GENERAL EFFECT OF TEMPERATURE

Generally, temperature extremes above and below the optima, lead to some disorders in the metabolic activities of plants. It is important to understand the normal temperature mechanisms and why temperature deviations cause plant disorders. In the plant systems at low temperature, molecular activity is very slow and there is not enough energy for metabolic processes to proceed.

When temperatures are high, molecular motion is extremely rapid and the complex protein molecules come apart. Only when the heat energy level is about 20° to 45°C, biochemical reactions can proceed normally. Thus, normal plant development is dependent on a temperature regime suitable for metabolic activity. Plant development is largely a function of the cellular biochemical reactions. These reactions are controlled by enzymes. The rate at which an enzyme reaction proceeds is a function of temperature. According to thermodynamic law, rates of most chemical reactions double with every 10 degree rise in temperature upto about 20 to 30°C. Above this, the reactions decrease because enzymes are gradually denatured or inactivated. The higher the temperature, the more rapid the denaturation. Besides this, the optimum absorption of water generally
takes place above 30°C, but high temperatures are also conducive to rapid water loss, and cause moisture stress. Thus seed germination, growth, flower formation and plant hardiness are all influenced by fluctuations in temperature.

The relative air and soil temperature is important to normal development and distribution of plants (Daubenmire, 1959). High air temperature has been reported to stimulate top growth of wood seedling.

Normal plant functioning depends on proper control of every cellular biochemical reaction. Temperature is the most critical control and every plant is best adjusted to a particular temperature regime. As the temperature deviates from optimum i.e. either at high or low temperature, the various enzymatic activities decline, reducing metabolic activity and suppressing plant development.

The temperature extremes which the plant cell can endure are determined largely by the capacity of the component molecules, especially the proteins and lipids, to maintain their structure. This capacity is to a greater extent a function of the sulfhydryl and hydrogen bonding. Hydrogen bonds tend to pull together much more tightly at temperature below 0°C than at higher temperatures. This pulling shrinks and distorts the protein structure which in turn reduces or destroys irreversibility of protein
structures, as well as their activity and ability to function normally. Moreover as the temperature increases, the bonding becomes looser, until at around 30°C bonds tend to come apart thus disturbing many enzymes and denaturing the molecules.

Indirect injury may occur at temperature just below the point of protein denaturation (Langridge, 1963). As the temperature increases, the reaction rates and metabolic activity increases proportionally. At 30°C the metabolic rate can be very high and due to increased activity, there is a great demand for the essential elements in the cell; and if these elements cannot be supplied quickly, or are supplied in insufficient quantity, then disorders or malfunctions can be expected in the plant system.

Cellular damage may also result from the formation of toxic substances in certain cells exposed to a localised high temperature. This toxic material may subsequently be translocated to other parts of the plant and cause more widespread injury (Yarwood, 1961). Thus, when the temperature exceeds the extremes to which the plant is adapted, growth is inhibited or suppressed. Plant species can acquire heat tolerance by hardening i.e. by exposure to gradual increase in temperature. Hardening is determined by the increasing viscosity of the cytoplasm. A gradual increase in cytoplasmic
viscosity results in a higher degree of water binding in the cells, which increases the capacity of molecules to adjust to the molecular deformation caused by high temperature.

Chinoy (1947, 1947a) compared growth and developmental processes of 260 Indian and exotic varieties of wheat differing in vegetative period and found that yield and 1000 kernel weight were negatively correlated with temperature during the ripening period. Pontes et al. (1972) noted that flowering was delayed at the higher temperature with little difference in carbohydrate level.

The closest correlation between grain yield and the dry matter accumulation in wheat plants is observed in the shooting, earing and milky ripeness stages. There was a tendency toward a direct association between the grain yield and the dry matter content in the wheat plants during the period of grain ripening and harvesting. A determination of the degree of correlation between yield and atmospheric temperature during grain formation and expansion shows that the temperature exerts an influence on the yield (Chinoy, 1968).

Smith and Jewiss, 1966; Smith, 1970; have observed that high temperatures result in poor growth of temperate species and also hasten flowering or maturity. Tropical grasses, on the other hand, have a high temperature optimum for growth and their response in chemical composition and digestibility to high temperatures might well be different.
from that of the temperate grasses.

**ROLE OF ASCORBIC ACID (AA):**

It has been shown that pretreatment with ascorbic acid (AA) accelerates germination process as well as growth and yield of many plants (Abraham, 1969; Chminoy, et al., 1969; Dave, 1969; Patel, 1971; Ghesani, 1972; Shah, 1973). Hausen (1936) and Soubert V. Hausen (1948) were able to demonstrate an appreciable growth stimulation in intact and decotylised seedlings of pea grown in culture medium to which ascorbic acid had been added. Havas (1935) also noted a favourable effect of low concentration of ascorbic acid. Similarly, Davies et al., (1937), Bonner and Bonner (1938), Rigs (1947) were also able to get the stimulatory effect of ascorbic acid in different seeds e.g. oat, pea etc. Guttenberg and Wiedow (1952) observed a slight stimulation in excised oat embryos with very low concentration (0.001 per cent) of ascorbic acid.

