Chapter I

REVIEW OF LITERATURE

In the life cycle of seed bearing plants, seed germination is the first and the most important event. Germination is easy to recognize but difficult to define. It is defined in different ways by different workers. According to common men and farmers, the germination is the emergence of the aerial parts of seedling from the soil. International Seed Testing Association has defined germination as "the emergence and development from the seed embryo to those essential structures which for the kind of seed in question are indicative of its ability to produce normal plants under favourable conditions", (Anonymous, 1959; Heydecker, 1960). According to Evenari (1961); seed germination is "the sum total of all the physiological processes occuring inside the seed which starts with the imbibition of water and ends with the protrusion of embryonic root". Botanists define germination as "the emergence of radicle from seed coat", (Lang, 1965). Heydecker (1973)recognised seed germination as the process of activation of a hitherto resting embryo. According to him the germination begins with the first metabolic process during imbibition. However, he admits the difficulty in determining the end point of germination. Seed analysts considered seedling capable of independent growth as the end point of germination. Thus germination means a different thing to different persons. From all these definitions multi-facet concept of the term germination

has emerged. For plant physiologists germination is a biochemical process representing a transformation from low metabolic to the high metabolic state. Such a transformation involves several complex biochemical processes. All these processes are generally programmed and controlled by environmental factors.

Thus germination, in general seems to be a complicated process when we try to define it. However, the plant physiologists have made it a simple process, when we look into the physiology and biochemistry of this dynamic process. The attempts by several plant physiologists and biochemists have contributed much in understanding this utmost event in the life of a plant. The following few pages throw light on such attempts.

1) Factors affecting germination :

Under optimum conditions, seeds show their ability to give rise to the normal sprouts. In normal process of germination, the embryonic organs like radicle, plumule and cotyledons develop into a functional root and shoot systems at the expense of the cotyledonary reserves. If conditions are not optimum, germination will not proceed. It means that this process is controlled and regulated by some other factors which may be internal (intrinsic) and external (extrinsic). The major internal factors which affect germination of seeds are

(i) Viability, (ii) dormancy, (iii) Hormonal balance and (iv) age. The external factors are (i) water (moisture supply); (ii) aeration, (iii) light and (iv) temperature.

(A) Internal Factors :

i) Viability and Vigour :

A viable seed is one which is alive and potentially capable of germination. The viability is the capacity to retain the ability to germinate. The length of time for which seeds can remain viable is extremely variable and depends both on storage condition (environmental) and on type of seed (genetical). With respect to the viability, seeds can be divided into two categories such as orthodox and recalcitrant, (Roberts, 1973). The orthodox seeds are those which can be dried to low moisture contents i.e. to 5% or less without damage. The seeds of most of the crop plants and weeds are orthodox. Those seeds which are killed if they are dried a little below the fully hydrated condition and even if they are stored under optimal conditions they loose viability in a relatively short time. These are of the other kind. The large seeds of woody perennials like cocoa, mango belong to this category. Roberts (1972) has expressed all the known data on viability by a simple mathematical relationship -

$$\text{Log } \bar{P} = K_v - C_{1m} - C_{2t}$$

where $\overline{P}$ is the mean viability period of seeds.

K & C are constants.

t is the temperature in °C.

m is the moisture percentage.

The storage conditions required to maintain viability for different seeds are different and hence it is difficult to formulate any general rule for favourable conditions. A variety of factors to which the parent plant is exposed during seed formation and ripening can also profoundly affect subsequent viability of seeds; after dispersion or harvest. Such factors include water supply, temperature, mineral nutrition and light. According to Saxena and Maheshwari (1980) the loss of viability in soybeans is mainly due to inactivation of hydrolytic enzymes as well as poor rates of biosynthesis of essential metabolites by embryo axis. Rudrapal and Basu (1979) observed that vigour and viability are associated with capacity to metabolize carbohydrates during subsequent germination.

Seed vigour has been defined by the Vigour Test Committee of the International Seed Testing Association as "the sum total of those properties of the seed which determine the potential level of activity and performance of the seed lot during germination and seedling emergence" (Perry, 1978). Seed vigour is different from seed viability or germinability in that the

latter indicates a 'yes' or 'no' situation while seed vigour means the growing ability of ordinary viable and germinable seed under somewhat suboptimal conditions, On the basis of biochemical tests seed vigour can be predicted. The ability of cell dehydrogenase to reduce tetrazolium chloride (Lakon, 1942) the initial activation of amylase, glutamic-pyruvic transaminase, RNase, and glutamate decarboxylase (Perl et al., 1978) respiration efficiency (Wood Stock, 1969) have been implicated in assessment of seed vigour. The energy status of seed during formation, storage and germination is important, in the expression of seed vigour in terms of the speed of germination and the rate of seedling growth. Hence Ching (1973) has predicted that ATP content can be considered as a useful biochemical index of seed vigour.

ii) Dormancy :

In many seeds it is observed that the viable seeds do not germinate inspite of favourable conditions. This inability to germinate is commonly termed as 'dormancy' and the seeds are said to be dormant seeds. The seed dormancy can be visualised as a suspended growth of seeds under favourable conditions. In a broad sense dormancy is described by Heydecker (1973) as the state in which ungerminated or resting seeds survive. The seed dormancy is critically important for the

survival of plants. Dormancy and breaking of dormancy ensures that the seeds germinate only at certain times of the year and in temperate climates after winter, when the young seedlings are likely to face less hazardous condition.

Dormancy is not just an inactivation of metabolism but is associated with many complex events. It is a defence mechanism against winter frost or summer drought and is a necessary part of the lives of many plants. It must occur at right time, must last for a sufficient time and must be relieved when the conditions are right for resumption of growth. The main mechanisms those cause seed dormancy or prolong dormancy by preventing germination can be of 'primary' dormancy- the dormancy of seed at the time of harvest or it can be of secondary or induced dormancy. According to Mayer and Poljakoff-Mayber (1963) secondary dormancy is induced in seeds if environmental conditions are unfavourable.

Embryo Dormancy :

There are several factors which are responsible for inducing dormancy in seeds. The embryo of mature seed is not capable of development even under optimum moisture and temperature conditions due to presence of some physiological "block". This is referred to as embryo dormancy. In number of seeds the

embryo is incomplete when they are shed and germination will not occur until further embryo development takes place e.g. Anemone nemorosa, Ficaria verna and Caltha palustris. The embryos of Fraxinus excesior are morphologically developed but undergo considerable growth after shedding (Wareing, 1969). Some mechanisms evolved, prevent the seed from germination immediately after shedding, even when other environmental conditions are right. This requirement, which suggests a further period of development after the seeds are shed and before they can germinate, is called 'after ripening'. When dormant seed is subjected to the action of some environmental factors e.g. light, temperature etc. the physiological block is removed and the seed becomes capable of germination by rupturing the seed coat.

Seed-coat Dormancy :

The physical properties of the seed coat and adherent structures may also be involved in dormancy. The seeds of families like malvaceae, leguminosae, convolvulaceae solanaceae and chenopodiaceae have testas impermeable to water when freshly shed and remain dormant in the soil until impermeable layers have been removed (Wareing, 1969). In many tree species the arrest of embryo development may be due to mechanical pressure from seed coat impermeable to water and gases (Quinlivan

1971; Webb and Wareing, 1972). In some cases seed coat may also contain inhibitors (Webb et al., 1973).

Various methods have been devised to overcome dormancy for better performance in the field. The dormancy due to hard seed coats can be overcome by modification of seed coats by cutting, milling to remove adhered appendages or scarification by abrasive or chemical means or by keeping seeds for small period in hot water. The most effective physical treatments include temperature (Pollock and Olney, 1959) and light treatment (Wareing and Saunders, 1971). During stratification (pre germination cold treatment) several changes occur in the hormones. Very high initial levels of hormones decline to low level at the time of germination (Webb et al., 1973). Sharma and Singh, (1980) have studied the effect of stratification, temperature and duration on the level of endogenous inhibitor and its relationship with dormancy in Peach seeds. The extracted inhibitors were found to be similar to ABA; and were very high in dormant seeds. However, during stratification at low temperature the inhibitors declined either to zero or non-measurable quantity. Chemical treatment with growth substances and other chemicals also effectively promote germination of dormant seeds (Esachi et al., 1978). These include growth substances like GA and kinetin. Gases like ethylene (Ketringe

and Morgan, 1969), chemical compounds like thiourea and potassium nitrate (Nikolaeva, 1969) and organic solvents like ethanol (Taylorson and Hendricks, 1979) promote germination. Such compounds vary in their effectiveness on different species and many a times combinations of the promoters prove more effective.

