studies in different months revealed that June-July was the optimum period with 95 percent seedling survival. Production of roots was also maximum when plants were transplanted during this period (Chatterjee et al., 1985).

Mitra and Chakravarti (1948) noticed that growth of ipecac plants improved with onset of rains and was found to be best during the month of August. In November, when temperature was around 18°C and mean humidity was 64 percent, the cuttings and young plants showed signs of decay. According to Chakravarti and Das(1966), plants raised from seeds produced more roots and TA (specifically, NPA) in comparison to the plants raised from cuttings. TA content of roots

increased from pre-flowering to fruiting stage although NPA content remained more or less identical. Guha and Mukherjee(1945) demonstrated that with proper conditions of cultivation and collection, the Indian ipecac roots would agree with the most recent standards laid down both in B.P. and U.S.P.

Kalyansundaram(1969) observed that rooting of ipecac root-cuttings was enhanced by treatment with IBA, boron and combination of boron and IBA. According to him, the average maximum length of roots was greater with IBA treatment than with boron whereas treatment with boron and combination of boron and IBA increased the number of annulated roots.

The alkaloid content increased upto the altitude of 500 m and declined thereafter. Plants growing at low altitudes contained higher percentage of TA but roots contained more cephaeline. Emetine content increased with increase in altitude (Chatterjee, 1977; 1980). Age of plants had distinct bearing on dry root yield as well as TA formation. Dry weight of root per plant increased with age till the 4th year whereas TA increased upto the 3rd year. This effect was found to be more pronounced in plants of lower altitudes (Chatterjee, 1977).

Decisive effects of roofing and mulching upon TA content was reported by Chatterjee and Bharati (1986) and Chatterjee et al. (1985). According to them highest TA content in the roots could be attained by maintaining the seed-beds without roof and mulching them with black polyethylene paper.

Experiments with different fertilizers showed that highest yield of roots was obtained in the medium supplied with 200 g of oil cake per unit area of 2.7 m x 1.22 m and application of superphosphate increased total alkaloids. Superphosphate application in combination with nitrogen fertilizers augmented the formation of NPA.

Banerjee (1968) working with mutagens, observed increase of NPA in comparison to PA in colchiploid plants. He established that the best method for inducing polyploidy was the application of 0.5 percent colchicine solution for 12 hours

by shoot tip plugging. Pharmacognostic studies of Bal and Dutta (1946) showed the presence of emetine and other alkaloids in the bark and their absence in the xylem. Progressive increase of alkaloid content with the age of the plants was due not to the increase in the percentage of alkaloids in the tissues but rather to the increase in total dry weight of the roots(Banerjee, 1974). Melville (1948) noted differential histological characters in commercial varieties of ipecacuanha.

Addor (1945) reported that the total alkaloid content in Brazilian ipecac was 2-2.7 percent of which 1.36 percent was emetine; 0.25 percent cephaeline; 0.4 percent psychotrine; 0.002-0.006 percent emetamine and 0.015-0.033 percent O-methylpsychotrine. The Columbian plants contained more alkaloid than the Brazilian but were poorer in emetine. Brazilian variety contained higher percentage of emetine (Janot, 1960). Analysis of roots of ipecac cultivated at Rangoon revealed 1.88 percent NPA and 0.22 percent PA (Ohn et al., 1970).

Sahu and Mahato(1982) devised a convenient method of HPLC analysis for routine simultaneous determination of emetine and cephaeline. Hatfield and Arteaga(1981), working on HPLC analysis, showed that alkaloid content of Panamanian ipecac was typical of that reported from Columbian and Central American drug in which the percentage of cephaeline exceeded that of emetine. From HPLC analysis, Subba and Sandberg(1982) reported that TA content was highest in the root, lowest in the leaves and intermediate in the stem whereas the ratio of emetine to cephaeline was identical in different plant parts. According to them, the yield of emetine and cephaeline can be increased by 20 and 66 percent respectively if whole plant is extracted.

Several workers have studied alkaloid chemistry of ipecac; its pharmacology, toxicology, alkaloid recovery from raw materials and estimation methods. Brossi et al.(1971) described chemistry, occurrence, preparation, spectral analysis, biosynthesis and pharmacology of ipecac. Brown et al.(1979) demonstrated stereoconservative synthesis of ipecac alkaloids from secologenin and Rohaly

and Szantay(1978) showed synthesis of ethoxy analogue of emetine. Preobrazhenskii et al.(1951) demonstrated that the method of emetine formation was like the formation of piperidine derivatives from glutaconates. Szantay(1967) too studied structure and synthesis of ipecacuanha and Adamskii(1969) studied the effect of temperature rise on the stability of alkaloids in ipecac tincture.

Battersby et al.(1978) and Nagakura et al.(1978) showed that desacetylisoipecoside was the true precursor of cephaeline and emetine. Sobiezewska and Borkowski(1970) worked on the determination of cephaeline in ipecac roots. Makauskas et al.(1976) improved alkaloid recovery by repercolation of the liquid extract and reduction in the size of particles in the extract of ipecac.

Ipecac roots were used in disease therapy as early as seventeenth century as an emetic and expectorant. Its use against dysentery was first recommended by Helvetius in the eighteenth century (Anonymous, 1971). Emetine when administered in small dose would act as expectorant and in larger dose, as an emetic. But the chief use of emetine was as a remedy of amoebic dysentery. Stewart (1949) studied the action of emetine upon Entamoeba histolytica, the causal organism of amoebic dysentery and Yeary(1972) demonstrated emetic properties of ipecac syrup in cats. Choudhury et al.(1957) clinically tested the preparation of bismuth-iodide complex of total alkaloid of ipecac in intestinal amoebiasis. Das Gupta (1952) also recommended chemotherapy of amoebiasis with emetine. According to Craig(1934), cephaeline possessed amoebicidal properties and Radomskii et al.(1952) found cephaeline as toxic as emetine. Boyd and Knight (1963) stated that alongwith emetine, cephaeline and 2-dehydroemetine also acted as expectorants. Cooney(1978) studied absorption of emetine in activated charcoal and justified the practice that ipecac syrup should not be given alongwith activated charcoal to counteract poisoning.

Different authors worked out methods for estimation of total alkaloid and emetine-cephaeline ratio. The major methods reported were thin-layer chromatography (Habib and Harkiss, 1969); column chromatography(Graf and Roensberg, 1970); TLC spectro-photometry (Habib, 1975; Elsayed et al., 1978), colorimetric and

spectrophotometric method (Saleh et al., 1979). A method was devised for the determination of emetine and cephaeline in which a H-column chromatographic procedure was used for the isolation of the alkaloids, which were measured individually by u-v absorption (Sharkay et al., 1971, Smith et. al., 1971). Seth and Ray(1967) worked out a simple method for the estimation of emetine based on the formation of chloroform-soluble coloured complex of emetine with 1,2-naphthaquinone - 4 - sodium sulphonate at a definite pH and temperature of which the colour intensity was proportional to the concentration of emetine.