There are a number of reports about the positive results by the ascorbic acid treatment on germination e.g. cress and mustard (Davies et al., 1937), pea (Ray, 1934), jute (Mitra and Dutta, 1951), lupine and tomato (Leea, 1955), tobacco (Dennison, 1940), legumes (Shrinivasan and Wadarekar, 1950), tung (Chatterji, 1960) etc. Khudairi (1968) showed that ascorbic acid enhances the percent germination as well as seedling growth in lettuce seedlings and concluded that
AA acts as a growth hormone up to 100 ppm. Chinoy et al. (1969) have found the germination pattern and various metabolic and enzymic changes are indication of the fact that AA at a higher concentration does not show any inhibitory effect. The application of 100 ppm solution enhances the fresh and dry weights of roots and leaves in green grass as observed by Sinha and Nandi (1972).

Chen An Mei et al. (1967) pointed out that if soybean seeds were soaked in the solution of ascorbic acid before germination more chlorophyll was found. According to Wetmore and Morel (1949) ascorbic acid reduces the toxic products generated by the action of phenol oxidase and it may influence the oxidation state and activity of $\mathcal{SH}$ enzymes and may possibly an important regulator in iron metabolism. Rabinowitch (1956) has discussed the role of ascorbic acid on photosynthesis as a hydrogen donor in chlorophyll sensitized diphosphopyridine nucleotide reduction. Toole et al. (1956) have reported that ascorbic acid may influence germination process through its regulating action in the oxidation reduction process of protoplasm.

Kogl and Haagnsmut (1936) did not find any effect of exogenous application of ascorbic acid in the increment of growth. Both Bonner and Devirian (1939) as well as Massa (1942) failed to observe any stimulation of growth by ascorbic acid. On the bases of his results Massa (1942) argued
that growth retardation caused by soaking of seeds in ascorbic acid solution may be due to inhibition of enzyme activity by ascorbic acid which depended on the concentration used.

On the basis of all the beneficial effects of ascorbic acid, a simple pretreatment method was developed by Chinoy (1962). It was found that this process accelerated germinative processes as well as growth and yield of plants.


ROLE OF HYDROGEN PEROXIDE (H₂O₂):

Ching (1959) had shown a stimulating effect of hydrogen peroxide (H₂O₂) on seed germination and subsequent
growth in a number of seeds with different food reserves e.g. starchy, proteinaceous and fatty (Barley, Legumes and Sesamum) which depended on the duration of soaking.

The effects of \( \text{H}_2\text{O}_2 \) on growth and metabolism has been differently interpreted as: (a) germination of Douglas fir is accelerated by \( \text{H}_2\text{O}_2 \) via increased rate of respiration (Ching, 1959); (b) catalase acts on \( \text{H}_2\text{O}_2 \) and molecular oxygen is released, which ultimately increases the rate of respiration and facilitates fatty acid oxidation (James, 1953; Neilands and Stumpf, 1955; Chance and Williams, 1956); (c) \( \text{H}_2\text{O}_2 \) stimulates the activity of lipoxidase which in turn, enhances the oxidation rate of unsaturated fatty acids (Bergstrom and Holman, 1948; Holman, 1948); (d) high concentration of auxins (growth inhibiting concentrations) are oxidized by a peroxidase which functions with \( \text{H}_2\text{O}_2 \) (Waygood and Maclachlan, 1956); (e) \( \text{H}_2\text{O}_2 \) treatment may act as an electron acceptor when there is an appropriate donor and thus the rate of respiration is increased (Barron et al., 1953; James, 1953; Dolin, 1955; Neilands and Stumpf, 1955); (f) \( \text{H}_2\text{O}_2 \) treatment leads to a greater production of ATP which activates many of the metabolic and enzymic processes and the latter are in turn essential for the mobilization of cellular components and energy for synthetic reactions in growth (Neilands and Stumpf, 1955; Chance and Williams, 1956; Bandurski and Ching, 1958).
In the presence of H$_2$O$_2$ peroxidase is capable of oxidising a whole series of phenolic substances (Mecko and Novacky, 1966; Seigel and Calston, 1967). It was shown by Simak (1968) that Abies seeds which do not germinate completely, started to germinate in H$_2$O$_2$ (1%) and developed normal plants. H$_2$O$_2$ was used as a growth promoter with GA, AA, KIN, KNO$_3$ and thiourea by giving presowing treatment as a moistening media (Abu Shakara, 1968). H$_2$O$_2$ can also overcome the dormancy effects (Jensen, 1968). Recently, Ghesani (1972) working with sesameum seeds found that H$_2$O$_2$ when added in low quantities to these media not only enhanced almost all enzymic activities, fresh weight, dry weight, but macromolecular content was also considerably effected. Best results were obtained when H$_2$O$_2$ was added together with low concentration of ascorbic acid.

As a mild oxidizing agent, it has been used as inhibitor of SH enzyme. H$_2$O$_2$ inspite of its high oxidation-reduction potential is a sluggish oxidising agent as molecular oxygen. Large number of oxidations attributed to this substance have been found to be due to the free radical formation which occurs on addition of catalytic amounts of Fe$^{+++}$ or Cu$^{+++}$. Neither nucleic acids nor adenosine triphosphate are affected by H$_2$O$_2$ (Barron et al., 1952). Depolymerisation caused by - x-rays on DNA did not occur in
the presence of $H_2O_2$ (Butler and Smith, 1950), whereas it took place readily in the presence of $OH$- radicals when $H_2O_2$ was added to $FeSO_4$. Further it was also observed that the effects of externally applied AA on growth were to a considerable extent caused by $H_2O_2$ originating during its oxidation. $H_2O_2$ gave growth responses that are similar to those caused by unstabilized AA (Aberg and Johannson, 1963).