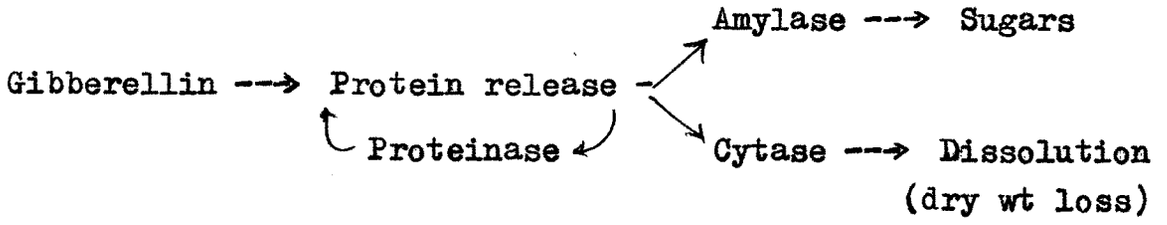
iii) Hormonal Regulation :

The adverse environmental conditions may prolong physiological material or even induce secondary dormancy as reported for wild oats (Andrews and Burrows, 1972), maize (Routchenko and Soyer, 1972) and other species. According to Ikenaga and Chashi (1972) the environmental factors apparently exert their influence through alterations of the relative levels of auxins in the seeds. Dormancy is balanced between growth inhibitors and promoters. It is controlled by a balance between endogenous inhibitory ABA and promoter GA. Abscisic acid is a potent inhibitor of seed germination and its presence as a major growth inhibitor in dormant seeds of many species lost it in the role of maintenance of seed dormancy (Pienizek and Grozovskava, 1967; Oegema and Flecher, 1972; Ho, 1979). Addicott and Lyon (1969) have suggested that ABA might play an inhibitory role in germination particularly in those seeds which undergo a period of dormancy. There are many reports of the existence

of ABA in seeds, of the reduction of ABA levels in dormant seeds during stratification and of the inhibition of germination by exogenous ABA applications. Evidence has been presented by Dure (1975) and HO and Varner (1976) that ABA inhibits synthesis of specific enzymes necessary for the initiation of germination by inhibiting their translation from mRNA. Thus preventive role of ABA in the process of germination is now very well established (Khan, 1971).

It is now almost unanimously accepted that endogenous gibberellins are the primary promoters of germination and other hormones like cytokinins may play a secondary role particularly during enforced quiescence or dormancy. Among the hormones gibberellins occupy a central place in control and regulation of germination. Wiberg and Kolk (1960) found that germination of freshly harvested i.e. non-afterripened barley seeds was strongly promoted by GA. Kaul (1974) has found that freshly collected seeds of Hemigraphis dura are light sensitive. They could be made to germinate in continuous darkness by using thiourea, ammonium nitrate, ascorbic acid and gibberellic acid. The occurrence of GA like compounds was demonstrated in many seeds. The role of GA in the formation and secretion of a great number of enzymes is the most throughly documented case of an endogenously produced hormone which controls seed

metabolism. Many workers consider the induction of de novo hydrolytic enzyme synthesis in cereal aleurone tissue by GA, as a post germinating phenomenon. According to Higgins et al., (1976), GA promotes the synthesis of RNA specific for α-amylase. The experiments of Simmonds and Dumbroff (1974) indicated the possibility that GA can regulate energy change in seeds. GA induced release of sucrose (Chrispeels et al., 1973) and inorganic ions (Jones, 1973) suggest that modifications of cell membrane accompany GA treatment. Thus it is clear that GA has the ability to regulate germination in many ways. Paleg (1960 and 1961) showed that GA increased the activity of starch hydrolysing enzymes in isolated barley endosperm resulting in the release of glucose and maltose. Substantial amount of proteins was released, resulting in considerable loss in dry weight. He assumes that gibberellin acts as a general endosperm mobilising hormone and proposes the following sequence of action.



The importance of cytokinins as regulators of germination has been accepted in recent years. The discovery that exogenous cytokinins concentrated the effects of various growth

and germination inhibitors on seed germination indicated that cytokinins may be a pre-requisite for germination in some species (Khan, 1967). It plays permissive role in seed germination, (Khan, 1971). The experiments of Thomas et al., (1975) have suggested that cytokinins may regulate seed germination through alteration of membrane permeability. It is well established that cytokinins act at the transcription and translocation level of nucleic acid metabolism in germinating seeds. It is also proposed that cytokinin action is mediated through a cyclic-AMP system. But this needs further experimental support. From these and other studies, Khan (1977) formulated an elegant hypothesis regarding regulation of seed dormancy by the balance among the three hormonal systems. The gibberellins play a primary role of promoter in germination, abscisic acid plays a 'preventive' role and the cytokinins play a 'permissive' role.

(B) External Factors :

Biological systems have to fit into their environment sufficiently well to ensure their own continuation. The observations on germination responses have established that control of the process is exercised through physical factors in the environment such as temperature, light and soil conditions. The relationships between the environmental factors and the germination of seeds vary from species to species, (Sen, 1977). The

environmental fluctuations can be promotive as well as disruptive. The unfavourable environmental factors cause various physiological disorders in germinating seeds which ultimately lead to disturbances in the developmental processes and the development of morphological abnormalities. Among the environmental factors which regulate seed germination; water, light, aeration and temperature are the prominent. The other factors affecting germination are soil and its related things, fertilizers, residues of fungicides, herbicides and insecticides.

i) Water :

Water plays an important role in the germination process. It is an important solvent and necessary liquid or colloidal medium for the biochemical reactions to occur. Being the best solvent, it provides the nutrients in a well simplified form. It is the water that provides O_2 for aerobic oxidation and not the molecular O_2 (Vartapetyan, 1966). During germination, as the hydration level of seed increases preexisting macromolecules and organelles are reactivated. These macromolecules include several enzyme systems of various storage tissue. Marcus and Feeley, (1964) have shown that water is essential for the seed swelling and thereby for the activation of RNA.

Simply water in the cell does not guarantee seed germination. Water absorption is the first and the most essential process for seed germination. In the initial stages, the process of water uptake is primarily by imbibition. Certain seeds like Xanthium, can take up water by this process even from relatively dry soil (Wareing, 1969). The availability of water in soil varies with the nature of soil, its chemical composition and temperature. Similarly availability of water to the germinating seeds, is limited by prolonged drought of arid and semiarid conditions.

There is an optimal substrate water status for better germination, with lower germination on either side of this optimum status (Gulliver and Heydecker, 1973). The increasing moisture tension of the medium resulted in slower and reduced germination, although slight positive soil moisture tensions have been reported to have promotive effects (Cavazza, 1953). Difference in the water and air regime of the germination environment may cause variations of 30% and over in the yield of barley, wheat and oats, (Haller, 1984). The maximum limit of moisture tension at which seeds can absorb sufficient water for germination differs from species to species (Hunter and Erickson, 1952). The rice is insensitive while other species and varieties are sensitive to an excess moisture tension (Cavazza, 1953; Sircar et al., 1955).

Prolonged drought causes considerable changes in the osmotic potential of the soil water due to evaporation. A varietal difference regarding drought resistance at germination stage was evident in some experiments (Richards, 1978). In experiments by Joyce et al., (1983), where the radish plants were subjected to different water deficits during the expansion of fleshy axis and cell division, it was observed that water deficit inhibited both cell expansion and cell division in all axis tissues and lignification of parenchyma during water deficit period. When water stress was not relieved, cell expansion in pericycle cells out stripped the recovery of cell division. Sahu (1977) has observed that weeds like E.colonum, E.stagnina, Paspalum sciobiculatum, Setaria glauca required higher moisture amount for germination then the seed of rice, wheat, jute, pea, lentil and mustard.

