CHAPTER– I

REVIEW OF LITERATURE

Tropical and subtropical plants exhibit a marked physiological disorders when exposed to low or non-freezing temperatures below about 10° to 12°C. This disorder was referred to as chilling injury and has been of great concern for many years with harvested plant parts because, lowered storage temperatures are generally an effective means of extending the post harvest life of fruits and vegetables. The phenomenon has not been well understood or recognised for its importance even though plant sensitivity to low temperature has been recorded for centuries.

Although it has been recognised for centuries the chilling stress is still very difficult to define quantitatively. As early as 1778, Bierkander reported that plants of eight species were killed at 1° to 2° C. Goppert (1830) obtained similar results. Hardy (1844) tested 56 species of tropical plants, out of which 25 were killed at 1° to 5°C. Molisch (1896) cited several early studies demonstrating that a number of plant species were killed at low temperatures above the freezing point. He suggested (1897) that this physiololgical harm should be referred to as "Chilling injury" (Erkaltung) to differentiate it from freezing damage (Erfrieren). Such injury to susceptible plants has also been referred to as low temperature injury. (Fidler, 1968) or cold injury (Plank, 1938), whereas, in apples it has been referred to as low temperature breakdown (Wilkinson, 1970).

Chilling injury appears to be a more preferable term (Ryall and Lipton,1972). Since it is not easily confused with freezing injury or with phenomenon related to cold or winter hardiness. Though it has been putforth that chilling sensitive plants tend to show response to critical temperature, below which injury often occurs, being 10° to 12° C.Chilling injury is the permanent or irreversible physiological damage to plant cells, tissues or organs which results from the, exposure of chilling sensitive plants to temperature below some critical threshold. This generalization does not apply in all cases, however, the optimum temperature for any plant process is not necessarily constant, but may vary with other conditions, even the species vary somewhat in tolerance with their region of origin. For example, rice when in flower and sugarcane suffer chilling injury at 15° C (Adir, 1968; Tsunoda _et al_., 1968). Similarly the germination of cacoa seeds is decreased at temperature below 14°C (Boroughs and Hunter, 1963). For temperate fruits, such as apples, the lower temperature limit is 0° to 4°C, whereas, 8°C for subtropical fruits such as citrus, avocado and pineapple and around 12°C for the more tropical banana (Wilkinson, 1970).

Furthermore, exceptional plants (eg.some fungi) may be killed at freezing temperatures in absence of freezing, that is in the undercooled state (Lindner, 1915; Onoda, 1937). On the basis of the above results a chilling temperature can be defined as any temperature that is cool enough to produce injury but not cool enough to freeze the plant.

The recent developments in many fields of plant physiology instrumentation and techniques in the last decade have provided the means of introducing a new insight on the elusive problem, leading to a more integrated concept of chilling injury.

The more advanced discussion of chilling injury was done by Levitt (1972) and Filder (1968). Chilling injury has also been considered in conjunction with other topics in review on the storage and postharvest physiology of fruits and vegetables by Biale (1950,1960), Hansen (1966), Miller (1946,58), Pentzer and Heinze (1954), Ulrich (1958) Wardlaw (1937,1961) Various symptoms of chilling injury are described by Lyons (1973). The symptoms which develop in the field vary from those shown by fruit of the same plant as a result of chilling in storage (Platt - Aloia and Thomson, 1976).

Chilling injury may occur in three different ways.

A) Direct injury

B) Indirect injury

C) Secondary stress injury (Fig.1)

A. DIRECT INJURY :

Seible (1939) divided plants that suffer chilling injury into two types according to the speed of the reaction. The first type (eg. Achimenes, Gloxinia) showed injured spots after hours at the latest after a day due to death of the protoplasm and infiltration of the intercellular spaces.

Seible's first rapid type is presumably direct injury. It is conceivable that the direct injury is due to qualitative, all or none, physical changes. The injury due to sudden chilling occurs so rapidly that it may be called as cold shock. Thus cold shock is obviously a direct chilling injury.

Sachs (1864) observed a cessation of cytoplasmic streaming at 10° to 12° C in root hair of cucumber and tomato, and Cohn described a pseudoplasmolysis in Spirogyra cells suddenly exposed to 0° C (Molisch, 1897). Greeley (1901) and Livingston (1903) observed the same phenomenon. This was explained

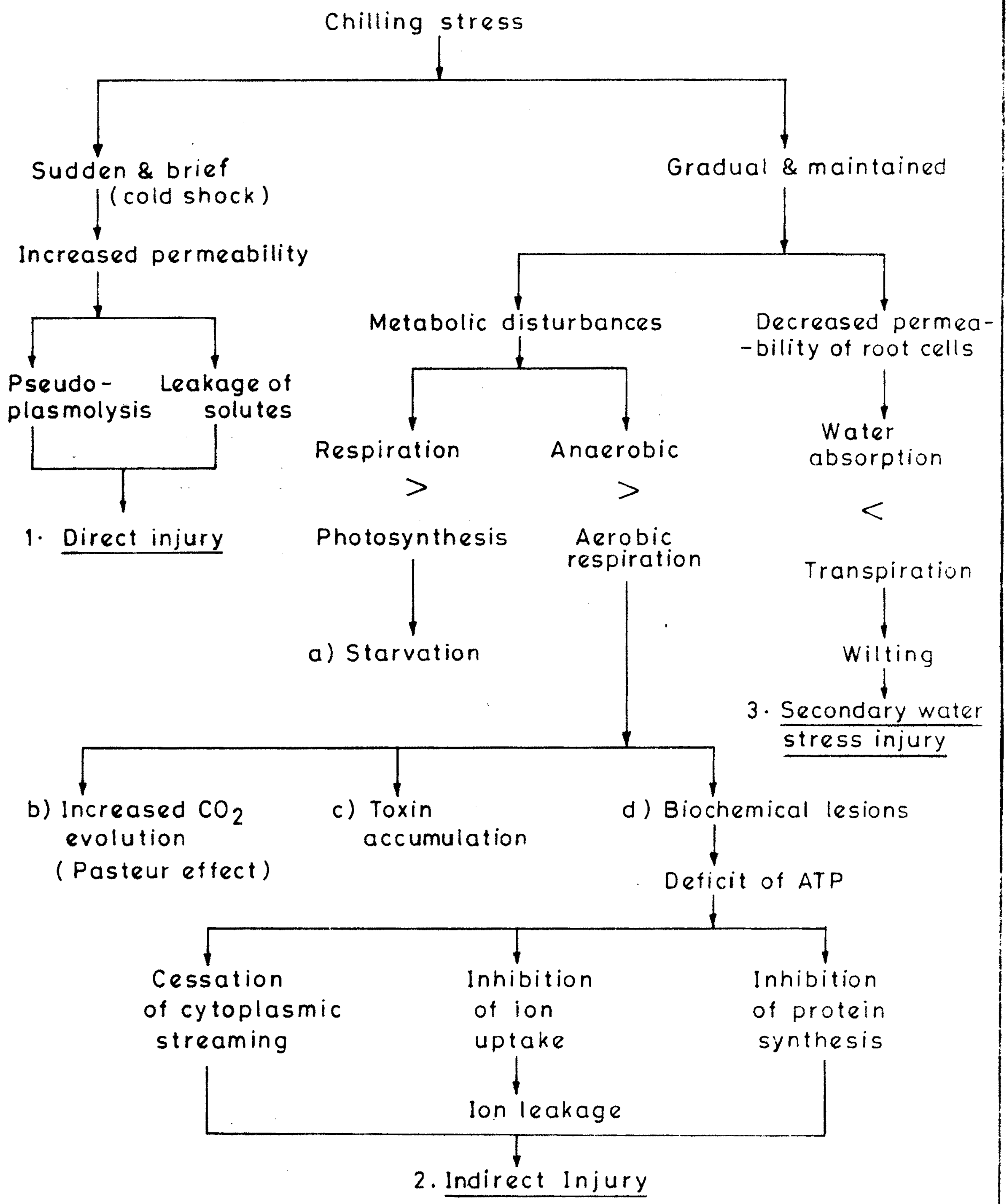

FIG. 1 – THE DIFFERENT KINDS OF CHILLING INJURY AND THEIR PHYSIOLOGICAL CHANGES THAT APPEAR TO PRODUCE INJURY.

by a sudden increase in permeability resulting in leakage of cell solutes. The postulated leakage has indeed been confirmed by Lieberman et al., (1958). Chilled sweet potatoes showed five times as much leakage as the controls, almost all of it being K^+. In this case, some what longer chilling periods were required than for cold shock. Direct injury therefore, may perhaps occur as a result of several hours chilling as well as when chilled abruptly for a few minutes.