**ASCORBIC ACID METABOLISM:**

The universal presence of ascorbic acid in an actively mobilizing cell suggests that it has an important physiological role in plant growth and development. Patel (1967) has shown that AA as well as ascorbigen (ASG) content is higher during early stages of germination and the increase in AA oxidase in ground nut is more pronounced after 6th day of germination Patel (1965). Stojskovska and Godisen (1969) noted in wheat seeds that an increase in AA content up to 5th day which decreased afterwards. Sreathamula (1970) showed a close correlation between growth, high AA content and percent moisture content in germinating ground nut seeds.

The role of ascorbic acid turnover in metabolism, growth and differentiation has been studied in this laboratory since last 25 years. Ascorbic acid (AA) is known to regulate the redox system at various stages of growth and development of plants. Its participation in photophosphorylation
and oxidative phosphorylation is fairly established (Amon, 1956, 1957, 1958, 1961; Mapson, 1958; Mitsui and Oi, 1961) AA brings about changes not only in the oxidative system but also in dehydrogenase system during vernalization (Sissakian and Filippovich, 1951, 1953). Chinoy and Nanda (1959) have shown that the synthesis of AA is catalysed by IAA. Further, a number of studies were carried out to test the validity of the above mentioned findings and the results clearly indicated that the biosynthesis of AA is catalysed by IAA, kinetin and gibberellic acid (Chinoy et al., 1965, 1967). A similar catalytic action of auxin and gibberellic acid has also been shown by Michniewicz (1960) and Burgovitzky and Popovici (1966). Lalaraya et al. (1972) reported a very high rate of ascorbic acid utilization in cytokinin induced cell division in expanding cotyledons.

Chailakhyan and Khlopenkova (1959) showed that ascorbic acid increased the growth of Perilla and Rudbeckia more than induced by gibberellin. Khudairi (1968) showed that AA can act as a growth hormone upto 100 ppm. In the same year, Chinoy (1968) working with AA pretreatment concluded that pretreated seeds gain appreciable advantage over the control in growth rate of the seedlings. Austin et al. (1969) had also shown that seeds treated with three alternative soaking and drying cycles gave 51% longer embryos than those of untreated seeds. According to Starchenkovo (1967) also
pretreated seeds show better growth of above ground part as well as of root.

Chinoy and Nanda (1959) were first to show that auxin (IAA) catalyzes the biosynthesis of AA in germinating wheat embryos. Similarly experimental data presented by Michinicwicz and his co-workers (1966) also support this. There is a very significant enhancement in the utilization of AA by kinetin treatment (Lalomya et al., 1972 and Patel, 1973).

NUCLEIC ACID METABOLISM:

Watson and Matthews (1966) discovered in Chenopodium shoot apices, a new RNase resistant RNA associated with DNA and called it floral messenger RNA. Shrinivasan and RAO (1971) found that nucleic acid content, RNA and RNA/DNA ratio were almost double the quantity in reproductive shoot than in the vegetative shoot in grape vine. Cytoplasmic male sterility is caused by the fundamental nucleic acid disturbances which are prominent during microsporogenesis (Dmitrieva and Khavzhinkaya, 1969). Matinyan (1969) found a marked increase in RNA content of corn roots accompanied by the onset of reproductive phase. DNA content constantly decreased as leaves turned yellow. Senescing radish leaves also exhibited typical downward drifts in nucleic acid content (Knight, 1969). The floral initiation is associated with the synthesis of nucleic acids (Sen, 1964). It has been shown that 5-flumuracil and 2-thiouracil inhibit
the initiation of floral formation when applied to leaves (Salisbury, 1963, 1965) suggesting that during floral initiation and cell division, nucleic acid synthesis must take place. Sage and Yamada (1965) have shown that nucleic acid metabolism plays an important role not only in photoperiodic induction but also in thermal induction of flowering.

During the early stages of germination, there is a net increase in the RNA content of peanut cotyledons (Marcus and Feeley, 1962; Cherry, 1963, 1963a). It has been suggested by Barker and Douglas (1960) that RNase of the growing tissues is involved in the synthesis of RNA. Mayer (1971) has also pointed out significance of nucleic acid and protein synthesis during germination. RNase activity also rose to a maximum between the initial and final phases of germination in storage organs of *Phaseolus aureus* after which it declines (Paul et al., 1970). Content of RNA in the cotyledons of *Vicia sativa* falls from 1 to 7 days and concomittant with in vivo degradation of RNA, RNase activity increases. Early increase in RNA content between 24 and 48 hours of germination in coleoptile and radicle is considered to be a reflection of cell division (Graffet et al., 1968). Vold and Cyphard (1968) found that soluble RNA present in the dry wheat embryo was hydrolysed during the first 15 hours after germination. Price (1966) demonstrated a considerable
increase in RNA content as a result of exogenous AA application. There is a several fold increase in the RNase of the wheat (Matsushita, 1959, 1959a) and barley endosperm (Ledoux et al., 1962) during germination. Mitra et al. (1966) working with germinating wheat seeds have revealed that during germination a loss of RNA and acid soluble nucleotides takes place from the endosperm with a concomitant increase of RNA and acid soluble nucleotides of the embryo and seedling proteins.

Semenenko and Krasilnikova (1963) with the help of P32 found a parallel increase in the dry weight of wheat embryo with increase in the absolute content of RNA and DNA. Stoletov et al. (1965) have shown an increase in the content of nucleic acids during germination. In Hedera helix, Millikan and Ghosh (1971) reported significant changes in nucleic acid content with maturation and senescence. Attempts to detect RNA, specifically related to floral evocation have not been rewarding in Xanthium (Ross, 1962; Cherry and Huystee, 1965). Laloraya (1965) found an increased RNA concentration at the time of reproductive differentiation. Bijven and Evans (1967) have also demonstrated an increased RNA content in the shoot apex of Lolium temulentum following flower induction.