The effect of water stress in germinating seeds of seven legume species has been studied by Oizumi et al., (1981). They found that the seeds were more susceptible in the advanced stage than in the early stage of germination. They also found increased germination percentage and promoted seedling growth probably due to breaking of the dormancy in case of highly tolerant species to water stress.

The compounds like mannitol and polythelene glycol have proved to be of a great value for stimulation of water stress conditions (Hoveland and Buchanan, 1973; Hegarty and Ross, 1978).

ii) Aeration :

Air and its various constituents affect germination directly or indirectly. Under normal atmospheric conditions (20% O_2 and 0.03% CO_2), seed germinates well. The process of seed germination involves many metabolic activities and requires expenditure of energy which is obtained mainly from oxidation. For the functioning of respiratory chain and for oxidative phosphorylation, oxygen becomes necessary prerequisite and for this reason the oxygen is usually a condition needed for germination. Its availability, many a times becomes a limiting factor for germination as in case of apple seeds (Come and Tissaoui, 1973). The presence or absence of O_2 in ambient atmosphere markedly affects seed germination. Oxygen uptake during germination at 30°C was investigated by Morohashi and Shimokoriyama (1972). They observed that O_2 uptake increased rapidly for 4-5 hours, remained constant for 1-2 hours and then increased again.

The requirement of oxygen is not the same in all types of seeds. Lower O_2 concentration or its deficiency disrupts many aspects of metabolism. The lowering of O_2 in atmosphere

hampers the respiratory process and causes morphological abnormalities, low oxygen level affects the uptake of mineral nutrients. It can also induce secondary dormancy in seeds possibly because of the interaction of oxygen concentration with germination inhibiting substances in the seed (Come, 1970).

During seed germination, respiratory rate increases. This increase in respiratory rate is a characteristic biochemical event of seed germination. This involves exchange of O_2 and CO_2. Good germination is dependent on adequate aeration. Increase in partial O_2 tension was found to promote germination in some seeds. Howell (1963) has reported no quantitative relationship between germination of soybeans and oxygen supply. But he concluded that the rate of growth of soybean roots was reduced in the absence of O_2, to half that obtained with the optimum oxygen level of 6 ppm in solution. In some seeds like Xanthium the process of germination is favoured by higher O_2 concentration (Thornton, 1935).

Like O_2, CO_2 is not directly participating in the metabolism of seed germination. However, CO_2 is found to exert effects opposite to those displayed by oxygen. Most of the seeds fail to germinate if the CO_2 concentration is greatly increased. Kidd (1914) showed that the seeds of barley and

cabbage are unable to germinate if CO_2 concentration is considerably increased. This inhibiting property of CO_2 is greatly exploited for the seed storage purpose.

Ethylene (C_2H_4) plays an important role in germination. It has been found that ethylene promotes seed germination of buckthorn (Vacha and Harvey, 1927; Balls and Hale, 1940). Seeds of some small plants such as clover (Trifolium subterraneum) have been shown to break dormancy in response to extremely low ethylene concentrations (Esachi and Leopoid, 1969). Ethylene and the compounds liberating ethylene have been reported to break dormancy of peanut (Ketringe and Morgan, 1969).

Eventhough N_2 is the major gaseous element among the constituents of air, it is inert and hence does not interfer the process of seed germination.

iii) Light :

The effect of light on germination is exceedingly complex. The effects of light have been recognised by many workers. Cieslar (1883) for the first time recognised the importance of light in germination process. The germination of many seeds is sensitive to white light. This phenomenon was termed as 'photoblastism' by Evenari in 1956. Broadly, seeds

can be divided into two classes - i) Light requiring (Positively photoblastic) and dark requiring (negatively photoblastic). The response of seeds varies with quality and quantity of light. Some germinate after being given a brief illumination and some are indifferent to the presence or absence of light during germination. Mukherjee (1967) reported that germination of the seed of Panicum coloratum was favourably affected by light and those of Panicum turgidum and Cenchrus setigerus preferred comparatively dark conditions for germination. The seeds of Cenchrus ciliaris and Panicum antidotale were found to be indifferent to light.

Excellent work of Borthwick and his co-workers (1952) has shown that red/far red photoreversibility in germination of lettuce is regulated by a photorecepter pigment, phytochrome. It exists in two photoreversible forms P_r and P_{fr}.

$$P_r \underset{\text{far-red light}}{\overset{\text{red light}}{\rightleftharpoons}} P_{fr}$$

Now it is an established fact that phytochrome is a metabolic switch involved in several metabolic processes (Smith, 1975). However, Malcoste (1969) has shown that P_r/P_{fr} ratio is different in positively and negatively photoblastic seeds. Germination in light requiring seeds depends on the presence of a

certain level of P_{fr}. In light requiring seeds phytochrome is in the P_r form. Red light converting P_r into P_{fr} form promotes germination. Far-red (FR) applied after red (R) irradiation converts P_{fr} form back to P_r inhibiting the germination. In case of negatively photoblastic seeds, germination may be due to the presence of a level of P_{fr} sufficient to bring about germination. In case of dark germinating seeds of tomato; Mancinelli et al., (1967), have demonstrated that a single short far-red (FR) irradiation inhibits germination and red applied after FR repromotes germination. This indicates that sufficient amount of P_{fr} to bring about the germination in darkness is normally present in the dark germinating seeds. Tewari (1965) reported that the levels of reducing sugars and total carbohydrates in the seeds fell when they were germinated in light. The levels were still lower in the seeds which were germinated in red light. Thus some seeds are light requiring while others germinate in darkness (McCullough and Shropshire, 1970) poses a new problem in understanding phytochrome action. The quality of light even during seed maturation is important in deciding the phytochrome state which further determines the germination behaviour (Smith, 1975).

Daily illumination have also been shown to affect germination. Kovrigo and Krustina (1966) have observed that the

seeds of weed plants, Dracocephalum thumiflorus and Matricaria discoidea germinate at the rate of 0.3 to 0.7% in darkness, while under the influence of illumination, the germination rate reached 69 to 92% depending upon the photoperiod. The seeds of Oryzopsis miliacea gave germination of only 13% in darkness but after 1 to 2 minutes of exposure to red light yielded a germination of 50% (Negbi and Koller, 1964). Sen and Chawan (1968) found that the seeds of Asteracantha longifolia were capable of germinating in light. The germination was favoured by blue or red light whereas total darkness delayed it. Thus it appears that the spectral zones of light also affect germination quite differently. The light having the wave length of 670 nm promotes germination while that of 700 nm inhibits it. However, the stimulation or inhibition depends on the exact period of illumination. Sharma et al., (1975) have shown that in blue red spectrum of light, germination percentage of Caesalpinia bonducella was highest whereas dark and green spectrum of light and adverse effects.

The effect of light quality and its interactions with various growth regulators on seed germination of Malus prunifolia revealed that red light promoted seed germination and far-red light negated (Lee MacRan and Byun Jac-Kyun, 1977).

Mayeux (1982) studied germination of false broom weed (Ericameria austrotexana) seed. He found maximum radicle elongation and fresh weight of 14 day old seedlings when germinated at continuous temperature of 15-30°C if light was provided for 8 hours daily. Germination was suppressed in the dark. Tewari (1965) observed that the maximum elongation of the radicle and plumule of the seedlings of Phaseolus radiatus took place in total darkness and to a lesser extent in continuous light. Red light strongly inhibited elongation.

iv) Temperature :

Seed germination involves many metabolic processes which are regulated by temperature directly. The 'cardinal' temperature points viz. minimum optimum and maximum for germination vary considerably from species to species. The minimum temperatures for germination are lower for temperate species than for tropic ones. The seeds of various alpine plants germinate at temperatures little above 0°C. Some germinate at constant temperature or within a specific range of temperatures depending upon the stage of development. The optimum germination of many seeds falls within the range between 15°C to 30°C. The experiment carried out by Knapp (1966) indicated that the optimum germination temperatures for mung bean seeds were 24-30°C. Sharma et al., (1975) recorded maximum germination in

Caesalpinia bonducella when temperature was elevated from 25°C to 30°C whereas no germination occurs at 0 to 20°C. The optimum temperature requirement for germination varies from species to species and even in the same species it differs from season to season (Chatterji and Mohnot, 1964).