These apparent increases in cell permeability as a result of somewhat longer chilling periods, seem to differ from the increase proposed by early investigators to explain chilling shock, the shock produced a rapid, spontaneous pseudoplasmolysis.

B. INDIRECT INJURY :

It is a slower quantitative, kinetic change in the chemical reactions of metabolism.

a. Solute leakage :

Slow chilling injury may require days or weeks of exposure to the temperature stress before its appearance. Many suggestions have been made as to the cause of injury. An increase in permeability has been indicated by increased leakage although the increase was only to three times the original value after 4 weeks at 0° C in case of mature-green chilling sensitive tomato fruits (Lewis and Workman, 1964). Chilling resistant cabbage showed no increase. Citrus leaves showed chilling injury after 5 weeks at 1.7° C (YelenosKy, 1978). Light is essential for the injury to occur, the leaves becoming bleached and leaking amino acids. Since O_2 uptake is also decreased, the increase in leakage may be due to a decrease in active uptake. It has long been known that even in chilling resistant plants, such as barley, active metabolism - dependent ion uptake is sharply curtailed by chilling temperature. Thus, indirect injury is due to a metabolic upset. Several types of metabolic disturbances are known.

b) Respiratory upset :

The respiratory upset due to chilling is in many respect analogous to injury due to low O_2 (Lyons,1973). " Low O_2 at normal temperatures may actually increase the rate of CO_2 evolution, as much as at normal O_2 concentration in plants injured by chilling. This is due to Pasteur effect - an increased rate of breakdown of substrate under anaerobic conditions due to inhibition of aerobic phase of respiration and the consequent acceleration of the anaerobic phase." Typically, during chilling of sensitive plants respiration increases initially, but as chilling proceeds the rate decreases (Tanczos, 1977). With prolonged exposure to low temperature, there is a similar rise in respiration upon transfer to warm temperatures but the rate remains elevated. The development of prolonged high respiration usually coincides with exposures causing visible symptom development.

In opposition to these chilling sensitive plants, chilling resistant potatoes show a different kind of respiratory change at chilling temperatures (1.7° C). They develop a greater involvement of the pentose phosphate pathway (Dwelle and Stallknecht, 1978).

In case of fruits, there is another possible cause of the respiratory rise associated with chilling injury - an increased production of ethylene, which is known to stimulate respiration effects of chilling on cytoplasmic streaming, may possibly be related to these respiration changes, since streaming depends on utilization of respiratary energy. In all the chilling sensitive plants tested (tomato, watermelon, honeydew, tobacco, sweet potato), streaming was just perceptible in the trichomes or ceased completely after 1-2 min at 10° C (Lewis, 1956). In the chilling resistant plants, on the other hand, e.g. carrot, radish, streaming continued at 0° - 2.5°C. Just what phase of the aerobic process is inhibited by the chilling has not yet been demonstrated, although some attempts have been made to measure individual steps in the process.

In the case of sweet potatoes, however, the chilling injury affects the oxidative activity as much as the phosphorylating capacity, and the P/O ratio remains constant (Minamikawa _et al_., 1961). The respiratory activity increased slightly during the first 10 days at 0° C then declined sharply. They concluded that the declining respiratory rate was limited by the oxidative system and not by the concentration of ADP and ATP. However, even if the P/O ratio is unchanged, this simply meant that both phosphorylation and oxidative activity are inhibited by the chilling stress. There will, therefore, have to be a decrease in ATP, compared to that in an unstressed plant with normal aerobic respiration.

c. Toxins :

Plank has explained injury by the accumulation of a cell toxin due to disturbances in the normal balance of biochemical processes (Smith, 1954). Injury would then depend on whether the rate of accumulation of the toxin exceeds the rate of its dispersal.

The toxin concept has been explained by the inhibition of aerobic respiration on chilling as follows.

chilling inhibition site

$$\begin{tikzcd}
\text{carbohydrate} \arrow[r, "-O_2"] & \text{Pyruvic acid} \arrow[r, "+O_2"] \arrow[dr, dashed] & CO_2 + H_2O \\
 & & \text{acetaldehyde} \arrow[dr, dashed] & \\
 & & & \text{ethanol} + CO_2
\end{tikzcd}$$

Any inhibition of the aerobic phase of respiration allows the anaerobic respiration to go to completion, producing toxic intermediates such as acetaldehyde, and end products such as ethanol. The initial increase in rate of respiration due to Pasture effect, would then be followed by an inhibition due to accumulation of toxins. It has long been known that continued anaerobic respiration kills most of the higher plants at room temperature. At chilling temperature, undoubtedly it would take longer period. Direct support of this concept has been produced by Murata (1969), who investigated chilling injury in banana fruit at 4° - 5°C. Acetaldehyde and ethanol content increased in chilled fruits after transfer to 20° C. There was an accumulation of α - keto acids in the peel and of browning substances (polyphenols) around the vascular tissue. Similarly apple scald is induced at chilling temperatures by the accumulated acetaldehyde and is inhibited by the antoxidant ethoxyquin (Lyons, 1973). A superficial scald of apples after prolonged storage at 0° - 4° C is caused by conjugated triene hydroperoxides, which are oxidation products of α - farnesene (Lyons, 1973).

Other toxic products may also conceivably arise at the chilling temperatures, for the inhibition of aerobic respiration might have a higher concentration of O_2 than normal in the tissues. This O_2 would be available to oxidases other than the cytochrome system normally metabolizing O_2 in an anerobically respiring unstressed plant. Peroxides are among the products formed by some of these oxidases. Inhibition of photosynthesis may also lead to peroxide formation. Chilling cucumber leaves showed the formation of photoperoxides which oxidized the substrate when exposed to light (Kislyuk, 1964).

d. Protein breakdown :

Protein breakdown at low temperatures without an equally rapid resynthesis has been suggested as a cause of injury, either due to deficiency of proteins or a toxicity by the products of hydrolysis - (amino acids, NH_3). Wilhelm (1935) produced evidence of such hydrolysis in the case of beans and tomato plants exposed to low temperature stress. Minamikawa et al., (1961) failed to detect any hydrolysis of proteins in the mitochondria of chilled sweet potatoes. They concluded that proteins are not likely to be degraded during chilling injury. Razmaev (1965) however, did observe proteolysis in chilling sensitive plants but not in others resistant to chilling (corn and wheat respectively). Although, the above experimental evidence is insufficient to prove a chilling induced protein breakdown, it is to be expected on the basis of the respiratory upset. Any decrease in the aerobic phase of respiration must result in a decreased oxidative phosphorylation. This would decrease the supply of ATP and therefore, the rate of protein synthesis, resulting in a net protein breakdown.

e. **Enzyme activity :**

A number of reports relating enzymatic activity to the chilling response suggest that enzyme systems affected by chilling temperatures are associated with membranes (Lyons et al. , 1979; Raison, 1980; Wang, 1982). In particular, it has been suggested that the respiratory activity of many chilling sensitive species decreases more than other reactions when the temperature is lowered and this causes imbalances in metabolism. Raison (1980) states that the changes in the thermodynamic and kinetic properties of the respiratory enzymes of these plants is not an intrinsic property of the enzyme protein but is induced by change in the molecular ordering of the mitochondrial membranes with which the enzymes are associated. The above notion is not accepted as an adequate interpretation by all. Quinn and Williams (1978) conclude that the temperature dependant changes are basically reflections of changes in protein conformation that alter the physical properties of the lipids rather than the other way round, as usually been argued". Graham and Patterson (1982), in their review of the experimental evidence, support the latter view, that of a direct effect of low temperature on proteins and enzymatic activity. They point to the fact that many observed rate dysfunction can be explained on the basis of changes in maximum rate (Vmax) and affinity constants (Km) in response to low temperature. The concept that temperature directly effects the activities of enzymes supports the view that there is no primary response to temperature but rather a multitude of responses.

f. **Biological lesions :**

A biological lesion is an abnormality in the metabolism of an organism leading to a deficiency of an essential intermediate metabolite. Theoretically, the most important biochemical lesion would be a deficiency of ATP due to the inhibition of aerobic respiration, for, such a deficiency could lead to leakage and protein breakdown. Unfortunately, adequate evidence of this deficiency due to chilling is lacking.