Thomas (1963) has shown that cells of the apical meristem of Xanthium cannot perceive and fix the floral
stimulus unless they are actively synthesising either DNA as suggested by Zeevaarts (1962) or RNA as shown by Bonner and Zeevaart (1962).

PROTEIN METABOLISM:

Varner (1965) observed that during germination, seed proteins are hydrolyzed in the endosperm (wheat) or cotyledon (pea) into peptides and amino acids which are translocated to the growing axis. The maximum rate of hydrolysis of the storage proteins coincided with the maximum rate of growth of seedling. During germination, seed storage proteins decrease while free amino acids and amides increase which are either used as substrates for the synthesis of new proteins or are further degraded yielding energy (Koller et al., 1962). Josef et al. (1966) have recorded a decrease in protein content in germinating seeds of *Phaseolus vulgaris* L. associated with a rise in protease activity in the cotyledons during early stages of seed germination. Rosario et al. (1968) have shown that amino acids are the precursors of storage proteins in the rice grain. Hence, a higher level of these amino acids will contribute to a faster and greater accumulation of protein
in the so called protein bodies. The greater capacity for amino acid incorporation in the developing grain of samples with higher protein content provides an interesting biochemical explanation (Cruz et al., 1970).

Lepeschkin (1935) concluded that the cause of death by high temperature usually has been explained as the result of denaturation of proteins. Maximov (1933) stated that the action of superaoptimal temperature, as explained by the coagulation of protein theory, is often not sufficient since death may begin at 40°C, a level which is far below the coagulation point of proteins. There appear to be proteins or enzymes which are relatively stable at high temperature. Koffler et al. (1957) have suggested that the relative heat stability of proteins or enzymes in thermophilic organism depends on more effective hydrogen bonding. Mitra and Sen (1966) have suggested that chloramphenicol inhibited the protein synthesis and flowering of pea. Schwabe (1969) also detected new proteins consequent to induction treatment and considered it as an important advance. Bernier (1971) noted that synthesis of RNA and proteins is necessary for flowering process. Protein component of bracts, flowers and flower parts is different from that found in the vegetative organ of the same species (Marushige and Marushige, 1962; Barber and Steward, 1968; ThanhVan and Trippi, 1969; Kahlem, 1970).
Mulliken et al. (1970) also found a loss in the reserve protein in *Abutilon theophrasti* accompanied by a rapid radical growth during germination. Proteinase activity in the cotyledons of *Cucurbita maxima* rapidly increased during germination to a maximum level at 2 days followed by a decline to the initial values (Wiley and Ashton, 1967). It has also been shown that during germination, seed-storage proteins decrease while free amino-acids and amides increase which are either used as substrates for the synthesis of new proteins or are further degraded yielding energy (Koller et al., 1962). Hormonal control of storage proteins have been investigated from time to time by a number of workers (Paleg, 1960a, 1961; Briggs, 1963; Varner, 1964; Varner and Chandra, 1964). Josef et al. (1966) have recorded a decrease in protein content in germinating seeds of *Phaseolus vulgaris* L. associated with a rise in protease activity in the cotyledons during early stages of seed germination.

Chloramphenicol inhibited the protein synthesis and flowering of pea (Mitra and Sen, 1966). A direct correlation between growth, RNA and protein levels has been found out in bean callus cultures (Bajaj, 1970). Shvedoskaya (1968) has stated that growth and morphogenesis of plants take place on the basis of the protein biosynthesis which is inseparably linked with the metabolism of nucleus in which nucleic acids play an important role.
CARBOHYDRATE METABOLISM:

Generally in most seeds carbohydrates are the main storage substances (Abraham et al., 1963). The final product of enzymic degradation of carbohydrate in the endosperm is glucose, which is transported to the embryo (Keys and Skews, 1961).

Cotrufo (1965) found that the carbohydrate content of the endosperm decreased with the emergence of the radicle in the germination of long leaf pine. Following the change, there was a gradual increase in the carbohydrates of the endosperm. The latter period of germination showed a decrease in the embryo. The reducing compound tended to follow the same trend as carbohydrates. Transport of carbohydrates from storage organ such as the endosperm or cotyledon to the growing part of the embryo has been reported by Koller et al. (1962) and Chinoy et al. (1969, 1969a).

In the mature seed, it was shown that oligosaccharides which were widely distributed decreased rapidly with the march of seed germination (Ching, 1963; Bond and Glass, 1963). There are several reports that non-reducing sugar decreases
rapidly during germination (Pazur et al., 1962; Tada and Kawamura, 1963). In soybean seedling, it declined rapidly during the first three days of germination. The depletion of reducing sugar was brought about earlier in the embryo axis than in the cotyledon (Abrahamsen and Sudia, 1966). Total sugar content is also shown to decline by many workers (Ingle et al., 1964; Saxena, 1969; Cook and Mitchel, 1969). Patel (1967) observed a rise in the total sugar till 7 days in the light grown groundnut seeds. There was depletion of sugars in cotyledons of cotton seedlings during different stages of germination (Kamalavalli et al., 1972). Besides this, they found that in germinating *Sorghum* seedlings starch disappeared as the germination advanced with the concomitant increase in total and reducing sugars. Rameshwar et al. (1971) observed starch degradation followed by the protein loss at high temperature and the most rapid loss occurred between 6th and 10th day in cotyledon of *Pisum*.