According to Maguire (1973) the temperature variations affect the seed metabolism in a number of ways. Come and Tissaoui (1973) have observed that the higher the temperature, the smaller is the quantity of oxygen available to the embryo. They have further speculated that the seeds with seed coats rich in substances like phenolic compounds capable of fixing dissolved O_2 on its way to the embryo, become highly sensitive to high temperature. Low temperatures may inhibit the catabolic activities in the seeds that require warm temperatures to germinate or may fail to activate preexisting enzymes. Excessively high temperature may inactivate some enzymes and denature proteins, upsetting the anabolic activities involved in producing the seedlings. The relation between enzyme activity and temperature is fairly well established for a number of enzyme systems. The effect of low temperature on the activity of some enzymes and germination of Siberian pea tree seeds has been studied by Nikolaeca and Yonkuerich (1979). They found the enhancement of peroxidase activity due to cold adaptation of the seeds. The

response of seeds to external conditions can also be modified with growth regulators (Raynolds and Thompson, 1971). Mahmoud and Hill (1981) have studied the salt tolerance of sugarbeet under different NaCl levels at various temperatures. They have shown that salinity had little effect on seedling emergence at 10°C to 15°C but was increasingly inhibitory at temperature increased from 25°C to 35°C.

2) Biochemical Events During Germination :

A study of biochemical changes occuring before and during germination would be very important because it would be elusing the stages of break down of reserve food material to basic substances. According to Koller et al., (1962) in all such studies two things should be borne in mind. Firstly it is not always clear whether metabolic events which have been observed are the results of its cause. Secondly, it is quite possible that the extent of given metabolic process may be masked because whole seed or seed extracts are normally used for experiments.

The metabolism of germinating seeds is amphibolic i.e., it is both catabolic in the sense of degrading reserve compounds to provide energy and raw materials for the early growth of the seedling and anabolic in the sense of producing machinery for protein synthesis and biogenesis of various organelles needed

for catabolic activity as well as the true anabolic synthesis of new cells and tissues (Ching, 1972). Major catabolic activities are localized in storage tissues such as the endosperm and cotyledons while anabolism takes place in the embryo axis. The metabolic activities of seed germination can be distinguished into three phases - imbibition (a phase of hydration), activation (a phase including respiration and metabolism) and a growth period.

The initial step towards seed germination is rehydration of seeds by water. The necessary water is imbibed by the seeds. The rate and quantity of water imbibed by seeds vary with the nature of seed coat, seed size, chemical composition of seed and imbibition temperature. The water potential of mature, dry seed is considerably lower than that of a surrounding moist substrate and naturally water moves from substrate to seed. The entry of water in seeds marks the reactivation of macromolecules and cell anganelles. In most cases cell enlargement rather than cell division occurs during germination. Hallman et al., (1972) have described the sequence of ultrastructural changes which occured as the embryo of rye germinated together with the sequence of biochemical events to which these changes are linked. Three phases of imbibition have been recognised. The first phase is a short phase of 10 minutes of physical

wetting; the second phase is of longer period (1 hour) of further imbibition and the third is a continuous phase of water uptake. The last phase is coincided with an increase in the rate of respiration and number of mitochondria and cristae. During first 15 minutes of imbibition reformation of keto acids from amino acids by deamination and transamination reactions occur and this is considered as a very early metabolic event (Collins and Wilson, 1975). Respiration involves oxidative breakdown of certain organic constituents. Morohashi and Shimokoriyama (1972) studied glucose and organic acid metabolism in early phase of germination. There is always a change in the patterns of respirable substrates in germinating seeds. In general, respiration during germination is considered to involve four phases. They have been summarised by Bewley and Black (1978) as follows :

Phase I : This is characterised by a sharp rise in respiration lasting about 10 hours. The respiratory quotient (R.Q.) is slightly above 1:0. The major respiratory substrate is sucrose. The rise in respiration during this phase progresses linearly with the swelling of cotyledon tissue.

Phase II : There is a lag phase between hours 10 and 25 after the start of imbitition. Hydration is completed and all pre-

existent enzymes are activated. There is rise in R.Q.upto 3:0, indicating anaerobic respiration. The lag phase may be due to limited supply of O_2.

Phase III : During this phase R.Q. falls down to about 1:0 indicating predominant aerobic respiration of carbohydrates.

Phase IV : In this phase there is marked fall in respiration with the disintigration of storage organs following depletion of the stored reserves. In germinating seeds, the three respiratory pathways namely glycolysis, the pentose phosphate pathway and TCA cycle are assumed to be active.

During seed germination, the storage tissues become the source of nutrients for the developing embryo. The principal and most wide spread storage carbohydrate of seed is starch. Two pathways of starch catabolism are known to operate in germinating seeds. One is hydrolytic and involves two amylases (Marshall, 1972) and the other is phosphorylatic pathway.

i) Hydrolytic pathway :

Amylose $\xrightarrow{\alpha\text{- amylase}}$ Glucose + α- Maltose + α- Maltotriose

Amylopectiin $\xrightarrow{\alpha\text{- amylase}}$ Glucose + α- Maltose + α- Maltotriose + α- Limit-Dextrin.

$$\alpha\text{- Maltotriose} \xrightarrow{\alpha\text{- amylase}} \alpha\text{- Maltose}$$

$$\alpha\text{- Maltose} \xrightarrow[\text{maltase}]{\alpha\text{- glucosidase}} \text{Glucose.}$$

$$\text{Amylose} \xrightarrow{\beta\text{-amylase}} \beta\text{-Maltose}$$

$$\text{Amylopectin} \xrightarrow{\beta\text{-amylase}} \beta\text{-Maltose} + \beta\text{-limit Dextrin}$$

$$\text{Limit Dextrin} \xrightarrow[\text{Glucosidase limit dextrinase.}]{\text{de branching enzymes}} \text{Glucose.}$$

ii) Phosphorylatic pathway :

$$\text{Amylose} + \text{Amylopectin} + \text{Pi} \xrightarrow[\text{Phosphorylase}]{\text{Starch}} \text{Glucose - 1-p} + \text{limit Dextrin.}$$

The rise in amylase activity in a seed during germination is primarily in α- amylase (Ota et al., 1953; Palmiano and Juliano, 1972; Lineback and Ponpipon, 1977; Gibbons, 1980). The soluble carbohydrates increase due to break down of storage carbohydrates. The increase in soluble carbohydrates has a direct relation with the growth of the seedling. The higher the metabolic level, the greater was the growth of the seedlings (Chattarji, 1969). Though glucose and maltose are produced after starch degradation, it is noticed that the major form in which the carbohydrates are transported to the growing axis is sucrose. In the biosynthesis of sucrose from glucose and other

sugars, nucleotides play a key role. The osmotic regulation of oc- amylase has also been reported by Jones and Armstrong (1971).

The other catabolic pathway of starch break down is catalysed by starch phosphorylase which results in the production of glucose-1-phosphate (Shain and Mayer, 1968). Sucrose is the major form in which products of carbohydrate (glucose, sugars, nucleotides etc.). Catabolism are transported into the developing seedling and hence plays a key role.

Seed germination is characterised by a decline in the protein content of the storage tissue and an increase in the soluble nitrogen component in the growing embryonic axis. This suggests that during germination proteins in the reserve tissue, are hydrolysed to amino acids which are translocated to the glowing axis (Ingle et al., 1964; Beevers and Guernsey, 1966). Proteins are broken down to amino acids and amides during seed germination with rise in amino acids and amides followed by de novo protein synthesis in growing parts of embryo (Ota et al., 1953). The break down (hydrolysis)of reserve proteins is catalysed by proteases to yield amino acids and units of peptides. An increase in protease activity during germination of pea and squash seeds has been reported by Beevers and Guernsey (1966)

and Wiley and Ashton (1967). Both decrease as well as increase in the activity of peptidases were evident during seed germination (Beevers and Splittstoesser, 1968; Prentice *et al.*, 1969). Some of the amino acids liberated during protein hydrolysis are utilised for synthesis of proteins in the growing parts ot the seedling. The actual synthesis of new protein during germination has been shown. Certain enzyme proteins are synthesised *de novo* in the seed, not only in the growing part of the embryo but also in the endosperm or cotyledons (Young and Varner, 1959; Young *et al.*, 1960). Deamination of other amino acids provide carbon skeletons for various respiratory and carbon cycles. As a result, ATP level rises during germination (Pradet *et al.*, 1968).