It has been suggested (Ketellapper and Bonner, 1961) that chilling injury in higher plants is due to biological lesion. Attempts were made therefore to prevent the injury by supplying the organism with a number of possible intermediates that might be deficient. Podin (1966) showed that a specific reaction (transformation of lutein to violaxanthin) does not occur below 10°C in the chilling - sensitive banana. Similarly, there was an accelerated loss of ascorbic acid in chilled sweet potato and pineapple (Miller, 1951; Miller and Heilman, 1952). Chilled tobacco plant exhibited symptoms of nitrogen deficiency and there was a four to five fold increase in chlorogenic acid and lesser increase in other substances. (Koeppe _et al_., 1970). In tomato fruit stored at temperature below 10° C, there was a large increase in activity of an enzyme involved in chlorogenic acid metabolism (Rhodes and Wooltorton, 1977). Consequently, the chilling temperature may have shifted the plant's metabolism from the normal pathway to an abnormal one leading to a deficiency in some intermediates of nitrogen metabolism. A parallel explanation has been proposed for cotton seed killed by exposure to 5°C for 12 hours during hydration (Christiansen, 1968).

g. Photosynthesis :

As chilling temperatures are imposed on sensitive species a reduction over and above a normal temperature related reduction in photosysthesis occurs (Wright and Simon, 1973). Loss of photosynthetic capacity due to chilling may impair growth and development of intact plants. Photosynthetic dysfunction is probably responsible for losses in productivity. It is intersting, however, to note that the chloroplast membrane may provide a possible assay for chilling sensitivity. Smillie (1979) has shown that the development of chilling injury in leaves and green fruit is correlated with changes in chlorophyll fluorescence or oxidation of cytochrome f.

h. Protoplasmic streaming :

Cessation of protoplasmic streaming (cyclosis) is one of the more striking physiological responses of chilling sensitive plants to chilling temperatures. Insensitive species exhibit only a steady reduction in rate as the temperature is lowered down to or near 0°C. A number of others have confirmed the observation of a rapid reduction in the rate of change of streaming in cells of chilling sensitive plants as the temperature drops below the threshold (Lewis, 1956; Das et al., 1966; Patterson and Graham, 1977). Lewis (1956) postulated that the cessation of streaming in sensitive species was due to the effect of temperature on one or more of the following

1. Cell lipids and their role in struture and action of protoplasm.
2. Energy supply from respiration to maintain protoplasmic streaming.
3. Energy utilization for streaming.
4. Protoplasmic viscosity.

Recent studies have greatly expanded our knowledge of the way in which cyclosis depends on the cytoskeleton and on the membrane systems which extend through the cytoplasm. (Hepler and Palevitz, 1974; Saxton et al., 1980). The importance of these structural elements of the cell in the effect of low temperature on cyclosis was studied by Woods et al., (1984). They employed a fluorescence probe to follow intracellular distribution of Ca^{++} and their results suggest that as the temperature is decreased into the chilling range, calcium is released from a lipid environment, presumably into the cytoplasm. Ca^{++} would strongly inhibit myosin activity, which, perhaps along with a reduction in ATP supply, would inhibit streaming.

i. Stimulation of Ethylene production :

Increased ethylene production at chilling temperature has been reported for a number of species (Vines *et al*., 1968; Christiansen *et al*., 1970; Wang and Adams, 1980). The increased ethylene production following chilling might be the result of an increased capacity of the tissue to make ACC (1-amino cyclopropane-1-Carboxylic acid), the immediate precursor of ethylene. Most of the workers have shown that ACC synthase activity and ethylene production remained low in the tissue during chilling and increased rapidly upon transfer to a warm temperature.

It has been shown that added Ca^{++} maintains ethylene biosynthesis in post climacteric apple slices (Lieberman and Wang, 1982) and that ethylene biosynthesis may be membrane bound. Recently, Legge *et al*., (1982) demonstrated that Ca^{++} treatment alters the micro viscosity of microsomal membranes, an effect which appears to be greatest at the membrane surface. He also suggested that the rigidification mediated by Ca^{++} prevents membrane deterioration by restricting lipid peroxidation and that preservation of ethylene production (biosynthesis) by Ca^{++} may reflect protection of the putative membrane-associated enzyme converting ACC to ethylene from peroxidative damage. These results along with those of Woods <u>et al</u>. (1984) may provide the foundation of a cohesive view of the Ca^{++} / membrane / cytoskeletion interaction in chilling and of the physiological consequences of that interaction.

C. SECONDARY STRESS INJURY :

The development of a secondary water stress at low temperatures was first indicated by Sachs (1864). He found that plants such as tobacco and cucumber begin to wilt if their roots are cooled to temperature below zero. Sugarcane is much more sensitive. It wilts if its root temperature drops to 15°C. This may lead eventually, to death and desiccation.

Kramer (1942) found that low temperature decreased the water absorption more in chilling-sensitive than in chilling resistant crops. He concluded that this decreased absorption was due to a chilling induced decrease in permeability. Evidence in favour of this interpretation has now been produced by Kaufmann (1975). The secondary water stress may also sometimes mimic the indirect chilling injury, by producing a metabolic upset, for instance, a decrease in photosynthesis due to stomatal closure (Crookston et al., 1974).

D. MECHANISM OF CHILLING INJURY :

Three basic changes are responsible for three kinds of chilling injury.

a. Metabolic disturbances leading to indirect injury.

i. Differences in energy of activation :

It has long been known that a marked temperature change may alter the end products of certain metabolic paths. Many plants for instance accumulate starch at normal growing temperature but replace the starch by sugar at low temperature. Some of these changes can be explained by the different energies of activation of different chemical reactions and therefore the differences in slopes of the Arrhenius plots. It can, however, be explained by the proposal of Selwyn (1966) that due to their larger energies of activation, some enzymes are inhibited more than other by the low tempereture, resulting in a relative enhancement of the latter and leading to an increase in a specific metabolic pathway.

In case of chilling resistant plants such shifts in metabolism induced by chilling temperature are noninjurious and reversible (elastic strain). Pollock and ApRees (1975 a) obtained evidence of a differential inhibition of enzymes at low temperature in potato tubers. At 2°C there was a greater inhibition of some of the glycolytic enzymes than of the sucrose synthesizing enzymes, leading to a net sucrose accumulation. This accumulation exceeded the activity of the sucrose synthetase, but was less than of sucrose phosphate synthetase (Pollock and ApRees, 1975 b). The carboxylation of RuDP was suppressed in plants acclimatized to low temperature. Instead the C_1 and C_2 moieties of RuDP were converted to glycine and serine via the glycolate pathway. whether or not injurious and irreversible, changes can be produced in chilling sensitive plants by such differences in activation energies, has not been demonstrated.

ii. Menbrane bound enzyme inactivation :

It was long suggested that chilling injury may be in some way related to a solidification of the membrane lipids. When a chilling sensitive plant is cooled to a chilling temperature the membrane lipids undergo a phase transition from the fluid to the solid state and this results in contraction of the membrane layer. The membrane bound protein molecules, therefore, are probably compressed and suffer a conformational change (Lyons, 1973). One immediate effect is a marked increase in the energy of activation of enzymes associated with the lipid membrane. This is observed by the sharp downward bend at the phase transition temperature of the Arrhenius plot. Presumably, there is an optimum protein conformation for normal enzyme activity. Just as solidification of the membrane lipid at chilling temperatures compresses the protein to an enzymatically less active state. So also to fluid a lipid, a membrane at high temperature would permit the protein to unfold to too great a degree for normal enzyme activity.