Chinoy et al. (1969) working with wheat seeds recorded the highest content of sugars in the seedling which went on increasing with the march of germination. At the same time they found that the starch of the endosperm decreased rapidly. Saxena et al. (1969) studied the effect of gibberellic acid and ascorbic acid on isolated barley endosperm and concluded that the AA was superior in accelerating the release of sugars. Ascorbic acid enhanced also the
production of reducing sugars.

The production of reducing sugar from starch was greatly increased when endosperm were pretreated with GA$_3$ (Paleg, 1965). Sandegran and Beiling (1968) came to the conclusion that reduction in germinating time (or in other words the hastening of starch hydrolysis) was due to the gibberellic acid effect on embryo growth. Gibberellic acid treatment of dwarf maize seedlings resulted in the increased reducing content (Katsumie, 1970).

Kinetin also plays an important role in carbohydrate metabolism. Boothby and Wright (1962) have suggested that growth responses induced by gibberellin or kinetin can be explained simply in terms of liberation of d-glucose and the amount of sugar released into the medium with kinetin was more than gibberellic acid. In an experiment with sunflower cotyledons kinetin and the embryo axis act similarly in bringing about an elevation in the level of reducing sugars in the cotyledon incubated in darkness (Gilad et al., 1970).

**AA-FR-PEROXIDASE**

Yamazaki and Piette (1961), using ESR spectroscopy, reported the formation of a free radical or ascorbic acid called monodehydroascorbic acid (MDHA) during its peroxidative oxidation. Further, it was shown that ascorbic
acid oxidase also involves free radical mechanisms. In 1963, Gurevich reported an enzyme from wheat and corn seedlings which brought about the substances with very low oxidation reduction potential like orthodinitrobenzene to yield yellow soluble product called orthodinitropherylhydroxylamine.

Intensive work has been carried out on the free radical peroxidase by Chinoy and his co-workers indicating its active participation in metabolically active cells (Chinoy, 1969; Pandya, 1969; Gurumurti, 1971; Singh, 1971; Shah, 1972; Ghesani, 1972; Shah, 1973). Chinoy et al. (1969) recorded stimulating effect on the activity of AA-FP-peroxidase by exogenous application of ascorbic acid. A marked enhancement in the activity of this enzyme has also been reported by Ghesani (1972) in sesamum seeds treated with ascorbic acid. A little quantity of H₂O₂ was also found to increase the activity of this enzyme.

PEROXIDASE:

Hackett (1963) and Brezhnev et al. (1970) have attributed a great importance to peroxidase in the respiratory process of plant. Catalytic function of peroxidase is one of the links of respiratory chain (Ivanova and Rubin, 1963; Rubin and Ivanova, 1963). It also participates in oxidative phosphorylation (Hackett and Ragland, 1962). Yang (1969) suggested the function of peroxidase as the electron
oxidising agent. There is a positive correlation between respiration rate, catalase and peroxidase activity in growing Citrus roots (Altman et al., 1966).

Yamazaki and Piette (1961) have shown from their free radical spectrum studies that free radical of AA called monodehydroascorbic acid (MDHA) is formed during its peroxidative oxidation and concluded that, during the oxidation of AA by AA oxidase and peroxidase, free radical mechanism is involved.

Siegel (1955) has pointed out the broad hydrogen donor specificity of plant peroxidases. There is a regular, steep increase in peroxidase activity in all parts of Sorghum vulgare and in roots and cotyledons of Phaseolus mungo from 4th day of germination (Gopalachari, 1964). The correlation of peroxidase with cell elongation in wheat coleoptile has also been worked out (Chappet and Zubouchet, 1970). Peroxidase has been considered as a constituent of terminal oxidation (Alexander, 1964), as an agent in the oxidation of metabolites by means of $\text{H}_2\text{O}_2$ by e product (Fruton and Simmonds, 1963) and as a key component of the IAA oxidising system (RAY, 1958; McCune, 1961). Peroxidase is also involved in the formation of amides (Halvøy, 1962; Monselise and Halvøy, 1962).

**CATALASE**

Micheniwicz and Stanislawski (1962) studied catalase activity during germination of wheat and observed increased activity of enzyme with increase in germination period. Boca
and Ondarza (1953) have found that catalase activity of germinating maize increased about 4-fold in 7 days. In soybean, there was a sharp increase in catalase activity which reached a maximum on the 4th day of germination (Holman, 1948). There is a reciprocal relationship of catalase activity to that of polyphenol oxidase and peroxidase which register a steep increase on the 4th day of germination in *Sorghum vulgare* and *Phaseolus mungo* (Gopalachari, 1964). In the endosperm of wheat and cotyledon of *Arachis* an increasing trend of catalase activity has been reported (Chinoy et al., 1969).

A high level of catalase and low level of peroxidase activity is related to a higher rate of protein synthesis (Verma and Huystee, 1970). There is a high catalase activity in tobacco leaves during their most vigorous growth (Krenke and Dubrovitskaya, 1949). Galston (1951) reported an inverse relationship between catalase activity of plant tissues and their rate of growth.

**PROTEASE ACTIVITY:**

Protease activity in *Cucurbita maxima* in the cotyledons rapidly increased during germination to a maximum level at 2 days followed by a decline to about the initial value after 7 days (Wiley and Ashton, 1967). Penner and Ashton (1967) have observed that during the course of germination of
squash seeds the level of proteolytic activity increased in the cotyledons through the 3rd day and then decreased. Besides this, they have also suggested that the presence of the embryonic axis during the first 32 hours of germination was a prerequisite for the development of maximum proteolytic activity. Rameshwar et al. (1971) have observed that the rapid loss of protease activity coincided with the decreased loss of cotyledon protein.