For the synthesis of metabolites like ATP coenzymes and nucleic acids and for early growth of seedlings, adequate supply of phosphorus, potassium, calcium and magnesium is essential. Hydrolysis of phytin in many seeds, by phytase to release phosphate is one of the most prominent events during germination (Bieleski, 1973). An increase in the activity of phytase during seed germination represents one of the significant features of phosphorus metabolism in germinating seeds (Matheson and Strother, 1969; Tronier *et al.*, 1971; Dimitrieva and Sobolev, 1979; Eskin *et al.*, 1983). Other forms of phosphates are in

smaller amounts in seeds and are hydrolysed by a variety of phosphatases (Mayer et al., 1971). Relation between acid phosphatase content and germination capacity of different species was studied by Arbestain Ribas, Ignacio (1977). These workers noticed a positive correlation between acid phosphatase activity and germination vigour of seeds. According to Borris (1978), the activity of acid phosphatase is only partially correlated with the hormonal control of embryo growth.

3) Germination under Saline conditions :

A) Salinity Problem :

Saline and sodic soils mostly occur in the arid and semi-arid regions of the world. Such soils are formed due to accumulation of undesirable concentration of salts in the upper layer of the soils. The high concentrations of the salts in the soil develop unfavourable characters which hamper normal germination of the seed and growth of the crop plants and gradually the soils become unproductive.

Saline soils contain excessive concentrations of soluble salts. The U.S. salinity laboratory (Richards, 1954) has published a terminology and discription of saline sodic soils. The electrical conductivity of the saturation extract (EC_e) of a saline soil is greater than 4 $mS \cdot cm^{-1}$ and the exchangeable

sodium percentage (ESP) is less than 15. The pH of the soil is usually less than 8.5. These soils are recognised by the presence of white or greyish white crust of salts on the surface or by oily looking surface devoid of vegetation.

Sodic soils have the conductivity of saturated extract less than 4 mS cm^{-1} at 25°C and the exchangeable sodium percentage greater than 15. The pH of the saturated soil solution is usually greater than 8.5 some times as high as 10. These soils are usually plastic sticky when wet and form large crust on drying. Sodic soils are found in semi arid and arid regions in small regular areas and often referred to as "slick spots", (Richards, 1954). Allison (1964) has used the term 'sodic' instead of 'alkali' because the latter has had various and somewhat indefinite meanings.

Salinity has posed a constant problem to crops either on salt affected soils or under saline water irrigation. Millions of hectares of land throughout the world is saline and unproductive. There are about 1000 million hectares of the land affected by salinity in the world (Massoud, 1974). In 1963, Planning Commission of the Government estimated that in India there are about fifteen million acres of saline land distributed in the satates of Punjab, Rajasthan, U.P. and the Deccan and

coastal areas. Central soil Salinity Research Institute of India, has reported that in Haryana 3 lakhs and 40 thousand acres of land is salty. Paliwal (1972) has reported that about 15% of the cultivated land in India is affected either by soil salinity or alkalinity.

The continuous irrigation leads to the building of salts in root range of the crops. During the last decade irrigation potentials in India have been significantly increased. With the increase in irrigation, the affected areas are likely to increase if proper irrigation and agricultural practices are not adopted, (Vaidya and Sahasrabudhe, 1970). About one third of irrigated soils amounting to almost 77 million hectares are said to be sufficiently affected by salinity so as to adversely affect crop growth (Eckholm, 1975). Unfortunately no cheaper methods of desalination of soil or water have been achieved to overcome the salinity hazards. According to Epstein et al., (1980) besides an engineering approach, the development of crops, tolerant to salinity is a better strategy for meeting the challange of salinity problem. To achieve this, it is of prime importance to understand the performance and the physiology of plant species and the cultivars under saline conditions.

It has been observed by several workers that most of the crop species which are glycophytic in nature differ greatly in their tolerance to salinity (Abel and Mackenzie, 1964; Mehrotra and Gangwar, 1964). Maas and Hoffman, (1977) have made an assessment of salt tolerance of 76 economically important species. Their survey indicated that the crop plants differ in their tolerance capacity and can broadly be classified into sensitive, moderately sensitive, moderately tolerant and tolerant groups. The varietal differences are also noticed by some workers with respect to salt tolerance potential (Iyengar et al., 1968; Paliwal et al., 1971; Torres and Bingham, 1973; Rizk et al., 1979; Tesu et al., 1979; Kumar and Bhardwaj, 1981; Fageria et al., 1981).

Salinity puts various problems to plants, at the population the individual, the physiological and the molecular level. The overall reflection of all the problems occurs in the reduction of growth. Salinity causes stunting in glycophytes. On the contrary the growth in halophytes is favoured by certain amount of salts (Strogonov, 1964). According to him the adverse effects of salt on growth is due to a change of the state of protoplasm in the cells. Eaton (1942) holds the view that water deficit induced by high osmotic pressure of the root medium is the factor restricting growth. A change in hormonal balance by salinity (Itai et al.,1968) also contributes to such effect.

The work on salinity for the last several decades has lead to the formation of two different schools of thoughts regarding the nature of salt injuries. The champions of Osmotic School (Bernstein and Hayward, 1958) claim that most of the adverse effects of salinity are related to the decreased osmotic potential of saline root media. On the contrary the champions of specific ion school (Eaton, 1942; Strogonov, 1964) propose that the adverse effects of salinity are caused mostly by the specific effects of individual ions. The work of Russian worker (Strogonov, 1964) indicated that each type of soil salinity creates specific condition for growth of plants and also determines the nature of anatomical and physiological changes which occur in it. According to Maas and Nieman (1978) each specific ion effects seem limited to certain susceptible plant species and rarely are a major cause of growth suppression. Among the two salinities, the responses of plants to chloride salinity are extensively investigated.

The osmotic adjustment of plants to saline media was investigated in detail by Bernstein and co-workers (1975). Their work indicated that there is no apparent correlation between salt tolerance and sosmotic pressure of expressed sap of leaves or roots. According to Bernstein (1961) the reduction in growth under salt stress conditions may be regarded as

a consequence of the requirement for osmotic adjustment and the primary mechanism for effecting it.

The major nutritional effects of salinity are those associated with cation nutrition. According to Greenway (1965) the regulation of ion concentration is relevant to salt tolerance of vascular plants, Epstein (1968) suggested that halophytes and salt tolerant plants have developed a mechanism for the preferential uptake of potassium from mixtures rich in sodium. This suggestion is supported by recent findings of Storey and Wyn Jones (1978) in members of gramineae. Stuiver et al., (1978) observed a difference in root membrane structure with respect to lipid composition which could be correlated with differences in membrane permeability to sodium and chloride in salt sensitive bean, less salt sensitive barley and the salt tolerant sugarbeet. The work of La-Haye and Epstein (1969) has shown that calcium ion has an ameliorating effect on plant growth under the conditions of salinity. Influence of salinity on uptake of micronutrients like iron, manganese and zinc has been investigated by Maas et al., (1972).

The plants subjected to salt stress show several morphological and anatomical changes. The structural changes include fewer and smaller leaves, fewer stomata per unit area, increased,

succulence, thickening of leaf cuticles and surface layers of wax reduced differentiation and development of Vascular tissue, increased development of tyloses and earlier lignification of roots (Strogonov, 1964). Strogonov further noticed that chloride salinity induces development of halosucculent features which are mainly due to increased elongation of palisade cells. Sulphate salinity on the contrary inhibits cell expansion and develops haloxeric features. The work of Blumenthal-Goldschmidt and Poljakoff-Mayber (1968) showed that salinity causes ultrastructural changes in cell organelle like chloroplast.