The phase transition of the membrane explains some of the metabolic disturbances at chilling temperature.

1. Starvation :

It occurs due to an excess of respiration over photosynthesis at chilling temperatures if the phase transition temperature,of the membrane lipids of the chloroplast occur at higher chilling temperature than that of the mitochondria. This possibility has received some support. Chilling can apparently injure the chloroplast synthesizing apparatus in mesophyll cells of C_4 grasses (sorghum and Papsalum) without affecting other organelles or preventing leaf growth. An exposure to low temperature (2° to 4°C) for a night was sufficient to produce chlorotic bands and to decrease the activity of C_4 enzymes. (Slack, et al., 1974).

2. Respiratory upset :

At chilling temperature the mitochondrial membranes of chilling sensitive cells are impaired due to the phase transition of the membrane lipids. Anaerobic reactions, on the other hand occur free in the cytoplasm, unconnected with membranes and therefore would not show bends in the Arrhenius plots found for the mitochondrial aerobic process. The phase transition of the membrane lipids therefore explains a) The disturbed respiration, b) The accumulation of toxins or c) The biochemical lesions in chilling sensitive plants at chilling temperatures.

3. Protein hydrolysis :

The suppressed mitochondrial activity would reduce the rate of oxidative phosphorylation and therefore of ATP production. Since ATP is required for protein synthesis but not for its breakdown, this ATP deficiency would shift the balance between the two, producing a net protein hydrolysis.

4. ATP is also needed for ion uptake, its deficiency would therefore lead to a net efflux (leakage) of ions. The leakage could also result from inactivation of ion pump enzymes due to a conformational change in the enzyme protein, for instance as a result of compression of the membrane on transition of its lipid to solid state.

5. Cytoplasmic streaming would be similarly inhibited by the decrease in ATP.

b. Permeability changes :

i. Increased permeability leading to direct injury :

The chill-induced phase transition of the membrane lipids from the liquid crystalline to the solid (gel) form involves greater order of the lipid molecules and therefore a contraction of the membrane. These changes are known to restrict the movement of small molecules through the membrane and therefore to decrease the permeability of the membrane to water and aqueous solutes.

If, however, chilling is sudden (cold shock) the contraction of the membrane and its contents may not be uniform and this could induce mechanical stresses, leading to the formation of fractures in the membrane. The membrane would therefore, become much more permeable permitting the leakage and other injurious phenomenon of direct injury.

ii. Decreased permeability leading to secondary water stress injury :

A gradual prolonged chilling, leads to a uniform solidification and contraction of the membrane, resulting in the decreased permeability.

The decreased permeability due to the phase transition is probably the basic cause of the secondary water stress injury. If only the roots are cooled below the phase transition temperature, this would produce the decrease in water absorption without a decrease in transpiration which is characteristic of secondary water stress injury.

The permeability change due to the phase transition of the membrane lipid may also conceivably lead to a metabolic inhibition.

All three kinds of injury can therefore be explained by the initial chill-induced but reversible strain the phase transition of the membrane lipids (Fig-2).

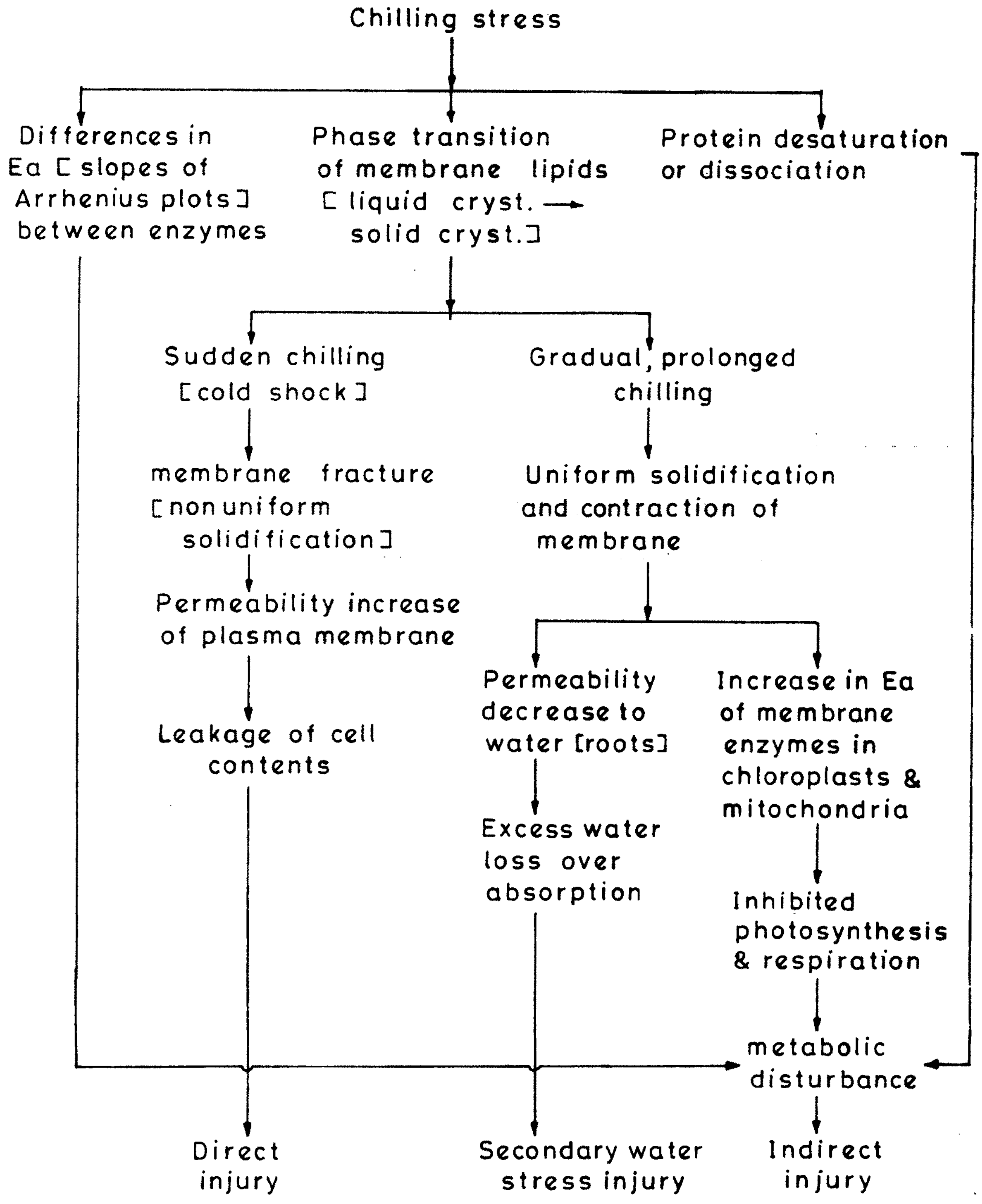

FIG. 2 — POSSIBLE MECHANISM OF CHILLING INJURY PROPOSED CHANGES LEADING OF THREE KINDS OF CHILLING INJURY ON EXPOSURE OF CHILLING SENSITIVE PLANTS TO A CHILLING STRESS.

A cold shock would induce direct injury by fracturing the solid membrane, permitting leakage of vital cell contents. A gradual chilling would induce the metabolic disturbances characteristic of indirect injury by sharply decreasing the activity of chloroplast and mitochondrial membrane - associated enzymes. The secondary water stress injury would be due to the marked decrease in permeability of the plasma membrane of the root cells to water, reducing the rate of water absorption below that of water loss from the shoot.

C. Mechanism of chilling injury :

A number of mechanisms have been proposed to accomodate the physiological and biochemical changes associated with chilling injury. Since a review of these changes indicates that chilling disrupts the entire metabolic and physiological processes, however, it would almost appear futile to explain the various phenomenon by some primary single change or master reaction controlling chilling injury. A single controlling response is found in the evidence that cellular membranes in sensitive plants undergo a physical phase transition from a normal flexible liquid - Crystalline to a solid gel structure at the temperature critical for chilling injury.