Proteases are the enzymes attacking protein by cleavages of the peptide bond in a protein molecule. An increase in activity seems to occur as germination proceeds. In 1961, Kudryashova published the voluminous literature which deals with this enzyme. For the first time, the proteases of germinated soybeans seedlings were studied by Ofelt et al. (1955). Tazakawa and Hirokawa (1956) showed changes in protease activity in soybean seedlings during germination. Activity was found to be very low in the seeds. It rose in the cotyledon for about 6 days and then dropped again. The shoot began to show protease activity after approximately six days. Penner and Ashton (1967) have observed that during the course of germination of squash seeds the level of proteolytic activity increased in the cotyledon up to 3rd day and then decreased. Embryo axis was found to be necessary for maximum activity. Dey et al. (1969) reported increasing trend of proteases in rice during
germination, while Rameshwar and Steponkus (1971) have observed that the rapid loss of protease activity coincided with the decreased loss of cotyledon protein. Bounce and Umana (1962) had found a variety of peaks of maximal activity of proteolytic enzymes with respect to pH. Investigations on the proteolytic enzymes in soybean, lettuce, peanut and other seeds have been published by Koller et al. (1962). The high dipeptidase activity is found in rootlet and epithelial layer of scutellum.

Briggs (1963) has suggested that there is a possibility of de novo synthesis of the enzyme in the aleurone layers of barley. Ashton et al. (1966) are of the opinion that storage protein is hydrolysed after 24 hours of germination in Cucurbita cotyledon, the hydrolysate being transported to embryo axis. Protease activity increases in cotyledon only after 2-3 days. Ihle and Dure (1970) have suggested that increased m-RNA synthesis is a prerequisite for the appearance and accumulation of protease in germinating seed. The mechanism by which the m-RNA controls protease synthesis is however not known.

Chinoy and Saxena (1971) have shown a slight increase in protease activity by exogenous application of ascorbic acid to Cicer seeds while Chesani (1972) working with sesamum seeds fed with AA in medium for germination found significantly enhanced activity of protease. It was
also shown that when hydrogen peroxide and ascorbic acid were added together in the medium, protease activity was further increased.

Yomo (1961), Paleg (1961) have observed that increased proteolytic activity in excised barley endosperm resulted from the addition of gibberellic acid. Varner and Ramchandra (1964) suggested that protease of the barley endosperm is also produced by de novo synthesis. Recently, however, Maslowski and Maslowska (1972) did not find any accelerating effect of gibberellic acid on proteolytic enzymes; the influence was rather negative. According to Penner and Ashton (1966) at least one proteolytic enzyme in the cotyledons of germinating squash seedlings is controlled by a cytokinin originating in the axial tissue.

INVERTASE ACTIVITY:

Hatch and Glazion (1963) found a strikingly close relationship between the invertase activity and growth rate. The results of Seitz and Lang (1968) also indicated that the invertase activity was correlated with growth, and further suggested that the enzyme was in some way essential for growth, by making sugars available for cell expansion. To elucidate the role of AA on germination Ghesani (1972) reported that AA helped in stimulating invertase activity to a considerable extent resulting in better growth.
To understand the mechanism by which gibberellic acid accelerates growth Paleg (1960), Hayashi et al. (1969), Varner (1964) as well as Varner and Chandra (1964) carried out experiments to show that it was through the promotion of invertase synthesis that gibberellic acid accelerated the growth. This would result in release of reducing sugars that could be used in the polysaccharide biosynthesis in elongating cells. Kaufman et al. (1968) have found that gibberellic acid induces invertase activity within the first 6 hours.

Gaylor and Glazsiou (1969) reported that both auxin and gibberellic acid increased the enzyme activity of invertase but not of peroxidase. The effects of two hormones were interpreted as causing stabilization of m-RNA for invertase.

HISTONE (BASIC PROTEIN):

The histones are linear structures normally elongated and wound into the grooves of DNA. Bonner and his associates (1963) have pointed out that the inhibition with the whole histones occurred at a histone:DNA ratio of 2 and it was interpreted by them as evidence in favour of the concept that DNA synthesis is shut off when the concentration of histone becomes high in tissues. Alfert (1956, 1957, 1958) and Alfert et al. (1963) have shown that the synthesis of
histones increased simultaneously with the synthesis of DNA. Irvin et al. (1963) have suggested that the synthesis of histones results in the shutting off of some further synthesis of RNA in an intermediate stage of cell division.

They have also suggested that the increase in histones resulted in an enhanced biosynthesis of DNA. During mitosis, the synthesis of histones ceases and extra histone formed earlier is either degraded or modified so that, after mitosis, the normal ratio of DNA to histone results. Another possible explanation is that histones lead DNA synthesis, or that histones represent an essential component of the chromosomes that must be laid down first (Busch, 1965).

However, it would appear that histones could be readily destroyed by the intranuclear proteases that are present in almost all histone preparations. Dounce and Umana (1962) have found that the maximal activity of proteolytic enzymes varied with pH. In view of the activity of these enzymes at pH 7.0, which is a physiologically meaningful pH, these enzymes serve to hydrolyze the histones. Besides this, Chalkley and Maurer (1965) have shown that in the absence of DNA replication, histones do not increase. Rasch and Woodward (1959) studied the differentiation of several plant species using cytochemical methods to demonstrate differences in the affinity of alkaline fast green for basic nuclear proteins. They were unable to detect any gross alterations in the basic
proteins of the plant nuclei.