Ipecac plants are, in general, hard enough for falling susceptible to most of the common diseases. But, as in the cases of almost all plants, it is not free from diseases. Two major diseases have been reported in this plant. The first and the more prevalent disease is the leaf blight caused by Alternaria alternata (Bharati et al., 1984); Chetia(1963), on the other hand, has reported the wilt disease caused by Fusarium sp.

Review of work done on high temperature

Temperature is one of the most important environmental factors controlling the activities and evolution of organisms and is one of the easiest variables to measure. According to Levitt(1980) the heat killing temperature for plant may be defined as temperature at which 50 percent of the plants are killed. The first systematic studies on high temperature stress in plants were carried out by Sachs(1860) who found that 51°C was the killing temperature. Though the plants could withstand the aerial temperature of 40-50°C for 10 minutes or more without injury, the same plants were killed when plunged in water at 49° to 51°C for 10 minutes. Nyland and Gohen(1969) observed that the effect of temperature - treatment would be 15 times more in aquatic medium compared to aerial application. Just(1877) observed that resting tissues could tolerate severe treatments as compared to actively growing tissues; this was confirmed later by Schneider(1910) who showed that dry seeds were able to tolerate temperature as high as 120°C in contrast to highly hydrated tissues that were killed by temperature below 50-60°C. Bieble (1962) recorded 60°-65°C to be the highest tempera-

ture tolerated by growing plants whereas Lange and Lange (1963) found heat killing temperature to be ranging from 44° to 55°C in thirty-nine species of plants.

Living tissues of 1-3-month old seedings of conifers of Western America were quickly killed when subjected to a temperature of about 54°C (Baker,1929). The thermal death point of most plant cells had been found to range between 45-55°C (Heilbrunn,.1924; Miller, 1931). But Belehradek and Melichar (1930) observed that cells would survive a temperature of 65.2°C for 3 seconds whereas temperature of 66°-69°C for 1 minute resulted in complete killing in Pinus strobus, P. resinosa, Picea glauca, Ulmus americana, Catalpa speciosa (Lorenz, 1939).

Toumey and Neethling (1924) demonstrated that sustained temperature of 49.4-50.5°C in the surface soil was sufficiently high enough to cause typical heat-lesions on white and red pine seedlings. Bukharin (1958) observed that wheat plants became scorched when the soil temperature was 50-53°C, injury becoming worse with the temperature exceeding 54.5°C. Cole and Steponkus (1968) observed that though shoot manifested visual symptoms of thermal injury, root was actually responsible for primary perception of high temperature which induced a deficiency of some metabolite synthesized in it.

Effect on germination : Porto and Siegel (1960) observed that air dry lettuce seeds were injured by exposure to 75°C as shown by reduced germination. Mukherjee et al. (1973) showed that germination and seedling growth increased with the increase in temperature but Ghosh (1978) found decreased germination percentage in rice due to high temperature treatment. Erzsebet and Szabo (1979) considered 20°-30°C to be optimal for germination of seeds of six American and six Hungarian soybean varieties. All genotypes of maize had lower germination at 30°C than at 32° or 27°C and a few genotypes exhibited no germination after 48 hours at 38°C. Prolonged exposure to temperature above 32°C reduced pollen germination of any genotype to levels near zero (Herrero and Johnson, 1980).

Optimum temperature-ranges for rapid mobilization of seed reserves and germination were 25-30°C in berseem clover and 20-35°C in Persian clover. Emergence and seedling elongation rates were highest at 30°C (Dalianus, 1980).

Biddington et al. (1982) found that a temperature treatment of 32°C produced irreversible embryo damage excluding membrane damage caused by drying. Germination of spinach seeds was inhibited at 35°C but pericarp removal promoted germination at 35°C (Suganuma et al., 1985).

Effect on growth : Gur et al. (1972) observed that temperature of 30°C and above was sufficient to reduce root and shoot growth and above 35°C for serious damage to leaf of apple trees. Intact barley coleoptile, subjected to high temperature stress, showed a significant increase in growth rate, the effect being more in increased duration of stress (Risueno et al., 1973). Relative growth rates were highest with root temperature of 25°C and root/shoot (weight) ratios were also greatest at 25° and 30°C (Combus and Nye, 1982). Hurewitz and Harry (1983) established the optimal root zone temperature for seedling growth to be 30°C based on fresh and dry weight and leaf area. Root production was not reduced by high temperature combined with low irradiance (Hunt and Thomas, 1985).

High temperature treatment stopped growth and new root formation alongwith abscission of lower leaves in Baldwin apples (Nightingale and Blake, 1934). Asana and Williams (1965) observed hastened senescence due to high day temperature and a significant reduction in stem weight at higher night temperature. Ordin et al. (1974) noticed inhibition of leaf growth in bean plants dipped in water at 46.5°C - 47.5°C. Feirabend (1977) observed that growth and expansion of leaves were little affected by high temperature but showed high degree of chlorosis. Dry weight of leaves declined with higher temperature in oats and peas and reached a maximum around 30°C in wheat and barley. In a late variety of maize, the leaf area increased by 58 and 73 percent at root zone temperature of 25° and 35°C while in early variety it increased by 19.9 and 24.5 percent (Stanev and Tsonev, 1980). Wardlaw et al. (1983) observed that shoot extension and leaf area development were stable with an increase in temperature from 20° to 30°C and growth was markedly reduced at temperature below 20°C and at 16°C there was no net gain in dry weight even after a period of more than 42 days. Al-Khatib and Paulson (1984) recorded acceleration of normal decline in leaf blade area and increase of protease activity during

senescence due to high temperature stress. In corn, Mackay and Barber (1984) found 2.6-5.1 times increase in root growth and 2.4-6.4 fold greater yield at 25°C when compared to that at 18°C.

Effect on stomatal function : Stalfelt (1962) studied the effect of temperature on opening and closing of stomata and found that 34°C caused complete opening in leaves whereas at freezing temperature and at 40°C opening did not occur or was very erratic. Barabal'chuk and Chernyavskaya (1974) also noticed increased transpiration by keeping the stomata open by 10°C rise in atmospheric temperature. Stoma became increasingly open at higher temperature until guard cells were lethally damaged (Rogers et al.,1981).

Effect on reproductive behaviour : High temperature given late in the panicle development induced floret abortion and even moderate temperature range after anthesis was associated with embryo abortion (Downes, 1972). Sato et al. (1973) found that high temperature just before or just after flowering caused sterility and 1000-grain-weight increased. High temperature applied just before or during flowering decreased pollen size and the storage of starch in pollen but when applied 1-2 weeks before or after flowering, decreased 1000-grain-weight. He also observed that grain quality was low with high panicle temperature. Herrero and Johnson (1980) noticed that high temperature during maize pollination resulted in poor setting of kernel. Increasing temperature from 21/16° to 30/25°C during the period of development from anthesis to maturity, reduced grain dry weight in wheat (Wardlaw et al., 1980). Prabha et al. (1982) observed that hot water treatments (50-55°C) given to the stigmatic ends of excised pistils just before pollination overcame the self-incompatibility barrier in Ipomoea fistulosa. In Triticum aestivum female fertility was reduced by high temperature treatment. Anthers on heat stressed plants were small and neither extruded nor dehisced normally and contained pollen grains mostly shrivelled having abnormal cytoplasm (Saini and Aspinall, 1982). Similar observations were made by Imaki et al. (1983) in rice plants. The number of fully ripened grains was low and most of the non-ripened grains were unfertilized. A small number of pollen grains was observed on the stigma and the dehiscence of anthers was incomplete. According to Gehrke et al. (1986) morphological abnormalities along with delayed flower initiation were the main symptoms of heat injury at 35°/24°C day/night temperatures. Mutters and Hall (1986)

also observed high temperature-induced male sterility and floral abscission in cowpea (Vigna unguiculata) which were more pronounced in long days than in short days.