The schematic pathway of the events leading to chilling injury in sensitive plant tissue has been given in Fig. 3. It is the modified presentation of the previous presentation by Lyons and Raison (1970) and Levitt (1972). As the temperature is lowered in chilling - sensitive species the membrane lipids solidify at the critical temperature and the change in state would be expected to bring about a contraction that causes cracks or channels leading to increased permeability. This effect would lead to an upset in ion balance as well as account for the ion leakage that results from chilling in some tissues. The phase transition also brings an increase in the Ea of membrane bound enzyme systems, leading to a suppressed reaction rate and establishing an imbalance with nonmembrane - bound enzyme system. Below the critical temperature the membrane bound enzyme exhibits a marked increase in Ea, establishing a major imbalance in the two systems. Then metabolites such as pyruvate, acetaldehyde and ethanol would be expected to accumulate at the interface between glycolysis and the mitochondrial system, and these compounds do indeed accumulate very early in chilling (Ogata <u>et al</u>., 1968; Pantastico <u>et al</u>., 1968 Murata, 1969). Similar events can be projected for the chloroplast, where the phase transition leads to suppressed activity and change in metabolites after brief chilling (Taylor <u>et al</u>., 1972). The external

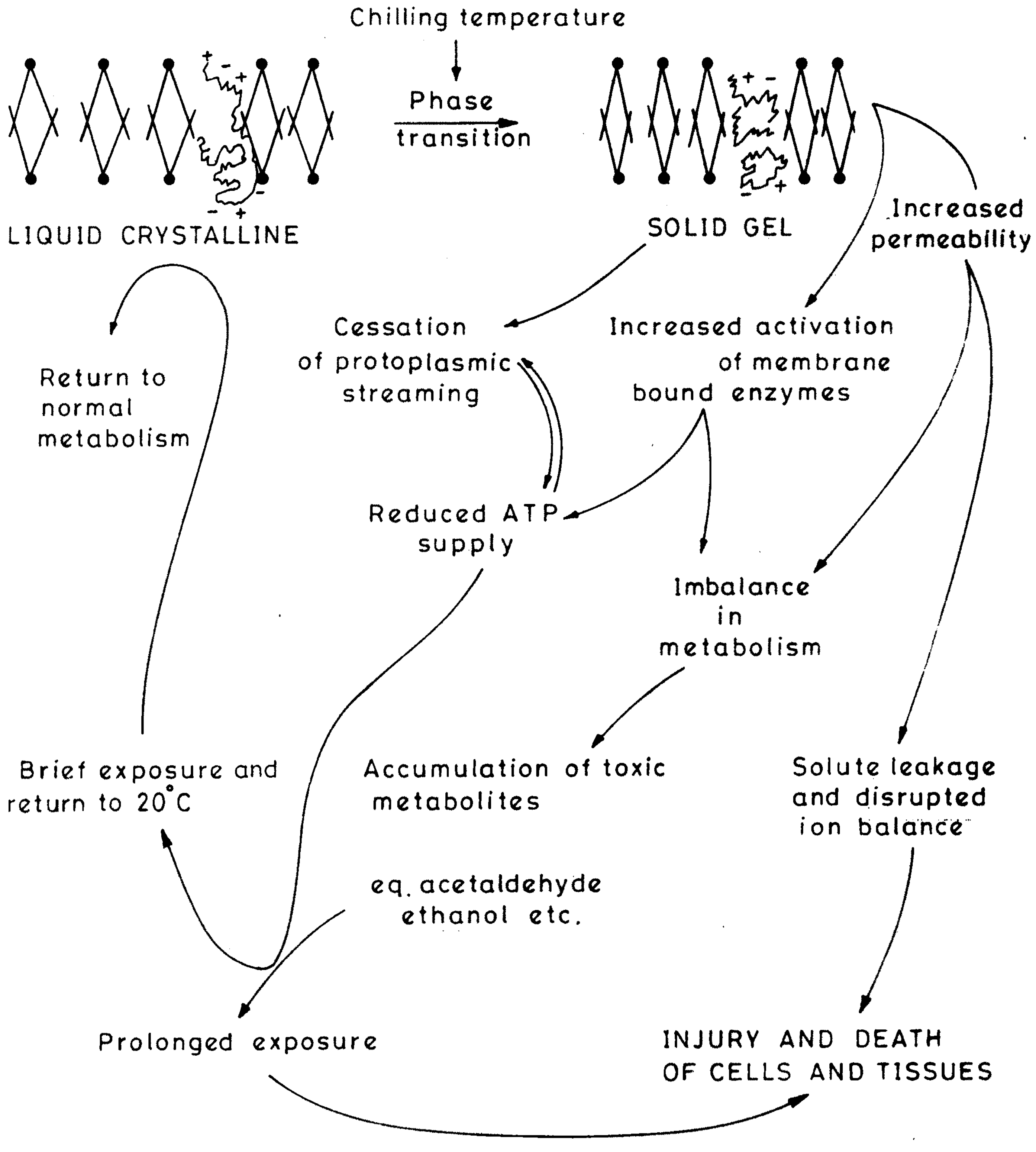

FIG. 3 – SCHEMATIC PATHWAY OF THE EVENTS LEADING TO CHILLING INJURY IN SENSITIVE PLANT TISSUES.

symptoms of injury and ultimate death of the tissue would reflect the cells inability to withstand increasing concentrations of these metabolites as a function of time.

A greatly reduced energy supply accompanying the suppressed mitochondrial respiration along with the possibility of altered activity of the membrane bound ATP ase system, would greatly upset the the normal energy balance of the cell. This altered energy supply, coupled with the rigidity of the membrane system following the phase transition, very easily accounts for the cessation of protoplasmic streaming.

The temperature induced phase change in the lipid portion of the membranes is completely reversible, Thus with a short chilling treatment followed by a warmer temperaure, respiration increase sharply, with the normal metabolism soon reestablished.

i. Recently published hypothesis :

1. Membrane alternation; The hypothesis that a physical phase transition of membranes from a flexible liquid - crystal to a solid - gel is the direct and primary response to low temperature in chilling - sensitive species (Lyons and Raisons, 1970) has been widely discussed in the literature. Despite the

unresolved questions, there appears to be substantial support for some role of membrane associated events in the chilling response. Wolfe (1978) and Melchior (1982) in their analyses of the experimental evidence, concluded that the fluidity and physical state of the membrane lipids can have a marked effect on the activity of membrane enzymes. Recently, analyses of composition and positional distribution of fatty acids in major leaf phospholipids from chilling - sensitive and insensitive species revealed a major difference in the fatty acid composition of phosphotidylglycerol (Murata et al., 1982; Murata, 1983). The occurrence of high proportions of dipalmitoyl and 1 - plamitoyl - 2 (trans - 3 - hexadecenoyl) in this compound was correlated with the susceptibility to chilling. Raison and Wright (1983) showed a transition exotherm beginning at about 10°C for polar lipids from chilling sensitive mung bean, whereas, none was evident above 0°C with polar lipids from chilling insensitive wheat or pea. The results obtained by them suggest that a transition exotherm in the polar lipids from chilling - sensitive species could be related to the presence of small amounts of high melting - point lipids. This finding supports the suggestion that a low temperature induced - phase transition could be the primary event in chilling injury. Furthermore, the studies of Murata and Yamaya (1984) demonstrated that among the major lipid classes from leaves of chilling - sensitive plants, only phosphotidylglycerol could induce phase - transition. Bishop (1983) in his review of membrane lipids and their role in maintaining the integrity of biological membranes, concludes that "although the membrane hypothesis of chilling sensitivity has been criticized, no other satisfactory explanation for the phenomenon has yet been advanced."

ii. Chilling injury not associated with lipid phase transition :

Although the phase transition of membrane lipids appears capable of explaining all the known kinds of chilling injury, it is possible that other mechanisms of chilling injury also exist. Two possibilities have been mentioned.