Woodward and Swift (1964) have pointed out that localized uncoiling of chromosomes may be induced by cold. Huang et al. (1964) have studied the stabilization of DNA against thermal denaturation by histones. The Tm of nucleohistone preparations is greater than that of DNA, where the Tm is the temperature at which the increase in O.D. of DNA is halfway between the initial and final optical density as the sample is subjected to increasing temperature. In their experiment, Tm for DNA was 70°C and that for nucleohistone was 84°C. When the nucleohistones were reconstituted with lysine rich histones, slightly lysine rich and arginine rich histones, the values for Tm were 81, 75, and 71°C. They have noted that there was an inflection point in their curve for nucleohistone at 69.5°C and suggested that 80% of the DNA complexed with histone while 20% did not.

Considering another function of histone, it is possible that the observed basic protein differences are indications of changes in chromatin structure resulting from diverse temperatures. Huang et al. (1962) have suggested that not all histone fractions are involved in the repression of RNA, but some function in a different way. Littau et al. (1965) found that arginine and lysine-rich histone fractions function differently in the control of chromatin structure. Changes in chromosome structure due to temperature treatment
have been recognised by Wilson and Boothroyd (1944) who observed by cytochemical techniques that somatic chromosomes of *Trillium* develop constrictions upon cold treatment.

Mechelke (1955) observed this phenomenon in the root tip chromosomes of germinating barley seeds. In the barley seedling basic protein variations observed may be an indication of specific basic protein changes necessary to control physiological processes unique at different temperatures. The variation observed in barley seedling basic proteins is a result of temperature treatment (Thomason, 1970).

**SULPHHYDRL CONTENT**

Vivario and Ledoux (1930) found that generally sulphhydryl (-SH) content was absent in the dry seeds; but if rapidly appeared after hydration of the seed. Hopkins and Ellicott (1931) concluded that glutathione can function in oxidation reduction process. Hopkins and Morgan (1943) showed that -SH content reached its maximum level within 4 hours after adding water to the dry seeds. Knox (1960) ascribed two physiological roles to glutathione viz. as a respiratory catalyst and in the control of protein thioldisulfide groups. Waelsch (1952) ascribed an additional role to glutathione as the outstanding SH carrier of living material. Seed germination is accompanied by an increase in the amount of glutathione (Spragg et al., 1962), which could serve to activate SH requiring enzymes (Narahara and Williams,
1959; Bird, 1962), Kurup and Sanadi (1968) found evidence to show that SH groups were involved in light dependent transhydrogenation (NADP from NADPH$_2$) and other energy linked reactions. Lam (1968) has also shown that a highly purified protein factor contains a SH group that is functional in the energy transfer.

Ascorbic acid-sulphydryl system (AA-SH) is involved in seed germination. Hopkins and Morgan (1943) found that SH compound reached maximum within four hrs after addition of water to dry seeds. Glutathione is present in plants and is required by some cells for growth (Racker, 1954). In germinating seeds and sprouting potato tubers, ascorbic acid and glutathione appear at the same time and place (Pett, 1936; Hopkins and Morgan, 1943).

Szent Gyorgyi (1928) was the first to suggest that glutathione (GSH) donated hydrogen to dehydroascorbic acid (DHA). Kohman and Sanborn (1937) concluded from their experimental work that reduction of DHA by GSH is enzymic in nature. Presence of an enzyme DHA reductase has been shown in many plant tissues by Vennesland and Conn (1954). They also point out that this enzyme catalyzes the transfer of hydrogen from GSH to DHA i.e. $2\text{GSH} + \text{DHA} \rightarrow \text{GS-SG} + \text{AA}$. A decrease in the ratio of AA/DHA and GSH/GSSG causes retardation in growth (Marre and Lepid, 1954; Marre et al., 1957); whereas an increase in these ratios is
correlated with growth stimulation. Retardation in AA oxidation keeps glutathione in a reduced form thereby accelerating growth. According to Purr (1933), ascorbic acid assumes a protective role against the oxidation of SH compounds in the organisms. By the reversible oxidation and reduction of AA, it is indirectly related to cell respiration and is also a determining factor in establishing the equilibrium between SH and SS compounds.

PROSING TREATMENT:

Generally plant processes may influence the heat resistance of its organs. Thus, the nutrition of a plant can be of importance to its heat hardiness (Illert, 1924; Sapper, 1935; Carroll, 1943; Julander, 1945). The previous water and temperature conditions may also effect it strongly and periods of drought lead to heat hardening (Sapper, 1935; Lange, 1955). The possibility of increasing heat hardiness through treatment at raised temperatures is of particular interest. The investigations of Alexandrov (1956), Alexandrov and Feldman (1958), Lyutova (1958), Lomagin (1961), Yarwood (1961) proved that the cells of higher plants react with a reversible increase of their heat-resistance, on short exposure to high
temperatures, which are supraoptimal, but not yet fatal. Alexandrov (1962) concludes that this heat hardening is caused by the damaging influence of temperature during the preceding treatment. Apart from this type of treatment of heat hardening, Lange (1962) also found a heat resistance adaption in case of Commelina africana and Phoenix dactylifera, during extended cultivation at raised temperatures within the optimal range.

Some authors have reported the influence of thermal treatment upon viability of grains, but they have not mentioned the moisture content of grains (Barton, 1961; Levitt, 1956), while others have not specified the length of the heating period (Ministry of Agriculture and Fisheries, 1954). Watson (1969) has stressed the need of further work to test whether or not the time temperature criterion of damage is valid over a wide range of temperatures and to establish the effect of moisture content and seed variety upon the rate of thermal damage to viability.