Effect on protoplasmic streaming and plasmolysis : The most sensitive indicator of high temperature-induced injury is protoplasmic streaming. In high temperature it slows down and then stops completely but can recover if the plant material is cooled down. It ceases completely at little above 50°C in most plant cells and between 48-50°C in leaf epidermal cells of various phanerogams. It was observed that at 54°C there occurred reversible cessation of cytoplasmic streaming in cells which resumed to normal within 6-8 hours (Alexandrov, 1964). Nielsen and Todd (1946) found that the permeability of cell membranes of potato tubers was increased by heating to sub-lethal temperatures of 43° to 45°C. Stalfelt (1962) too observed increased membrane permeability with increase in temperature. Benzioni and Itai (1973) observed in tobacco leaves that exposure to 47.5°C for 2 minutes increased the leakiness of the membrane. Hendricks and Taylorson (1976) observed leakage of amino acids in imbibed seeds of eight plant species exposed to 30-35°C. Continuous heat shock at 45°C caused leakage of amino acids, soluble sugars and electrolytes into the medium which increased continuously during a 5-hour treatment. However, a brief heat shock of 47.5°C induced leakage at a slower rate than continuous heat shock at 45°C (Chu-Yung et al., 1984).

Effect on organelle integrity : The nucleus has been found to be the most heat-sensitive part of the cell, especially at dividing stage. Allium cepa exhibited nucleolar segregation in meristematic cells following a shock at 44°C (Risueno et al., 1973). Longer heat treatment followed by growth at 38°C gave rise to chromatin degeneration and chromosome lesions in sensitive varieties of wheat (Das, 1973). Heating for 5 minutes at 50°C decreased the nuclear volume by about 15 percent in leaf cells adjoining stomata in Tradescantia fluminensis and treatment at 59°C reduced them to less than half the original size (Barabal' chuk and Chernyavskaya, 1975). Daniell et al. (1969) observed disorganisation of tonoplast and chloroplast membrane at the thermal death point. Isolated vacuoles showed thermal damage at the temperature range greater than 50°C.

Heating of whole leaves and subsequent isolation of vacuoles showed that tonoplast integrity was not affected by heat stress in situ upto 45°C (Weigel, 1983).

Effect on protein stability : Protein denaturation was the earliest explanation of heat injury (Belehradek, 1935) and this is accepted still today. The death of cells by heat treatment has been explained as a result of denaturation of proteins (Lepeschkin, 1935). According to Alexandrov (1964) the temperature high enough to injure the plants directly causes denaturation of proteins with an irreversible coagulation of protoplasm. In seedlings of Pennisetum typhoides, after exposure to 48°C for 22 - 24 hours, ammonia nitrogen was found in detectable quantities which injured the plants (Lahiri and Singh, 1969). Lahiri et al. (1973) stated that the decomposition of protoplasmic protein, the disturbance of protein - lipid complex and the release of toxic intermediate and final decomposition products were the main causes of injury.

Many workers have observed relative increase in synthesis of certain special type of protein after heat shock (Loomis and Whuler, 1980). Key (1981) too observed new set of proteins known as heat shock proteins (hsp) formed in seedling tissue of Glycine max (cv. Wayne) when the growth temperature was increased from 28° to 40°C. Cooper and Twan-Hua (1983) observed that when temperature was raised from 25° to 40°C, protein synthesis continued and hsp made their appearance after 20 minutes in maize seedlings. Shifting the temperature back to 25°C caused decrease in synthesis of heat shock proteins. According to Voinikov et al. (1986), the heat-induced changes in protein synthesis in maize cell suspension were accompanied by the synthesis of hsp and cessation of formation of proteins, characteristic of normal temperature conditions. Nicotiana plumbaginifolia protoplasts grown at 25°C and transferred to 40°C synthesized new temperature dependent polypeptides (hsp) distributed in a wide range of molecular weight (Restivo et al., 1986). Similar observation was made by Chrispeels and Greenwood (1987); Deshaies et al. (1988); Kishore and Upadhyaya (1988). Exposures of cells to 38°C resulted in the formation of both types of proteins (Kanabus et al.,1984). Nover and Schraff (1984) noticed synthesis of 30 acidic and 18 basic hsp in suspension cultures of tomato subjected to supraoptimal temperature of 35°-40°C. Nebiolo and White (1985) found greater

incorporation of S^{35}-methionine in corn seedling mitochondria due to the rise in temperature from 27° to 37°C. This increase was in part manifested in the enhanced synthesis of a 52-kilodalton protein which was found in supernatants of pelleted mitochondria after heat shock.

In rice, SN/PN ratio in the straw was increased when both panicle and straw or straw only were treated with high temperature. When temperature around panicle was lowered, panicle accumulated more nitrogen than the straw (Sato and Inaba, 1973). According to Walgenbach et al. (1981) mean concentrations of total nitrogen (TN), total soluble nitrogen (TSN) and soluble non-protein nitrogen (SNPN) increased in leaves, stems and total forage with the increase of day/night temperature from 18°/10° to 26°/18°C. The dry matter of the shoot sprout, germinated after 20 hours at 25°C, had a much higher content of total nitrogen and total soluble nitrogen than the dry matter of the rootlets. The percentage of salt soluble protein fraction (globulin) of the total soluble nitrogen and the percentage of the residual nitrogen fraction of the total nitrogen were distinctly higher in the material of the shoot sprouts than in the rootlets (Deesbach and Schipper, 1981). Donovan et al. (1983) stated that high temperature did not increase grain nitrogen level in whole plants. The proportion of total ear nitrogen translocated to the grain was higher at 15° and 21° than at 25°C.

Like protein, nucleic acids also undergo denaturation at high temperature (Peacocke and Walker, 1962) and according to Byfield and Scherbaun (1967) denaturation of mRNA is actually responsible for the decrease in protein synthesis. When subjected to heat stress (35°C for 15 hours), the RNA content was reduced more in two sensitive species, Phleum pratense and Poa pratensis among four grass species, according to Baker and Jung (1970).