1. A metabolic disturbance due to innate differences between enzymes in energies of activation,
2. Protein denaturation due to low temperature - induced weakening of the hydrophobic bonds.

These two kinds of chilling injury apparently occur in tomatoes stored at chilling temperature (Rhodes and Wooltorton, 1977). Simon et al. (1976) have produced evidence in favour of the second of these kinds of injury, in chilling - sensitive cucumber and mungbean seeds. There was little indication of leakage from the seeds and the Arrhenius plots of respiration were linear without bends, and therefore presumably no phase transition of the lipids occured. So they concluded that this kind of chilling injury is due to protein denaturation.

E. CHILLING SUSCEPTIBILITY :

Chilling injury is a characteristic of plants of tropical or subtropical climates, although plants of the temperate zone can also have a sensitivity. Early works of Sachs (1865), Ewart (1895-97) and Molisch (1896) described the influence of chiling temperatures on a number of species of tropical and semitropical plants. Sellschop and Salmon (1928) demonstrated the sensitivity to chilling temperatures of a number of crop plants, including cotton, cowpea, peanut, corn and rice. When these species were subjected to chilling temperatures 2° to 4°C for a period of 12 hours, induced injury in some. Seible (1939) described symptoms of chilling in several species exposed to low temperatures for only a few hours. Similarly Spranger (1941) described the symptoms of chilling injury on vegetative tissues from a number of ornamental plants. Susceptibility has been described in many horticultural crops of economic importance.

Varietal differences in susceptibility to chilling injury have also been reported. For example Smith and Millet (1964) Showed that the average period for sprouting at 12° C ranged between 18 and 46 days among 10 tomato cultivars.

There are a number of factors that influence the relative susceptibility of vegetables to chilling injury. In addition to genetic diversity among the vegetable species, cultivars within a sensitive species can differ in symptom expression. Watada and Morris (1966) demonstrated that snap bean cultivars differed significantly in symptom development following a chilling treatment. Similaly, Apeland (1966) found that it took the cucumber cultivar, Ohio M.R. 200, only 10 days to develop symptoms at 12.5° C following a 4 day treatment at 4° C, whereas the cultivar Markateer required 47 days. King and Ludford (1983) showed that differences in chilling sensitivity among five tomato cultivars as measured by electrolyte leakage could be discerned prior to the development of visible symptoms. Another factor involved is the stage of phsiological development. Tomato fruit are quite susceptible in their mature green stage when a chilling treatment will disrupt and prevent the normal ripening process (McColloch et al., 1966). Recent studies by Autio and Bramlage (1986) demonstrated that as ripening progressed chilling sensitivity declined and then increased again during the late stages of ripening. Similarly fully mature Honey Dew melons were shown to be less susceptible to chilling injury than less mature melons (Lipton, 1978).

The conditions under which the commodities are grown may also influence the extent and development of chilling injury (Kader, et al., 1974). For example Abdel - Maksoud et al., (1974) reported that some winter grown tomatoes were more susceptible to chilling injury than summer - grown fruit of the same cultivar. Like wise Apeland (1966) found greenhouse grown cucumbers to be more sensitive than the same cultivars grown outdoors. Similarly, Palmer (1971) cited studies indicating that banana fruits maturing at higher field temperatures are more susceptible than those maturing in a cooler climate.

Chilling - sensitive vegetables are warm - season crops of tropical or subtropical origin. For postharvest considerations, all warm - season crops are susceptible to chilling injury during storage.

F. CHILLING RESISTANCE :

All plants from temperate climates routinely survive exposure to chilling temperatures and, therefore are fully chilling resistant. These chilling resistant plants can be further classified into chilling avoidance - plants which have the mechanism to avoid chilling injuries and chilling tolerant - plants developing the natural mechanism to tolerate chilling temperatures.

Following are the various mechanisms putforth for chilling resistance, (fig 4).

a. Increased unsaturation of fatty acids :

Chilling resistance is due to an ability to maintain the membrane lipids in the liquid crystalline state at chilling temperatures. Early workers observed that plants of warm climates contained more saturated fatty acids than plants of cooler climates (Lyons, 1973). Plants such as cotton and bean, become resistant to chilling temperature on exposure to temperatures above chilling. This hardening is presumably due to the observed increase in unsaturation of their fatty acids (Wilson and Crawford, 1974a),. which lowers the phase transition temperature below the previously injurious chilling temperature. Both the membrane fluidity and its normal high permeability to water are retained at chilling temperature. Similarly, the leaves of several species show a parallel decrease in unsaturation of lipids and chilling resistance accompanying the rise in the phase transition

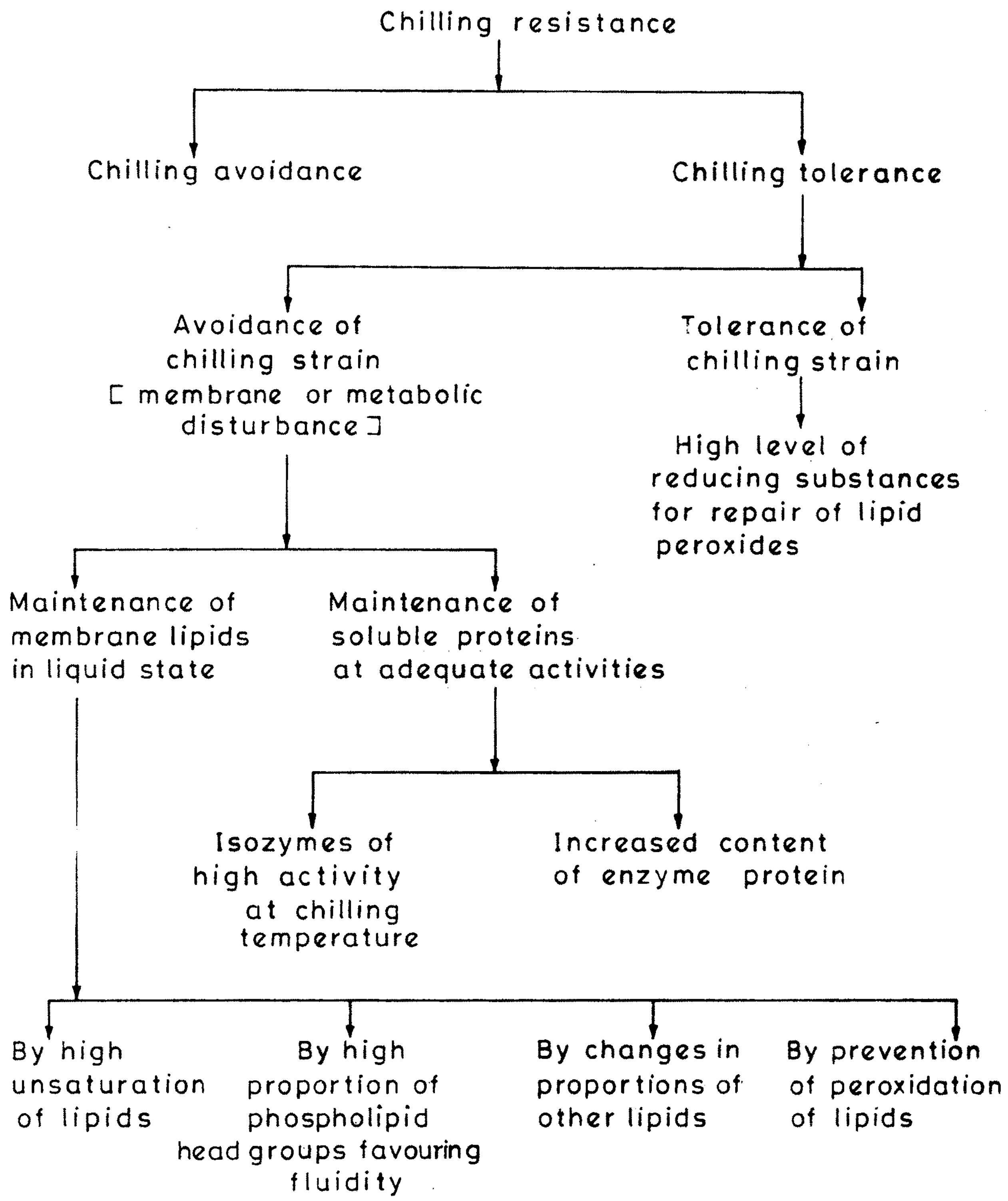

FIG. 4 – POSSIBLE MECHANISMS OF RESISTANCE TO CHILLING TEMPERATURES.

temperature during physiological ageing at 25°C. It may therefore, be concluded that a) chilling resistance is dependent on a downward shift in the phase transition temperature and b) this is commonly brought about by an increase in the membrane content of unsaturated fatty acids. One of the major factors according to Harris and James (1969), was an increase in availability of oxygen, which is the rate limiting factor for desaturation.