Salinity impairs protein synthesis thereby accumulating ammonia, diamines, amides and toxic amino acids. In halophytic plants, free proline has been found to vary with salt concentrations (Stewart and Lee, 1974; Treichel, 1975). Although proline contents in glycophytes are normally negligible, the concentration increases markedly in plants subjected to salinity (Chu et al., 1976). Salinity also induces accumulation of a quaternary ammonium compound glycine betaine in some members of graminae (Storey and Wyn Jones, 1975). The accumulation of keto acids and their incomplete utilization in the synthetic processes is also favoured by salinity. The balance of photosynthetic pigments under saline conditions is upset and the pigments like anthocyanins and carotenoids accumulate.

The consequence of the change in ionic balance in salt stressed plants is the shift in ionic interactions with the enzymes and intermediates of metabolism at several levels. The excess of salts in the medium may affect the synthesis of the enzymes or exert an allosteric effect on the enzyme proteins. Effect of salt stress on enzyme activities has been studied by several workers (Hasson-Porath and Poljakoff-Mayber, 1969; Weimberg, 1970; Waisel, 1972). However, in in vitro studies it is observed that the enzymes from salt sensitive and salt tolerant species do not differ markedly in their responses to salinity (Greenway and Osmond, 1972). According to Jennings (1976) ATPase can behave differently in halophytes and glycophytes. According to Flowers (1972) a compartmentalization of enzymes at a distance from salts in halophytic cells may help in keeping normal enzyme activities under saline conditions.

A decrease in net photosynthetic rate due to salinity was evident in the experiments of many workers. However, based on the studies on twelve crop plants, Nieman (1962) suggested that photosynthetic assimilation of CO_2 is not the growth limiting factor in salinized media. Gale et al., (1967) proposed that the adverse effects of salinity on photosynthetic rate are mediated through its influence on light reactions of photosynthesis. Nieman and Clark (1976) suggested that salt stress

limits photophosphorylation. According to Strogonov et al., (1970), salinity affects the strength of the forces binding the complex of pigment protein-lipid in the chloroplast structure. They further stated that the change in chlorophyll content due to salinization is related to salt tolerance capacity of the plant. Udovenko et al., (1974) have indicated that inhibition of photochemical activity of chloroplasts due to migration of these organelles into the lower part of the cells of palisade and spongy parenchyma cells is responsible for such effects. Lapina and Bikmukhametova (1972) stated that a decrease in the photosynthetic ability under saline conditions is mainly due to the growth retardation and the diminished assimilation area of the leaves. Based on the work of photosynthesis in salt stressed grapevines, Downton (1977) suggested that the salinity induced decrease in photosynthetic rate, can be largely attributed to increased residual (mesophyll) resistance to CO_2 fixation. According to Passera and Albuzia (1978) the reduction in $^{14}CO_2$ assimilation rate at higher concentrations of salt may be due to increased rate of photorespiration.

Salt stress also brings about significant changes in the pattern of photosynthetic carbon metabolism. Bidwell (1958) was the first to show that photosynthetic pattern in marine algae was significantly different from higher plants and it was

characterized by the synthesis of number of organic compounds uncommon in higher plants. Joshi et al., (1962) and Webb and Burley (1965) studied the effect of NaCl on dark CO_2 fixation by Marine and terrestrial plants. They observed a shift in the pattern of labelling from organic acids to amino acids in the marine plants. It was shown by Winter and Willert (1972) that a C_3 plant, Mesembryanthemum crystallinum responds to salination with the development of crassulacean acid metabolism. A C_3 to C_4 shift due to salinity was predicted by Shomer-Ilan and Waisel (1973) in a grass Aeluropus litoralis. Kennedy (1977) and Joshi and Karadge (1979) observed an increase in labelling of C_4 acids in Portulaca oleracea subjected to NaCl salinity. Sankhla and Huber (1974) studied the effect of NaCl salinity on $^{14}CO_2$ fixation in seedlings of Pennisetum typhoides. They found that high concentrations of NaCl (1.7×10^{-2} M) increased $^{14}CO_2$ incorporation in organic acids (especially malate) but less radioactivity was detected in amino acid fraction (alanine). The studies of Downton (1977) and Passera and Albuzio (1978) showed that salt stress leads to the accumulation of label in intermediates of the glycolate pathway. Thus it is apparent that photosynthetic carbon metabolism is variously altered in different plant species when they are subjected to salt stress. The translocation of photosynthates is also severely affected by salt stress (Strack et al., 1975).

It is evident from the foregoing discussion that salinity has far reaching effects on the plant metabolism which ultimately results in impairement of growth and loss in overall productivity. This is true for most of the crop species. At the same time we can notice that at salt concentrations injurious to conventional strains, certain plants can perform well, although not as well as plants not subjected to salt stress. Saline regions now ruled out for culture of most of the crop species might be usable for selected species of such plants which can provide food, fiber and other usable biomass on a permanent basis.

The species and varieties of plants exhibit varying degrees of salt tolerance with respect to the stage of development. Nadeem and Shah (1978) have indicated that lentils were more salt tolerant at germination than at later growth stages. According to Pearson et al., (1966) the salt tolerance of rice diminishes after germination. Contrary to this Kaddah et al., (1973) observed that salt tolerance of rice continues to increase after the seedling stage with no decrease during flowering and seed set.

It is generally believed that the plants are more sensitive to salinity at germination than at later stages of growth (Kling, 1954; Bhumbla et al., 1968). According to Bernstein

(1974) such generalization is not correct as the germination failure is caused by exceptionally high concentrations of salts in the surface (2-3 cm) of the soil where the seeds are planted.

Hence, it is not reasonable to compare salt tolerance during germination stage with that during later stages of growth. According to West and Taylor (1981) generally there is a poor correlation between salt tolerance at germination and that at later stages of growth. The species and varieties differ in their salt tolerance with respect to the germination and seedling growth. This emphasizes the importance of crop selection on the basis of salt tolerance in areas where salinity is a problem.

B) Germination Under Saline Conditions :

In the life cycle of plants there are three important stages when considered in relation to salt tolerance.

i) Germination and seedling growth.
ii) Vegetative growth.
iii) Maturation and fruiting.

The first phase of germination and seedling growth is critical under saline conditions. The ability of a given variety

to germinate and establish the seedling is frequently the limiting factor in crop production. Germination being the first and the most important phase, it is of utmost importance to study the effects of salts on this process.

The high concentrations of salts in the soil, develop unfavourable characters which hamper normal germination of seed and growth of the crop plant. The presence of high concentration in soil solution increase the osmotic pressure of the medium considerably, thereby affecting the water uptake process. Many workers have reported the retarding effects of high osmotic pressure of salt solution on germination of seeds.

Hayward and Wadleigh (1949) have mentioned that there is an evidence that certain salts or ions may be toxic to the embryo or seedling, occuring significantly in high concentrations. This toxicity may be reflected in reduced germination and is frequently accompanied by abnormalities in the growth and development of the seedlings. These studies indicate that germination is retarded or inhibited by the presence of soluble salts in the soils.

The early work of Buffum (1896 and 1899) showed that "the retarding effect of a salt solution on the germination

and seedling growth is directly proportional to its osmotic pressure when the solutions are strong". Similar conclusions were drawn by many workers where they found that high osmotic pressure inhibits germination, (Magistad, 1945; Dewey, 1960; Maliwal and Paliwal, 1967). Uhvits (1946) observed that germination was inhibited when NaCl solution of 12 to 15 atm osmotic pressures were used. She also found that the reduction and retardation of germination were greater on NaCl than on mannitol substrates.

Sorour et al., (1977) observed that seedling emergence was delayed in Triticum aestivum by increasing the salinity levels. The low and medium salinity levels (3000 and 6000 ppm) exerted suppressing effects on seedling emergence. Sionitt et al., (1973) have shown reduction in germination of safflower as salinity increased from 0 to 2% of NaCl. They have also shown variations among the safflower varieties during germination.

Narale et al., (1969) have found that NaCl when exceeds the electrical conductivity 8.9 m $S \cdot cm^{-1}$, the germination in rice is adversely affected. Sarin and Narayanan (1968) have also shown that salinity depressed and delayed the germination of wheat. Singh et al., (1971) have reported that in Phaseolus aureus variety RS-5 that germination was not affected at sali-

nity levels upto 22.5 meq but which was slightly decreased at 50 meq and markedly decreased at 100 meq.