Effect on photosynthesis : Photosynthesis is very much sensitive to changes in heat. Direct comparison have shown that photosynthesis is inhibited at a temperature several degree below that required for inhibition of other processes like protoplasmic streaming (Alexandrov, 1964), respiration (Bjorkman, 1975) and direct resistance to loss of ions across the tonoplast membrane (Berry,1975). The rate of photosynthesis decreased immediately after heating to 38°C in fir and to 42°C in maple and complete cessation of photosynthesis occurred

at 47°C (Baur, 1972). The inhibitory effect of high temperature on photosynthesis is completely reversible if the temperature is not too extreme. A 2-minute shock at 46°- 51°C reduced $C^{14}O_2$ fixation in detached leaves of Nicotiana rustica which continued for 2.5 hours but recovery was complete 45 hours after treatment. Benzioni and Itai (1972) similarly found that treatment at 40°C for 5 minutes was strongly inhibitory but 24 hours later the rate was restored almost to that of the control (Yardanov et al., 1975). When photosynthesis was completely inhibited by a short term heating (10-15 minutes at 37.5 to 45°C) all the plants were able to renew photosynthesis after 17 hours in dark at room temperature (Egorova, 1976).

Santarius. (1973) found cyclic photophosphorylation to be rapidly inactivated by heat in isolated chloroplast. Pearcy (1977) also observed that heat treatment at 46°C of leaves from plants grown under moderate temperature resulted in a marked reduction in photosystem activities of chloroplasts isolated from them. The combination of high root zone temperature (25-30°C) with high leaf zone temperature (35°C) led to higher photosynthetic rate in maize plants than in sunflower plants (Stanev and Tsonev, 1980). The photosynthetic function of isolated lettuce chloroplasts proved to be markedly thermolabile. Its photosynthetic CO_2 fixation and light-induced chlorophyll fluorescence were drastically reduced at temperature between 40° and 50°C (Joachim, 1983).

High temperature injury is manifested also in the form of decrease in chlorophyll content. Nightingale and Blake (1934) found in apple tree that supraoptimal root temperature caused a reduction in chlorophyll content. Friend et al. (1962) reported chlorosis of wheat seedlings subjected to high temperature. Similar reduction in chlorophyll accumulation in high temperatures had been reported in grape-vine roots, cucumber cotyledons (Chkuaseli and Kotaeva, 1964; Robeiz, 1967) and watermelon seedlings (Onwueme and Lawanson, 1972). Tsoneva (1973) observed that wheat plant at 34 - 35°C accumulated chlorophyll but had active chlorophyllase. Feirabend and Mikus (1977) observed that temperature above 28°C prevented chlorophyll accumulation in Avena sativa, Hordeum vulgare and Triticum aestivum. Soybean and Elodea, when subjected to sub-lethal temperature, showed loss of chloropyll and swollen chloroplasts and at thermal death point, disorganisation of chloroplast membranes occurred (Daniell et al.,1969).

In maize, however, chlorophyll content rose alongwith rise in root zone temperature from 15° to 25°C and 34°C and maximum chlorophyll accumulation was observed between 30 and 34°C (Stanev and Tsonev, 1980).

Effect on respiration : High temperature stress leads to deviations from normal respiratory behaviour. Exposure to high temperature at short intervals of time caused metabolic changes in plant tissue which resulted in an increase in respiration and amylase activity (Itai and Benzioni 1973) and decrease in anabolic processes like CO_2-fixation (Benzioni and Itai,1972). Ulrich (1941) showed that respiration rate was associated with rise in temperature and more than double increase in the rate was noted for a 10°C rise in temperature. The fact that the intensity of respiration strongly increased during exposure to heat, indicated heat-promotive nature of the process (Lange, 1965); but at higher temperature respiration finally ceased altogether due to plasma injury (Sullivan and Kinbacher, 1967). In Crepis biennis, Kuijper (1910) found that respiration decreased with time even at 30°C. A temperature of 41°C for 2 hours induced 50 percent decrease of respiration but the total protein content was not affected by the treatment (Nikulina, 1985).

According to Levitt (1980), the heat injury is due to toxicity which is mainly a result of respiratory disturbances that occur gradually at moderately high temperature. Maxie (1957) suggested that heat injury might be due to toxic products of anaerabic respiration. Gur et al. (1972) found direct evidence of heat-induced formation of toxic products in apple trees.

Protection against heat injury : The first substances tested as possible protective agents against heat injury were salts and sugars. The heat stability of Tradescantia leaf epidermal cells increased after a preliminary treatment of leaves with hypertonic sucrose, glucose or lactose solutions (Feldman, 1962). Gorban (1968) reported an enhancement of the heat stability of wheat coleoptile cells upon immersion into 1 percent sucrose solution. Working with isolated spinach chloroplast membranes, Santarius (1973) found that raffinose was the most effective sugar to protect membranes from heat and cold denaturations.

Calcium, magnesium and manganese ions also were found to have stability effects on protein (Hagihara et al.,1956). It was found by Henckel (1964) that $CaCl_2$ increased the protoplasmic viscosity and thereby the temperature for coagulation of protoplasm increased. He suggested that increase in heat resistance caused by zinc was due to the increase in protoplasmic viscosity. Thermostability of Tradescantia leaf epidermal cells was increased by 1.8°C by $CaCl_2$ solution and the effect was accompanied by slowing down of protoplasmic streaming and suppression of phototaxis of chloroplast. Thermostability of Tradescantia cells could be increased by $MnCl_2$ and to a lesser extent by $MgCl_2$ (Alexandrov, 1977).

It was shown that supply of adenine could prevent thermal death of pea plants (Galston and Hand, 1949; Galston, 1959). Ketellaper (1963) carried out experiments with lupin, kidney bean and pea and observed that depending on the plant and the temperature, either vitamin B or ribosides were found to be effective in protecting the plants from thermal injury. Langridge and Griffing (1959) exposed homozygous races of Aradiopsis to supraoptimal temperature and showed injury effects which were partially erased when supplied with vitamins, yeast extract or nucleic acids. Biotin and, to some extent, cytidine were found to be specifically effective to prevent heat lesions. Yeast cells released a protective agent when heated, that showed a distinct thermoprotective effect (Rudenok and Konev,1973).

Among the phenotypic changes in the surviving seeds produced by heat, the most conspicuous one is the delay in germination. The addition of GA promptly relieved the delay and inhibition and resulted in a much higher survival (Ben-Zeev and Zamenhof, 1962). In addition to delay in germination, the flowering of Brassica napus was also completely inhibited for at least two months. This inhibition could also be relieved by the addition of GA. The thermodormancy of seeds caused by exposure to 75°C can also be prevented by soaking the seeds for 15 minutes in a solution of kinetin (Porto and Siegel, 1960; Ben Zeev and Zamenhof, 1962). Some positive evidence has been obtained by using kinetin as protective substance in the case of the proteolytic kind of metabolic heat injury.

Wheat seedlings, pre-treated with kinetin or ethanol, when subjected to heat shock at 45°C for 2 hours could survive the incubation temperature of 34°C

(Skogqvist and Fries, 1970). The plants from seeds treated with $Al(NO_3)_3$ or $Co(NO_3)_2$ and subjected to action of high temperature (40-50°C) were more heat resistant (Shkolnik et al.,1965).