Another possible reason for the negative results is suggested by Wilson and Crawford (1974b). The acclimatization produced no effect on the composition of glycolipids or of the total fatty acids. It was the phospholipid fraction alone, representing only 25% of the total leaf fatty acids, that has its degree of unsaturation positively related to the chilling tolerance of the species.

b. Other lipid factors :

The importance of factor other than unsaturation in lowering the phase -transition temperature of the membrane lipids, may be the fact that unsaturation is a double edged weapon. According to christopherson (1969), the formation of lipid peroxides from the unsaturated fatty acids of membranes is probably highly injurious. It is therefore possible that the membrane lipids are normally protected against such peroxidation by the high reducing power maintained in actively metabolizing cells. When the cells are cooled to chilling temperatures, their metabolism slows down and their reducing power is decreased.

The unsaturated fatty acids of plants are sensitive to oxidation was shown by Van Hasselt (1974). The unsaturated lipids of Cucumis leaf disc at 1°C undergo photooxidative degradation. This damages the reducing capacity of the leaf discs (Van Hasselt, 1973). The mechanism of protection of Cucumis leaf chloroplasts against low - temperature photo - oxidative damage was explained by a protection of the electron transport pathway from the reducing to the oxidizing side of the photosystem. Protection against photo - oxidation by α - tocopherol has also been suggested by Dekok _et_ _al_., (1978).

c. Soluble Proteins :

Although the phase - transition of the membrane lipids from the crystalline to the solid (gel) state, appears to be the predominant injury mechanism have indicated that the activity of soluble enzymes may also be involved. The adaptation to chilling temperatures by the replacement of labile forms of an enzyme eg. glutamate dehydrogenase in corn roots and peroxidase in corn leaves (Brach et al., 1976), by a more stable isozyme (Alekhina and Sokolova, 1974) or more simply the counteraction of the lowered activity of an enzyme (per unit protein) by an increase in concentration of the protein (glutamine synthetase in corn roots, - Alekhina et al., 1975). In soybean a single mitochondrial enzyme (NADP - ICDH (Iso - citrate dehydrogenase) is apparently responsible for the respiratory control at chilling temperature (Duke et al., 1977). The occurrence of this kind of resistance may account for some of the negative results, for instance when chilling resistance is not related to unsaturation of the fatty acids.

G. HORTICULTURAL MANIFESTATION :

The symptoms of chilling vary with the plant tissue and the severity of injury. There are a number of commonly occurring visual symptoms which have been described for chilling injury in plant materials, Morris (1982) has recently summarized those visual symptoms which translate into postharvest losses. They are as follows.

1. Surface lesions : Pitting, large sunken area, necrotic areas and external discolouration. This symptomology of chilling injury has since been observed and described for almost all commercially important

horticultural commodities sensitive to low temperatures. If bananas exposed to mild chilling when green, develop a smoky or dull - yellow appearance to the peel and further chilling turns the peeling to dark brown or black. Cucumber gives an excellent example for typical surface pitting. Development of the symptoms of pitting shows the importance of relationships that exist among the factors of commodity characteristics, the presence of mechanical damage, severity of chilling treatment and relative humidity of the atmosphere.

2. Water (Soaking of tissues) : The disruption of cell structure and accompanying release of substrates favours the growth of micro-organisms. This is a commonly occuring symptom in leaves and is followed by wetting and desiccation.

3. Internal discolouration (browning) of pulp, vascular strands and seeds.

4. Failure of fruits to ripen in the expected pattern following removal to ripening conditions. Biale (1941) reported that avocado fruits stored at chilling temperature neither ripened normally nor developed the typical climacteric rise in respiration associated with ripening.

5. Abnormal pattern of ethylene evolution has also been shown in chilled banana (Murata, 1969) grapefruit,

sweet oranges, avocados and leaves (Cooper et al., 1969; Cooper and Reece, 1969).

6. Break down of tissues.

7. An accelerated rate of senescence.

8. Increased susceptibility to decay by surface pitting, necrotic areas and general weakening of the tissue. Injured tissue is readily infected by decay organisms.

9. A shortened storage life or shelf life due to the above responses. So abbreviated storage life in itself can be considered a symptom of chilling injury in horticultural commodities.

10. Compositional changes : Especially in relation to flavour and taste.

11. Loss of growth (sprouting) capacity especially important with stored propagules.

12. Development of superficial scald : A brown discolouration giving the skin a cooked appearance -in some varieties of apples stored at 0°to 4°C,`wooliness' of the fleshy fruits like peaches and plums refers to mealy texture and discoloured appearance (Davies et al., 1936, 1937; Boyes, 1952).

From the above symptoms, it is clear that abbreviated storage life in itself can be considered a symptom of chilling injury in horticultural commodities.

Symptoms of chilling in vegetative organs vary with the tissue involved, but under severe conditions the ultimate result is impairment of function and death. Chilling of sensitive grass species has been shown to cause a reduction in photosynthesis (Taylor and Rowley 1971) and changes in chloroplast ultrastructure (Millerd et al., 1969; Taylor and Craig, 1971). Common symptoms of chilling injury in developing vegetative tissues are necrotic lesions, increased susceptibility to decay organisms, cessation of growth and ultimately death.

H. ALLEVIATING CHILLING INJURY :

A number of workers have investigated methods for overcoming the symptoms and horticultural manifestations of chilling injury. Chilling injury can be easily avoided in sensitive species by simply limiting storage or handling to temperatures above the critical threshold; but as indicated previously, refrigeration is the most manageable way to slow down metabolic processes, control fruit ripening and deterioration and hence maximize storage life.

a. Manipulating the storage environment :

Temperature conditioning, by exposure to temperatures slightly above the chilling range for various periods has been shown to lessen the severity of chilling injury (Wheaton and Morris, 1967; McColloch, 1962). However, these temperatures slightly retard or delay symptom development. Alternating temperature can prevent symptom expression under certain time / temperature regimes. The beneficial effect of warming after a period of exposure to chilling may be related to either i) the restoration of normal metabolism so that potentially toxic compounds accumulated during chilling can be removed or the availability of some essential factor that became deficient can be resupplied or ii) repair of damage incurred to membranes, organelles or metabolic pathways before degenerative changes occur. This intermittent warming to interrupt the chilling treatment has received particular attention in the storage of temperate zone fruits (Smith, 1947) but has also applied experimentally with vegetables. [Lieberman _et al_., 1958; Creencia and Bramlage, 1971; Ilker and Morris, 1975; Marcellin and Baccaunaud, 1979 and Wang and Baker, 1979].

Modifying surrounding atmosphere of certain fruits subjected to low temperature disorders, to some extent alleviates or delays the symptoms associated with chilling injury. Reduced O_2 and elevated CO_2 can overcome the impact of low temperature injury on the ripening process, (Wade, 1979). Reduced chilling injury as a result of elevated Co_2 levels before or during a chilling treatment were reported for okra, (Ilker and Morris, 1975).