There are two ways in which saline soils may affect germination (i) There may be enough soluble salts present in the seed bed to build up the osmotic pressure of the soil solution to a point which will retard or prevent the intake of necessary water and (ii) certain constituents may be toxic to the embryo and seedling (Hayward and Wadleigh, 1949).

According to Udovenko and Alekseeva (1973) germination retardation under saline conditions is mainly due to a lower mobility of water in salinized medium. Sinha (1982) found that seed germination in S. aegyptica was inversely related to increasing levels of water stress, salinity and alkalinity. Excepting the very high levels of stress, he found good germination responses.

Bharadwaj (1957) and Sarin (1958) have reported that the salinity injury is often noted at the initial stages of plant growth itself. Highly saline soils inhibit germination, altogether leading to poor stand of crops. It was considered to be due to the osmotic effect of the hypertonic soil solution but it could also be attributed to an upsetting of the metabolism

of the germinating seed due to accumulation of toxic ions (Cl, SO_4 etc.) in the seedling or an imbalance of the accumulated ions. According to Pearson et al., (1966) the salt tolerance of rice diminishes after germination. It is generally believed that the plants are more sensitive to salinity at germination than at later stages of growth (Kling, 1954; Bhumbla et al., 1968). Allison (1964) has pointed out that rice is quite a tolerant during germination but becomes sensitive during seedling stage. On the contrary Kaddah et al., (1973) observed that salt tolerance of rice continues to increase after the seedling stage with no decrease during flowering and seedset. Ayers (1952) found that barley was one of the most salt tolerant plants. He reported that there was significant differences in salinity tolerance amongst different varieties of barley. Maliwal and Paliwal (1967) determined that amongst barley varieties NP 113 and NP 13 are highly salt and alkali tolerant. In general barley was more salt and alkali tolerant at germination stage than wheat.

Salinity causes several metabolic disorders in the germinating seeds which can be immediately recognised by changes in the pattern of key enzymes and metabolites. The disorders ultimately reflect in growth retardation and failure of emergence of normal seedlings. Many enzyme systems like amylase, catalase,

acid phosphatase, protease transaminase are affected by salt accumulation in seedling, (Bharadwaj, 1965; Sarin and Narayanan, 1968; El-Fouly and Jung, 1972; Huber and Sankhla, 1974; Prisco and Vieira, 1976; Sheoran and Garg, 1979). Weimberg (1970) has made excellent studies on the salt responses of enzymes in Pea seedlings. He studied the effect of 4 different salts (NaCl, Na_2SO_4, KCl and K_2SO_4) on 18 enzymes and found no significant effect of all the 4 salinities on enzymes except three enzymes.

Davis et al., (1974) have found that treatment of NaCl to Myriophyllum spicatum seedlings results in disruption of cell wall and membranes and in dispersed photosynthesis, respiration and Na accumulation. Salinity induced growth abnormalities in germinating seedlings may also be due to hormonal imbalance as there are reports of ameliorating seedlings (Babu and Kumar, 1975; Boucaud and Ungar, 1976).

4) Scope of Present Investigation :

The literature on metabolic processes taking place during germination is voluminous. Yet it is not possible to explain all the aspects of this process. It is noteworthy that most of the germination studies are centered around the cereal crops like rice, wheat, maize, barley etc. Considerable work has been

done on pulse-crops by various research centres throughout the world. There are intensive investigations on the metabolic, processes of pea, mung bean, soybean, gram etc. Among the legumes relatively little attention has been given to Phaseolus aconitifolius and Crotalaria juncea, so far their salt tolerance ability is considered. In the present investigation therefore an attempt has been made to have preliminary idea about the response of these plants under the salt stressed conditions during germination.

Germination process represents a shift from low metabolic state to high metabolic state. To understand the influence of salt stress on germination and seedling growth two salts have been employed in the present investigation. This investigation has also been extended to study the effects of salt stress on some of the enzyme activities. The enzymes like amylase (an enzyme playing a key role in starch breakdown in germinating seeds); acid phosphatase (an important enzyme involved in phosphorious metabolism of germinating seeds); peroxidase and catalase (playing significant role in respiratory process of the germinating seeds), and nitrate reductase (an enzyme involved in reduction of NO_3 to NO_2 in nitrogen metabolism) have also been studied.

A) The plants selected :

Legumes are second to the cereals as a source of human food and provide the much needed proteins to our predominantly vegetarian population. Legumes have been an important crop ever since man started domesticating plants and have been part of our cultural heritage. The reasons for the domestication and importance of pulses are many :

1) Grain legumes have a low water content and impervious seed coats. These features are valuable during transportation and storage.
2) They can be easily cultivated and mature rapidly.
3) They have an important place in the crop rotation.
4) The per acre production of protein is higher for pulses than for cereals and they provide protein rich food for man and live stock.
5) The legumes are important not only due to their food value as pulses but some also provide fodder, fibre and wood.

In most of the foods of plant origin, the protein content is low in quantity and poor in quality. The production of high yielding and high quality varieties of pulses, cereals and millets would therefore, offer the least expensive and most

practical way of diminishing the threat of protein malnutrition.

Pulse Crops :

Pulses are the cheapest source of protein in the Indian diet. There has been a declining trend in the per capita availability of pulses since 1959, when the daily per capita consumption was 75 grams. By 1971 this had been dropped to 50.3 grams per capita per day. Present consumption standards are very low. To meet these demands the production of pulses should be increased. Naturally, attention will have to be concentrated on improving the yield per acre in the conditions of limited acreage on pulses. This can be done either by introducing the high yielding varieties or by the application of fertilizers, or by increasing irrigated acreage or by introducing pulse crops of short-duration or by growing salt and drought tolerant varieties. With this idea and background in mind for the present work, two leguminous plants are selected. Phaseolus aconitifolius is chosen because of its importance as a pulse crop while Crotalaria juncea is selected for its diverse utility.

Genus Phaseolus :

The genus Phaseolus which supplies a large number of significant pulses to the mankind, comprises 230 species of

which 20 are cultivated for their edible pods or seeds (Zhukovsky 1962). Based on the origin the species can be divided into two main groups, the 'Asiatic' and the 'American'. The Asiatic species have yellow flowers, keel with lateral horn or spur; smaller and cylindrical pods without a beak; have small seeds and broad spur like stipules. The plant body is pubescent. The cultivated species are P.mungo L., P.aureus Roxb., P.aconitifolius Jacq., P.calcaratus Roxb., P.angularis Wight., and P.trilobus Ait. The American species have large flat pods with a beak, large seeds and small cuneate stipules. They include P.vulgaris, P.coccineus (multiflorus); P.lunatus; P.acutifolius Gray var. latifolius Freem., P.caracalla and a few other minor species.

i) Phaseolus aconitifolius Jacq.

P.aconitifolius is probably a native of India (de Candole), Pakistan and Burma where it grows wild. Bailey also considered that probably it is native of India. However, the absence of a sanskrit name and of different names in modern Indian languages, points to recent cultivation of this legume. At present it is largely grown in India, Sri Lanka, China, Pakistan and the South Western United States, particularly in Texas and California. In India the Legume is cropped over an area of about one million ha, giving a yield of 0.44 million t of beans (Kochhar, 1981). The major states producing this bean are Rajasthan, Uttar Pradesh, Punjab and Maharashtra.

P.aconitifolius is an important pulse crop of semi arid regions. It is an annual prostrate herb (Fig.1). On account of its mat-like spreading habit the terms 'mat bean' or 'moth bean' have been suggested as the English synonyms. The main axis is about 15-30 cm in height, somewhat angular and pubescent and has short internodes. A large number of primary branches, 30-150 cm long; arise form the main axis which trail horizontally and generally radiate outwards. The internodes of the primary branches are larger. The leaves are alternate and trifoliate, stipules are peltate, 1-2 cm long with lanceolate lobes, stipels are small. The leaflets are 5-12 cm long and divided into 3-5 narrow lobes or 3 acute lobes. The terminal leaflet in some forms is divided into 5 acuminate lobes while internal ones are generally 3 lobed.