When tobacco leaves were dipped in hot water (47°-49°C) for 2 minutes, senescence was accelerated which was retarded by applying cytokinin (Mothes, 1964). Kinetin increased resistance of Nicotiana rustica leaves to heat and prevented yellowing for long period (Engelbrecht and Mothes, 1964). Kinetin applied during or before heat stress diminished the effect of heat on catabolic processes like the increase in membrane permeability (Benzioni and Itai, 1973), changes in lipid composition (Skogqvist, 1974), the rise in amylolytic activity and O_2-consumption (Benzioni and Itai, 1973) and also prevented accelerated senescence (Mothes, 1964).

Development of tomato fruit was checked by high temperature treatment but application of auxin nullified this effect (Iwahori, 1967). Gorban (1962) found that cells of growing Kalanchoe leaves were less resistant to heat than non-growing leaves in lower layer. If, however, the plant was treated with maleic hydrazide, this difference disappeared after termination of leaf growth due to an increase in the thermostability of the cells in the upper leaves. ABA pre-treatment did not reduce heat damage while post-treatment enhanced recovery but in lesser extent than kinetin (Itai et al.,1978).

Review of work done on low temperature stress

A chilling temperature can be defined as any temperature that is cool enough to produce injury but not cool enough to freeze the plant (Levitt, 1980). In most cases plants do not suffer chilling injury until the temperature drops below 10°C. But rice (during flowering) and sugarcane may suffer injury at 15°C (Adir, 1968; Tsunoda et al.,1968). As in the case of other stresses, the injury increases with the increase in the degree of chilling. But in some cases injury is greater at a higher chilling temperature. Chilling-sensitive plants appear to have a critical temperature around 10-12°C, in most cases. But this generalisation does not apply in all cases (Wilkinson, 1970).

Symptoms due to chilling injury vary with the type of plant tissue and the severity of injury (Lyons, 1973). The symptoms usually develop more rapidly if the tissue under stress is transferred to a non-chilling temperature. The most apparent symptoms of general concern are surface pitting, necrotic areas and external discolouration.

Crop plants like cotton, cowpea, peanut, corn and rice manifested injury after 24-48 hours at 0.5° to 5°C (Sellschop and Salmon, 1928). Episcia, Achimensis and Gloxinia showed injured spots after a few hours or after a day of cold treatment due to death of protoplasm (Seible, 1939). On the other hand, Tradescantia, Solanum and Coleus became soft and wilted only after 4-5 days from the day of chilling. Soybean embryos were injured by imbibition at 2°C for as little as 5 minutes (Bramlage et al., 1978). Levitt (1980) calls such injury as cold shock which occurs so rapidly. Although chilling injury is primarily observed in plants from tropical or sub-tropical climate, certain plants from temperate climates may also be injured due to chilling (Toda, 1962).

Effect on germination : Germination of cacao seeds decreased below 14°C (Boroughs and Hunter, 1963). Obendorf and Hobbs (1970) found that imbibition of low-moisture soybean seeds at 5°C caused a reduction in survival and dry matter accumulation. Germination of chilling sensitive cucumber and mung-bean seeds decreased with temperature drop from 20° to 14°C. Below 11°C, germination was slight but at 10°C it was non-existent (Simon et al., 1976). Stewart and Bewley (1981) observed that germination of soybean, Glycine max cv. Biloxi seeds was unaffected by chilling at 4°C for 1 hour whereas that of cv. Fisteby was reduced. Seeds of Malus domestica, treated at 5°C, germinated whereas those treated at 15°C, did not germinate (Eichholtz et al., 1983). Warrington and Kanemasu (1983) suggested minimum temperatures of 9°, 8° and 7°C for germination, initiation of tassels and anthers respectively. Chilling injury was sustained by dry pollen of Typha latifolia but upon hydration at 0°C, injury was evidenced as poor germination, low vigour and depressed respiration. Vitality was irreversibly lost by the cold hydration. Decreased vigour and increased leakage started below 20°C and complete loss of vitality occurred below 10°C (Hockstra, 1984).

Effect on growth : When vegetative plants of Lolium temulentum grown at 20°C were transferred either to 5° or 2°C (at 8-hour photoperiod), a marked

decline in vegetative growth rate was noticed; but this reduction was not associated with increased mortality and was reversible if plants were returned to 20°C (Pollock et al., 1983). Exposure of cucumber seedlings to chilling reduced vegetative growth of both shoots and roots (Rikin et al., 1976). Growth in terms of fresh weight and dry weight increase was reduced during the cold treatment at 10°C for 24 hours in wheat seedlings (Triticum aestivum) (Nordin, 1977). Growth of Digitaria decumbens was severely reduced by night temperature of 10°C or below. Reduced growth was accompanied by an accumulation of starch granules in the chloroplasts of plants exposed to 10°C night temperature (Hilliard and West, 1970). Seedling growth of maize was reduced by 50 percent under 30°/8°C and 50°/10°C compared to plants under 15°/10°C day/night temperatures. The treated plants when inbred produced significantly reduced shoot, root and total plant dry weight (Landi and Crossbie, 1982).

Chilling of cotton seedlings at the time of seed hydration resulted in abortion of the radicle tip whereas chilling applied after germination but during early seedling growth caused damage to the root cortex (Christiansen, 1963). Hatfield and Egli (1974) observed that hypocotyl elongation was extremely slow at 10°C and the rate increased as the temperature was increased from 20°-30°C. Dry matter content of all cultivars of wheat and rye increased markedly during growth at 20°C (Pomeroy and Andrews, 1975) whereas chilling at 5°C resulted in loss of fresh weight(Whitaker and Wang, 1987). Relative growth rate and net assimilation rate decreased with lower night temperature (Grzesiak et al., 1981). In jojoba (Simmondsia chinensis)at 6°C, there was no net gain in dry weight over a 42-day period (Wardlaw et al., 1983). The roots of Triticum aestivum, Secale cereale, Hordeum sativum and Avena sativa grown at 5°C tended to be slightly shorter and thicker than those grown at 15° and 25°C (Al-Ani et al., 1983).

Effect on reproductive behaviour : When shoots of soybean (Glycine max) plants were chilled at 10°C for 1 week, they showed early abortion of flowers and a delayed resumption of flowering caused a 78 percent reduction in seed production (Musser et al., 1983). A constant low temperature of 12.5°C led to a strong delay in flowering in all genotypes of Pisum (Gottschalk, 1985).

Foliage temperature of 0-3°C in wheat, at the time when the pollen was in the stage of first nuclear division, resulted in sterility (Toda, 1962). Nishiyama and Satake(1979) observed that in the field, spikelets on the upper part of panicle in rice were more susceptible to cold for sterility - induction at the bolting stage than those in the lower part. Temperature below 16°C, especially at the beginning of flowering, caused floret sterility (Terres et al., 1981). Male sterility could be induced by cooling rice plants at the young microspore stage (Nishiyama, 1982). Fertility of flowers decreased with increasing length of cooling treatment or with decreasing temperature in rice plants (Satake and Koike, 1983).