Surface pitting, one of the major symptoms of chilling injury, due to water loss can be alleviated by maintaining a high relative humidity around the commodity during storage, by using film wraps or modified atmosphere storage. The severity of symptom development can be alleviated in some cases by simply decreasing the vapour pressure deficit and thus slowing the process of water loss (Wardowski _et al_., 1973).

b. Hypobaric storage :

Burg and Burg (1966) described a system of fruit storage under reduced atmospheric pressure which greatly extended storage life at non-chilling temperatures. They have shown that low-pressure storage at 50 to 200 mm Hg with continuous air changes to remove volatiles and could relieve symptoms of chilling injury in avocados, bananas, grape fruit, peppers and tomato. Tolle (1969) applied the term "hypobaric" to low pressure storage.

c. **Chemical treatments :**

A number of chemicals have been applied exogenously in an effort to decrease the incidence of low temperature disorders, including chilling injury.

Ilker and Morris (1975) found that treatment with solutions of calcium and potassium salts could afford some protection against chilling injury in okra.

Treatment of cucumber and bell peppers by the antioxidants ethoxyquin and sodium benzoate reduced the expression of symptoms of chilling injury and maintained a greater amount of unsaturation of the polar lipids (Wang and Baker, 1979). Jones et al., (1978) treated banana fruits with several chemicals including dimethylpolysilozane, mineral oil, and safflower oil and reported alleviated symptoms from mild chilling treatments. Ethanolamine treatment has been shown to alter the properties of membrane phospholipids in tomato seedlings (Waring et al., 1976) and also reduce the ultrastructural injury induced by chilling. (Ilker et al., 1976). Tseng et al., (1986) reported that foliar application of mafluide provided singificant protection from chilling injury in corn plants and suggested that the treatment modulated permeability of the plasma membrane in the chilling environment. Other substances used in sprays (eg,2,4-D and $KCl + NH_4 NO_3 +$ boric acid) have been reported to protect cucumber leaves against chilling injury (Slomonovski and Pomazova,1967). Artificially induced chilling resistance has been reported by application of the chemical substance picolinic acid and Dexon to cotton plants (Amin, 1969). More recently rice seedlings injured by exposure to 10°C recovered when treated with thiourea and to a lesser extent with potassium thiocyanate and cystine (Ghosh and Chatterjee, 1975). Waxing was suggested by Platenius (1939) as a method for reducing chilling injury. Waxes applied to the surface of chilling sensitive tissues have aleviated chilling symptoms in some instances. Waxing reduced surface pitting on chilling - sensitive fruits held at 0.5°C and 70% relative humidity (Morris and Platenius, 1938).

More recent reports examining possible approaches to alleviating chilling injury conclude that the most likely solution occurs through genetic modification. Periodic attempts have been made by plant breeders to introduce chilling resistance into chilling - sensitive crop plants. Much of this effort has been focused on improving seed germination at low temperatures. The major effort has been with tomatoes (Smith and Millet, 1959).

There has been some success in using all suspension cultures for recovering chilling resistant variants.

I. Plants Under Investigation

Amaranthus blitum L. var oleracea Hooker (Plate No. 1 and 2) is widely distributed throughout India, Ceylon and tropical countries. The plant is grown almost, throughout the year in rainy and summer seasons. Especially in India it is mostly cultivated in summer season.

It is an annual C_4 plant belonging to family Amarantaceae. Amaranthus is an erect glabrous herb, 30 - 60 cm in height. It has fleshy well developed tap root system. Stem is thick fleshy, branched, reddish in colour. Leaves are obtuse, entire, thick reddish green in colour. Axillary buds are modified into tufts of small leaves. Flowers are terminal or axillary. Spike type of inflorescence contains small unisexual greenish flowere. Fruit is a capsule and seeds are minute.

PLATE I : Amaranthus blitum L. var. Oleracea, Hooker (Tambda Pokla) growing in pot soil culture.

PLATE II : Amaranthus blitum L. var. Oleraces, Hooker. Habit.

PLATE - I

PLATE - II

Leaves and tender twigs are used as vegetables, as they contain large amount of proteins minerals, vitamins `A' and `C'. It is another popular leafy vegetable. The plant is rich in Amaranthin.

Table 1 : Some important constituents of Amaranthus blitum

CONSTITUENT	NUTRITIVE CONTENT	CONSTITUENT	NUTRITIVE CONTENT
Moisture	085.70 g	Proteins	004.00 g
Fats	000.50 g	Minerals	002.70 g
Fiber	001.00 g	Calcium	039.00 mg
Calories	046.00	Oxalic acid	772.00 mg
Magnesium	247.00 mg	Iron	025.50 mg
Phosphorus	086.00 mg	Potassium	341.00 mg
Sodium	230.00 mg	Sulphur	061.00 mg
Copper	000.33 mg	Vitamin `A'	092.00 I.u.
Chlorine	088.00 mg	Riboflavin	000.10 mg
Thiamine	000.03 mg	Vitamin `C'	099.00 mg
Nicotinic acid	001.00 mg	Vitamin `B'	000.10 mg
		Carbohydrates	006.30 g

I.u. : International unit.

value per 100 g fresh tissue.

Medicinal Value : As the iron content is more in this Leafy vegetable it is used in anemic condition and it adds as a constituent of a balance diet. It is used to maintain the skin texture. Stem being fibrous - used for clear motion, so used for easy digestion. It is also used during dysentry and digestive troubles.

Trigonella foenum - graecum (Linn), (Plate No. 3 and 4) is a native of southern Europe and Asia. In India it is grown mainly in Punjab and Kashmir. Now a days it is cultivated at all times of the year, all over Maharashtra.

`Fenugreek' is an annual herb, a C_3 plant belonging to family Leguminosae of subfamily - Papilionaceae. It is a herbaceous plant with a height of about 10" to 12". Tap root system is with prominent root nodules. Stem is erect, smooth, green in colour unbranched. Leaves are trifoliate, with fleshy ovate leaflets. Flowers are white coloured, papilionate in form. Fruits are Long slender pods with a pronounced beak, 6-7 cm in length.

Leaves and young pods are utilized as vegetables. Whereas seeds are mostly used in spices and condiment. Leaves and young pods are utilised as vegetable. Leaves contain large amount of proteins, minerals and vitamins `A', `B' and `C'. The plant contains an alkaloid trigonelline.

PLATE III : *Trigonella foenum-graecum* (Linn.) (Fenugreek) growing in pot soil culture.

PLATE lV : *Trigonella foenum-graecum* (Linn.). Habit.

PLATE - III

PLATE - IV

Table 2 : Some important constituents of *Trigonell foenum-graecum*.

CONSTITUENT	COTENT	CONSTITUENT	CONTENT
Moisture	086.10 g	Protein	004.40 g
Fats	000.90 g	Minerals	001.50 g
Fiber	001.10 g	Calcium	360.00 m
Calories	049.00	Oxalic acid	013.00 mg
Magnesium	067.00 mg	Fe (Iron)	017.20 mg
Phosphorus	051.00 mg	Potassium	051.00 mg
Sodium	076.10 mg	Sulphur	167.00 mg
Copper	000.26 mg	Vitamin `A'6450 C	49,000 I.u.
Chlorine	165.00 mg	Carbohydrates	006.00 g
Thiamine	000.05 mg	Vitamin C-54	099.00 mg
Nicotinic acid	000.70 mg	Vitamin B	.16 mg

I.u. : International unit.

values per 100 g fresh tissue.

Medicinal value : Leaves are used as vegetable as well as fodder. Leaves are aromatic useful in external and internal swellings and burns, prevents hair falling off (yunani). Seeds are hot, tonic, antipyretic, anthelmintic, appetiser, astringent to bowels, cures leprosy, "Vata", vomiting,bronchitis, piles; removes bad taste from mouth; useful in heart diseases (Ayurveda). The seeds are mucilagenous aromatic, diuretic, nutritive, tonic and carminative, much used in colic flatulence, dysentry, diarrhoea, dyspepsia with loss of appetite, dropsy, enlargement of spleen and liver. Seed infusion is given to small-pox patients as a cooling drink. Seeds are made in to gruel and given to increase flow of milk, powdered seeds are used in veterinary practice.

Indian preparations - several confections under the name of Methi Modak are used in case of dyspepsia and in diarrhoea of woman in child birth and in rheumatism.