Under certain conditions, the application of thermal energy to seeds may be beneficial (Gerzhoi et al., 1958), and dry seed is very resistant to thermal damage (Barton, 1961). Considering the above cited literature for the prethermal treatment, the present investigation has been carried out with exposures of dry seeds at 70°C(D).
The presowing treatment of the seed was first developed by Tincker (1925). It was observed by Kar (1944) that pretreatment of jute seed showed earlier sprouting, greater elongation of the stem and early recovery from wilting. Henckel (1961) and Henckel and Badannova (1959) explained the effect of presowing treatment primarily on changes occurring in physico-chemical properties of the cytoplasm, the greater amount of bound water and more intense metabolism.

May et al. (1962) claimed that by subjecting seed to one or more cycles of wetting and drying before sowing, the yield of crop grown under drought conditions can be increased. Henckel (1964) observed that three cycles give the best results in terms of rate of germination. In hardened seeds more than 50% elongation was recorded, compared with that of unhardened ones. The increase in the length of embryo axis was mainly due to cell division during hardening period.

From his results Chinoy (1968) concluded as follows:

1. Pretreated plants gain an appreciable advantage over the control in the seedling by acceleration in the dry weight production as well as relative growth rate (RGR) of the seedlings.
2. Results indicated the superiority of the pretreated seeds over untreated ones in their capacity to extract moisture from atmosphere more efficiently.

In general pretreatment of seeds favours germination, increases resistance to drought, cold and salt as well as bacterial, fungal and viral diseases and maintain seed production of super quality.

ROLE OF ASCORBIC ACID IN CROP PRODUCTION:

The role of ascorbic acid in crop production can best be understood by reviewing its function in some of the important physiological activities of the plant. Biosynthesis of AA takes place in the germinating seed as well as in other parts of the plant from sucrose (Chinoy and Nanda, 1969; Chinoy, Grover and Nanda, 1958, 1961). Simultaneously the bound auxin is released and is translocated to the embryo where it catalyzes the biosynthesis of ascorbic acid from sucrose and other precursors (Chinoy, et al., 1965, 1967).

Cold temperature treatment increased the concentration of free AA as well as bound form of ascorbic acid (ascorbigen). A similar effect was seen in the case of endosperm also (Chinoy, 1964-67a; Patel, 1967). With the emergence of the shoot above ground the relationship between AA and photosynthesis assumes great importance, Further we have now considerable evidence regarding the role of ascorbic acid
in oxidative phosphorylation, both in the early as well as late stages of development of the pre-fertilized carpels, the uptake of oxygen is appreciably faster in media containing sucrose and AA compared to that in distilled water. In the developing kernels, although the uptake of oxygen goes down in all the three media sucrose and AA have an enhancing effect upon respiration. When this result is considered in relation to ascorbic acid and ascorbigen contents of pre-fertilized carpels as well as developing kernels the relationship between respiration and AA-metabolism becomes clear. Incubation of carpels and developing kernels in sucrose and AA generally enhances the biosynthesis of AA and ASG. A mass of data which was accumulated during the course of the last ten years in our laboratory on the interaction between AA and auxin, exogenous application of AA to the shoot apices of plants, biosynthesis of AA by different organs of plants as well as on the AA content and its utilization during the course of the growth period of different crop plants such as Triticum, Hordeum, Avena, Secale, Triticale (of Arne Muntzing), Cicer, Cajanus, Arachis, Linum and other grown under different photoperiodic and vernalization treatments also points conclusively to the important role of ascorbic acid in growth and development (Chinoy, Grover and Sirohi, 1957; Chinoy, Nanda and Garg, 1957, 1958a; Garg, Chinoy and Nanda, 1957, 1958; Chinoy,
Our laboratory experiments on seed germination have shown that pretreatment with AA stimulated a quicker and higher percentage of germination, more efficient water absorbing capacity, ability to better withstand against the atmospheric drought and more vigorous growth than the untreated seeds in a number of varieties of wheat, oat, barley, sesamum, ragi, maize and other crop plants. Further, under wilting treatments, AA pretreatment stimulated the plant to resist the adverse conditions of drought better than the control plants (Chinoy et al., 1966; 1970c; Acharya, 1968; John, 1969; Jani, 1969; Chinoy, 1969b). It also been shown that the seeds pretreated with AA when grown under saline conditions, show comparatively a higher degree of tolerance to salinity (John, 1965; John and Jani, 1969).

From the foregoing review it becomes clear that the minimolecule of ascorbic acid has multimolecular and submolecular functions in conjunction with the macromolecules of the living cell. It has also revealed the importance of AA in various physiological processes of the plant. Hence its application to agricultural practice was considered of value. It was noted that AA when fed to...
germinating seeds was readily absorbed and incorporated in the main metabolic stream of the cell. Keeping this point in view a simple presowing treatment of seeds with AA solution has been developed in this laboratory (Chinoy, 1967). Extensive data on growth, metabolism and yield of AA pretreated plants have been obtained during the last several years. Large scale field experiments have been carried out on the experimental farm of this laboratory as well as with the cooperation of farmers in various parts of Gujarat and in other states of India (Chinoy, 1967, 1968, 1969b; Chinoy et al., 1966, 1969, 1970, 1975; Abraham et al., 1969). The results show an increase in yield from 7-50 percent and increases resistance to diseases.