Effect on stomatal function : In Phaseolus vulgaris and Pisum sativum, a reduction in stomatal aperture and the maintenance of a positive leaf turgor were the responses to chilling. Leaves of chilled non-hardened Phaseolus vulgaris plants maintained open stomata throughout the chilling treatment despite a severe wilt developing after 7 hours at 4°C. But chill-resistant Pisum sativum showed rapid closing and subsequent re-opening of stomata (Eamus et al., 1983).

Effect on protoplasmic streaming and plasmolysis : As early as 1864, Sachs observed that protoplasmic streaming ceased at about 10-12°C in root hairs of cucumber and tomato plants. But the streaming continued down to or near 0°C in chilling resistant species. Lewis(1956) found that streaming ceased or was just perceptible after 1 or 2 minutes at 10°C in petiolar trichomes of chilling sensitive plants such as tomato, water melon, honeydew, tobacco, sweet potato and it ceased completely at 5° or 0°C. In chilling resistant plants such as radish, carrot etc. streaming continued even at 2.5°-0°C.

Change in membrane permeability in response to chilling temperature has often been investigated as a possible cause of chilling injury. Kramer(1942) showed that low temperature reduced water absorption more in chilling sensitive than in chilling resistant crops. He concluded that the decreased absorption was due to a chilling-induced decrease in permeability. This interpretation has been confirmed by Kaufmann(1975) who found in cold sensitive citrus plants that cooling markedly lowered the permeability of the roots to water. Exposure to 0°C for 4 weeks caused a three-fold increase in cell membrane permeability

of mature green tomato fruits (susceptible to chilling injury) but a similar treatment had no effect on membrane permeability of cabbage leaves (Lewis and Workman, 1964). Cacas et al. (1965) suggested that membrane damage in cotyledonary tissues of cacao was a direct result of cold treatment.

The major physiological damage due to chilling injury has been attributed to the phenomenon of increased permeability due to the stress condition. Chilled sweet potatoes showed five times as much leakage as the controls, almost all of it being leakage of K^+ (Lieberman et al., 1958). Increased leakage of K^+ was observed in as little as 3-6 hours after exposure of bean and corn root tips at 1°C (Wheaton, 1963) and 5-14 hours after exposure of Coleus petioles at 0.5°C to 4°C (Katz and Reinhold, 1965). Two steps of K^+ leakage were observed in leaves of Passiflora exposed to 0°C, a relatively slow rate followed by a high rate during which most of the electrolyte was lost from the tissue (Patterson et al., 1976). When roots of wheat seedlings were cooled to 1°C for 24 hours, the potassium efflux increased by 50 percent of the rate of unstressed plants due to increased permeability of the root cell membrane (Nordin, 1976).

Christiansen et al.(1970) found an increased exudation from the roots of cotton seedlings in response to chilling temperatures which could completely be prevented by adding calcium or magnesium. In the case of leaves of Phaseolus vulgaris, increase in the rate of electrolyte leakage was observed only when the chilling treatment of 5°C was combined with partial dehydration of the leaves (Wright, 1974). The increased leakage of electrolytes has also been shown in cotton roots (Christiansen et al., 1970) and cotyledons (Guinn, 1971). Yelenosky (1978) observed that there was leakage of amino acids in citrus leaves after 5 weeks at 1.7°C. Since O_2-uptake was also decreased, the increase in leakage might be due to a decrease in active uptake. In rice, an unexpectedly large K^+ uptake occurred following cold stress. The anomalous ion transport in this thermophillic plant was considered to be a passive influx or exchange made possible by the change in permeability of cell membranes as a result of cold stress (Zsoldos and Gulyas, 1979). The promotion of electrolyte leakage was independent of the plant growth stage, the effect being stronger in less cold-resistant varieties (Zauralov and Lukatkin, 1985).

Effect on translocation : Studies on the transport of water and ions in chilling-sensitive plants indicated that transport also was reduced in response to low temperature (Drew and Biddulph, 1971; Jensen et al., 1961). Hartt (1965) reported that translocation in sugarcane ceased completely at 5°C. According to Geiger (1969), starvation of non-photosynthesizing plant parts resulted due to the inhibition of translocation by chilling temperature.

Effect on organelle integrity : Low temperature treatment (1°C) resulted in thickened cell wall and increased the tissue dry weight in Dicranum elongatum (Karunen and Liejenberg, 1981). Mitochondria also swelled, matrix became clear and the compact packing of cristie was lost. Several plastids achieved division; chloroplasts became elongated and ramified (Leddet and Geneves, 1982). Packs of cisternae in endoplasmic reticulum had been found to be induced by treatment at 5°C for several weeks (Dereuddre, 1981). Davis and Wilson (1984) observed that chilling the roots of Episcia reptans at 5°-15°C for 1-50 hours produced ultrastructural changes such as mitochondrial swelling, tonoplast discontinuity and sub-cellular deposition of crystalline compounds. Millerd et al.(1969) and Taylor and Craig (1971) found changes in chloroplast ultrastructure in a sensitive grass species. Temperature of 5° and 1°C induced a swelling of chloroplast stroma and deformation of chloroplast thylakoid was observed (Balagurova et al., 1983). Low temperature might prevent full formation of chloroplasts rather than act by blocking the synthesis of the chlorophyll molecules in tomatoes.

Effect on photosynthesis : Low temperature caused a sharp drop in photosynthesis due to damage of the chloroplast thylakoids at chilling temperature (Levitt, 1980). Lee and Estes(1982) observed altered chloroplast ultrastructure and a sharp decline in photosynthesis in the leaves of late-maturing corn following exposure to controlled day/night temperature of 15°/10°C in green-house. Phaseolus vulgaris exposed to 5°C for a single night exhibited severe reduction in photosynthesis accompanied by a parallel drop in transpiration, a rise in stomatal and mesophyll resistance to CO_2-uptake and a decrease in leaf water potential (Crookston et al., 1974). Pasternak and Wilson(1972) working with Sorghum vulgaris, a C_4 species, found that when only the shoots of the plant were cooled to 5°C for one night, stomatal opening and photosynthesis were reduced the

following day. Bingham(1971) reported retardation of photosynthesis of corn (Zea mays) (C_4) which had their leaves chilled at 5°C for 8 hours while the roots were maintained at 26°C. A single night exposure to chilling temperature (8-10°C) is sufficient for significant retardation of photosynthesis, stomatal opening and impairmant of physical and biochemical integrity of chloroplasts (Moss, 1965; West, 1970). According to McWilliam et al.(1982) reduction in photosynthesis due to chilling was rapid but the transpiration rate decreased slowly over the first 2-4 hours due to the closure of stomata. Leaves of Zea mays chilled for 6 hours at 5°C when returned to 20°C, showed a 45 percent decrease in the apparent quantum yield of photosynthetic O_2-evolution (Baker et al., 1983). Low temperature during juvenile growth resulted in reduction of respiration and photosynthesis in Zea mays(Crevecoeur and Ledent, 1984).

Low temperature reduced chlorophyll synthesis (Melvin, 1977; Bardhan Roy and Biswas, 1980); but no photodestruction of previously formed chlorophyll had been observed in mature leaves (Wardlaw et al., 1983). Exposure of cucumber cotyledon discs and isolated thylakoids of cucumber and spinach to 4°C in light resulted in a rapid inactivation of the thylakoids. The chloroplasts of chilling resistant spinach, however, were unaffected by exposure to 4°C in light (Melvin, 1977). A higher ratio of chlorophyll 'a' to 'b' was observed at lower temperature regime suggesting that the production of chlorophyll 'b' suffered more in lower temperature regime(Bardhan Roy and Biswas, 1980).

Effect on respiration : Anomalous respiratory behaviour during or after the chilling of sensitive plant tissues has been reported by a number of investigators (Eaks and Morris, 1956; Jones, 1942; Lewis and Morris 1956; Murata, 1969; Platenius, 1942). Reports of faster respiration in tissues injured by low temperature had been made in sweet potato, tomato(Lewis, 1956) and cucumber(Eaks and Morris, 1956). Another respiratory phenomenon of importance, in relation to chilling injury was the greatly exaggerated respiration-rate observed at warm temperature after transfer from a chilling treatment. Chilled cotyledons of cacao seeds showed initial respiration higher than normal but after 6 hours it was below normal (Ibanez, 1964). Murata(1969) observed the accumulation of acetaldehyde and ethanol (products of anaerobic respiration) in banana fruit

at 4-6°C. Exposure of maize to 2°C caused a sharp and continuous fall of soluble sugar in the root tips, which was accompanied by reduced respiration and cessation of growth (Crawford and Huxter, 1977).

Changes in mitochondrial physiology have been of interest because of the central role of this organelle in the respiratory process. Liebermann et al.(1958) found no difference in mitochondria resulting from 4 weeks of storage at 7.5°C. After the fifth week, activity began to decline in the mitochondria from the chilled roots; and by tenth week, the chilling treatment yielded completely inactive mitochondria. Similarly, Minamikawa et al.(1961) found a decrease in oxidative activity at 25°C of mitochondria isolated from sweet potato roots chilled at 0°C. Electron micrographs of mitochondria, derived from the chilled sweet potato, showed a large proportion in an extremely swollen state, not observed in mitochondria from healthy tissue(Yamaki and Uritani, 1973). When mitochondria were isolated from healthy but chilling-sensitive plants and exposed to chilling temperature, the immediate and direct effect was suppression of respiration before the onset of injury in opposition to the initial increase in whole tissues (Lyons, 1973).

Effect on protein metabolism : Protein metabolism is of paramount importance in the development of chilling injury. The manifestations of chilling injury have been generally ascribed either to protein deficiency or generation of toxic products from protein hydrolysis. Wilhelm(1935) observed protein hydrolysis in beans and tomato plants exposed to low temperature. Razmaev(1965) found proteolysis in chilling sensitive plants but not in chilling resistant plants. The effect of low temperature exposure on protein synthesis by mitochondria appeared to be different from that by the ribosomal fraction. The temperature of imbibition did not seem to have any effect on mitochondrial and ribosomal protein synthesis (Nagaraja and Patwardhan, 1978). Chilling at 100 percent relative humidity caused an increase in free amino acid content of the chilling-sensitive species (Rosinger et al., 1984). Under conditions of decreased temperature(2°C) for 20-30 days, the water content increased and the protein content decreased (Chel'teova, 1985). As in high temperature stress some new types of proteins (cold shock induced proteins) had been reported in barley (Hordeum vulgare) exposed to 6°C (Marmiroli et al., 1986).

Singh(1984) stated that the activities of pre-existing enzymes in imbibed seeds actually increased during the period of chilling. The activities of four enzymes studied (viz., amylase, protease, peroxidase and IAA-oxidase) in the imbibed chilled seeds were higher than in the unchilled seeds imbibed for the same period. Pollock and Ap Rees(1975) noticed greater inhibition of some of the glycolytic enzymes compared to sucrose synthesizing enzymes leading to a net sucrose accumulation in plants chilled at 2°C. Cotton seedlings chilled at 5°C showed a decrease in ATP concentration (Stewart and Guinn, 1969) as well as RNA, protein and lipid soluble phosphate (Guinn, 1971).

Protection against chilling injury : Artificially induced chilling resistance by application of growth regulators and chemical substances has been reported by different authors. According to Kuraishi et al.(1966), cytokinin prevented chilling injury in peas when sprayed every four days on the plant before exposure to chilling at -2°C for 3 hours. Abscisic acid was reported to increase plant resistance to sub-zero temperature in Acer negundo (Irving and Lanphear, 1968) and Medicago sativa (Rikin et al., 1975). Treatment with abscisic acid by direct application to the leaves or by incorporation in the root medium improved leaf resistance to sub-zero temperature in tobacco plants (Boussiba et al., 1975). Rikin and Richmond(1976) observed that application of ABA to chilling sensitive cucumber seedlings reduced chilling damage expressed by increased leakage of cellular substances, tissue dehydration, appearance of necrosis and inhibition of overall growth. On exposure to chilling for 24 hours, seedlings not sprayed with ABA showed typical chilling injury. On the other hand, in seedlings pre-treated with ABA, chilling injury was far less severe (Derek, 1986). The relative leakage from the cotyledons sprayed with ABA before chilling was only 50 percent of that in the untreated seedling. ABA (10^{-5}M) decreased chilling injury when applied in light as pre-treatment before the onset of chilling. Lower light intensity resulted in increased chilling injury and a decreased effect of ABA in prevention of damages incited by chilling in Gossypium hirsutum (Rikin et al., 1981). 2,4-D and mixture of KCl, NH_4NO_3 and boric acid, used in sprays were reported to protect cucumber leaves against chilling injury (Solomonovskii and Pomazova, 1967). Picolinic acid was also found to help recover the plant after chilling

to 15°C(Amin, 1969). Since these substances were respiratory inhibitors, they were considered to prevent the respiratory disturbances normally induced by chilling. Rice seedlings injured by a 10-hour exposure to 10°C recovered when treated with thiourea and to less extent with potassium thiocyanate and cystine (Ghosh and Chatterjee, 1975).

Cotton seedlings chilled at 5°C showed a continuous decrease in ATP concentration. By applying AMP to the seeds, it was possible to protect cotton seeds against chilling injury in field (McDaniell and Taylor, 1976).

Chilling resistance of citrus could be induced by nutrient treatment (Del Rivero, 1966). Buckwheat revealed an increased resistance to both chilling and cold shock (-3°C for 5 minutes) when potassium supply was increased, but an increase in calcium increased resistance to the former and decreased resistance to the latter (Korovin and Frolov, 1968).

Review of work done on ion uptake with tracer elements

Various authors reported their findings on the relation between temperature and ion uptake capacity by using labelled compounds. Baba(1955) observed that absorption of various inorganic nutrients was restricted by temperature, higher or lower than the optimum. Heat treatment caused reduction in active uptake of solutes inhibiting phospholipid biosynthesis (Benzioni and Itai, 1973). Mackay and Barber(1984) found 2-4 fold higher P-uptake at 25°C than at 19°C. Klein and Ferguson(1986) observed uptake of Ca^{2+} by suspension-cultured plant cells and protoplasts by exposure to 38°C compared to 25°C. Tissues pre-treated at 38°C showed increased uptake even upon return to 25°C.

Wehner and Watschke(1984) observed that in turf grass, incorporation of labelled leucine declined in 69 percent plants when heated at 43°C compared to plants held at 27°C. Corn seedling mitochondria incorporated a greater amount of labelled methionine with rise in temperature from 27 to 38°C(Nebiolo and White, 1985).

Export of radioactive carbon from tomato leaves was inhibited by application

of heat stress resulting in marked decrease in leaf starch levels (Dinar et al., 1983). Incorporation of P^{32} into phospholipid was lowered at 47-49°C temperature due to loss of membrane integrity (Dabtskaya et al.,1972). Ghosh and Chatterjee (1974) also reported reduction of P^{32} uptake and incorporation in rice seedlings treated with high temperature. They established that 5-minute pre-exposure to high temperature slightly increased P^{32} uptake whereas 15-minute pre-exposure decreased the total uptake. With increase in age of plants, incorporation of P^{32} increased in root tissues and decreased in leaves (Chatterjee et al., 1976).

According to Lin (1981) more C^{14}-assimilates were translocated from leaf to culm or panicle at high temperature than at low temperature. Kofka et al. (1982) reported that radioactivity in young leaves decreased by 63-75 percent, in older leaves by 24-26 percent and in the oldest basal leaves by 25 percent after saturation of hop leaves with $C^{24}O_2$ for 24 hours.

Mondal and Choudhury (1985) found that among the leaves the third leaf exported the minimal P^{32} to the grain in all cultivars of rice. Despite uniform amount of P^{32} having been applied, the relative export from fed leaf was different in different cultivars and this variation in export potential possibly induced different patterns of leaf senescence in different rice cultivars.

According to Webb (1967), transport of C^{14}-labelled sugar was sensitive to the change in temperature. The basipetal and acropetal movement of translocated C^{14}- labelled compounds in the phloem tissue was almost completely inhibited at 0°C. At 10°C, a partial inhibition occurred while an extremely variable degree of inhibition occurred at 15°C. A strong inhibition of ion uptake was observed at temperature below 10°C in excised barley and corn roots (Carey and Berry, 1978). Paull(1982) showed that uptake of leucine decreased progressively at 11°C in chilling-sensitive varieties of tomato (Lycopersicon esculentum). Koshiba et al.(1983) observed slower incorporation of 34-leucine into protein in excised axes of Vigna mungo during the first 6 hours of cultivation at 15°C than at 27°C. Similar observation was made by Marmiroli et al.(1986) in barley where a temperature step-down from 24° to 6°C reduced the incorporation of labelled amino acid.

Roychoudhury and Sen(1964, 1965) found that GA_3 stimulated the incorporation of P^{32} into DNA of coconut milk nucleii. GA_3 also enhanced the incorporation of C^{14}-adenine and C^{14}-uridine into RNA of isolated barley aleurone(Chandra and Varner, 1965). Working with different growth regulators and retardants, Ghosh(1978) established that cystine and kinetin feeding prior to high temperature exposure enhanced total uptake of P^{32} and increased incorporation in shoots and roots. GA_3 pre-feeding did not appreciably improve the total uptake of P^{32} except when the interval between temperature treatment and P^{32}-feeding was prolonged. According to her, thiourea pre-treatment decreased the uptake and incorporation. Mondal and Choudhury(1985) observed lower retention of P^{32} in leaves and its export to grains with ABA treatment; the amount of P^{32} translocated from mother to daughter shoot was less than that of panicles of the control plants of all cultivars. Osborne(1968) also observed that ABA decreased incorporation of leucine into protein and incorporation of adenine into RNA.

SCOPE OF THE PRESENT STUDY

Organised and large-scale cultivation of ipecac does not exist anywhere in the world except in Darjeeling Hills of India. With already more than 100 hectares under commercial cultivation, ipecac has attained the distinction of being the most prospective crop in Darjeeling Hills and has great potential to contribute as a major source of economic development of the hills.

In spite of the fact that ipecac has been growing commercially at the foothills of Darjeeling District since several years, no comprehensive report on its growth and developmental physiology is available with exception of some recent reports by Chatterjee and his students. The foregoing review will point out to the fact that this very important medicinal plant has not received due recognition from plant physiologists. The present study dealing with detailed analysis of growth and developmental features of ipecac is a pioneering endeavour in this direction.

The present set of experiments is the first systematic approach to elucidate the growth and developmental pattern of ipecac in Darjeeling Hills. This study has thus, considered the objective to determine the responses of ipecac plants to synthesize alkaloids in relation to their physiological conditions of growth and development under varying conditions of experimental diversions. Changes in some biochemical parameters in leaves during different developmental phases have also been investigated and a relationship between these biochemical changes with patterns of synthesis of alkaloids in the root has been sought.

Though conventional studies on pharmacognosy of this important drug plant have been conducted by a number of researchers, several interesting areas of investigation are yet to be undertaken. Since ipecac plants have distinct preferences for specific topographical as well as environmental factors for its optimum growth and development, the present pharmacognostic studies have covered detailed macroscopic and microscopic evaluation of plants in relation to TA content growing at different regions of the hills. Another objective of the

present pharmacognostic approach has been to index morphological and anatomical features of ipecac with the quality of root drug in terms of higher potency.

The cultivation of ipecac at the foothills of Darjeeling is restricted to elevations between 500 and 1000 m where the temperature ranges from 15°C minimum to 35°C maximum. Beyond this temperature range the plants do not thrive well; thus, restricting its commercial cropping within a narrow altitudinal belt of the hills. Hence, it was considered to be an interesting exercise to investigate the effects of exposure to high and low temperatures on developmental physiology of ipecac plants. With this objective the study of high and low temperature stress has been undertaken.

The review of literature mentioned in the preceeding pages will establish the fact that temperature injury is rather a complicated phenomenon and recovery from the injury depends on a multitude of factors including physical and chemical conditions of the plants. Though recently studies on stress physiology have been taken up in hand at the Research Station, Mungpoo, Darjeeling, search of literature brings out that temperature injury of medicinal plants including its protective mechanism has not, at all, been studied in India. Such a situation has prompted to take up the present investigation in which the effects of high and low temperature on growth and development including alkaloid formation have been analysed. Studies have also included detailed investigations on growth irregularities as well as some biochemical imbalances in ipecac plants that have undergone high and low temperature treatments. Besides, experiments include the effects of temperature stress on ion-uptake phenomenon and for technical convenience, radioactive phosphorus has been used in the uptake study. Experiments have also been designed to afford possible protection to the plants subjected to stress conditions.