The inflorescences are axillary long (5 to 10 cm), peduncled, capitate racemes of several small yellow papilionaceous flowers about 9 mm long, light yellow coloured standard is more or less hooded and broadly emerginate at the apex. The wings are bright yellow, the cleft is spirally coiled around the keel whereas the right one is slightly twisted and encloses the keel. The keel is characteristically twisted. The stamens are 9 and 1. The basal ovary is minutely hirsute having a twisted style and a flat-stigma, bearded on the lower side. The flowers are

normally self-fertilized. The pods are buff to yellow brown coloured with short stiff hairs. They are about 2.5 to 5.0 cm long., 5 mm wide and 3 mm thick have short stiff bristles and the beak is short and curved. There are 4 to 9 seeds in a pod. The seeds are small 5 mm long and 2-3 mm wide. They are yellow to brown or mottled black, somewhat reniform in shape with rounded or truncate ends. The hilum is linear and white, less than 3 mm long. Seed germination is epigeal. The mature dried seeds contain approximately 9.3% moisture; 23.0% protein; 0.7% fat; 59.0 carbohydrates; 4.0% fibre and 4.0 ash.

In India, moth bean is grown as a hot season crop. It is the most drought resistant of the kharif (summer crop) pulses and is largely grown on dry, light, sandy soils in the arid and semi-arid regions of the country. The crop requires a uniform high temperature and thrives best in an area with a well distributed rainfall of 75 cm per annum. Heavy rains are detrimental.

In India, the young and tender pods of moth bean are eaten as a vegetable, while the ripe seeds are eaten cooked either whole or split (dal). The whole grain after frying in a little fat is mixed with other savoury dishes to make 'dal moth'. Being drought resistant, it is an important crop for arid and semi-arid regions to provide food for men and feed for the cattle.

Fig.1 : Germinating seeds and seedlings of *C.juncea* and *P.aconitifolius*

A. *Phaseolus aconitifolius*

B. *Crotalaria juncea*

Fig. 2 : Growth and development of C.juncea and P.aconitifolius seedlings (120 h) under NaCl salinity.

A) C.juncea B) P.aconitifolius

a) 1) Control 2) 20 mM 3) 60 mM
4) 100 mM 5) 200 mM 6) 300 mM

b) 1) Control 2) 20 mM
3) 60 mM 4) 300 mM

Fig.3 : Growth and development of C.juncea and P.aconitifolius seedlings (120 h) under $CaCl_2$ salinity.

a) A) P.aconitifolius B) C.juncea

1. Control, 2. 20 mM, 3. 60 mM,
4. 80 mM, 5. 100 mM, 6. 200 mM &
7. 300 mM.

b) A) P.aconitifolius B) C.juncea

1. Control, 2. 20 mM, 3. 60 mM,
4. 80 mM, 5. 300 mM.

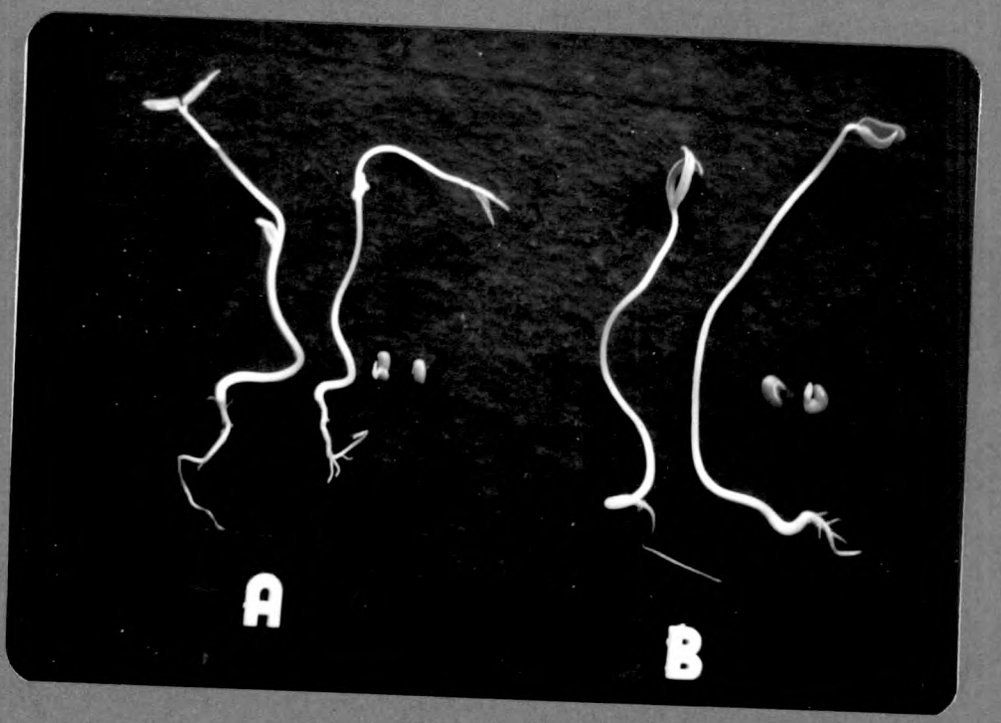


FIG : 1

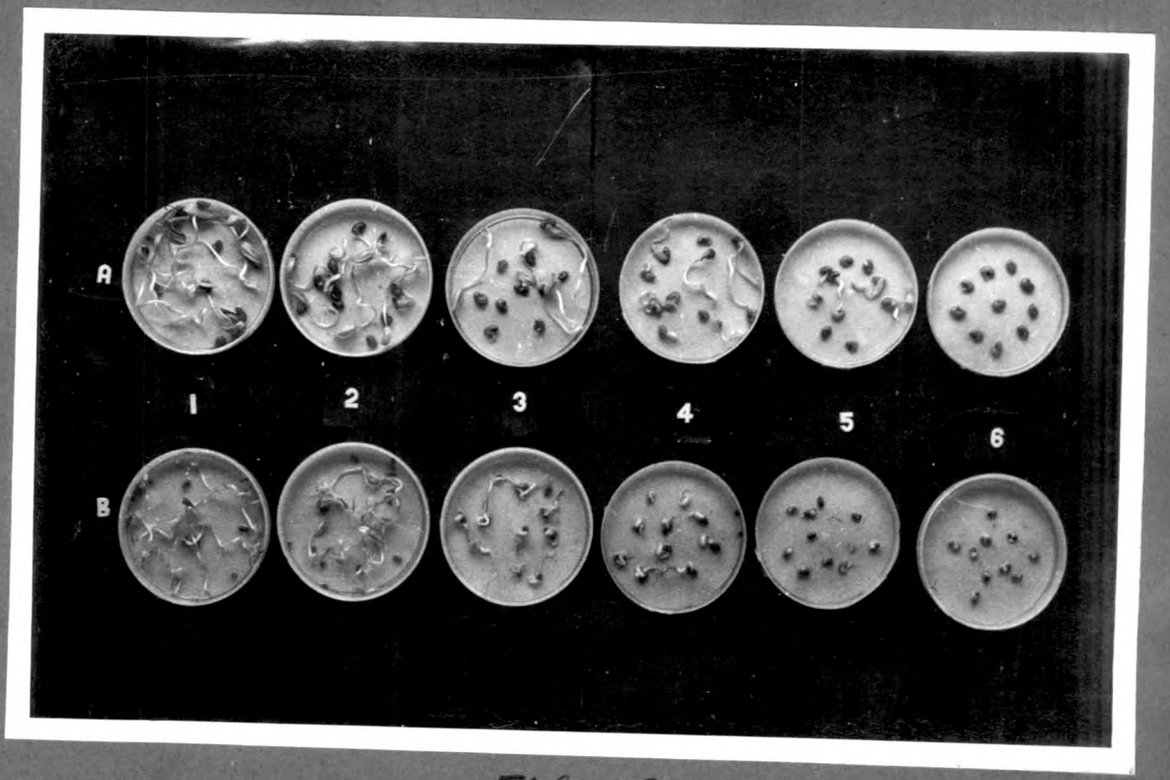


FIG : 2-a

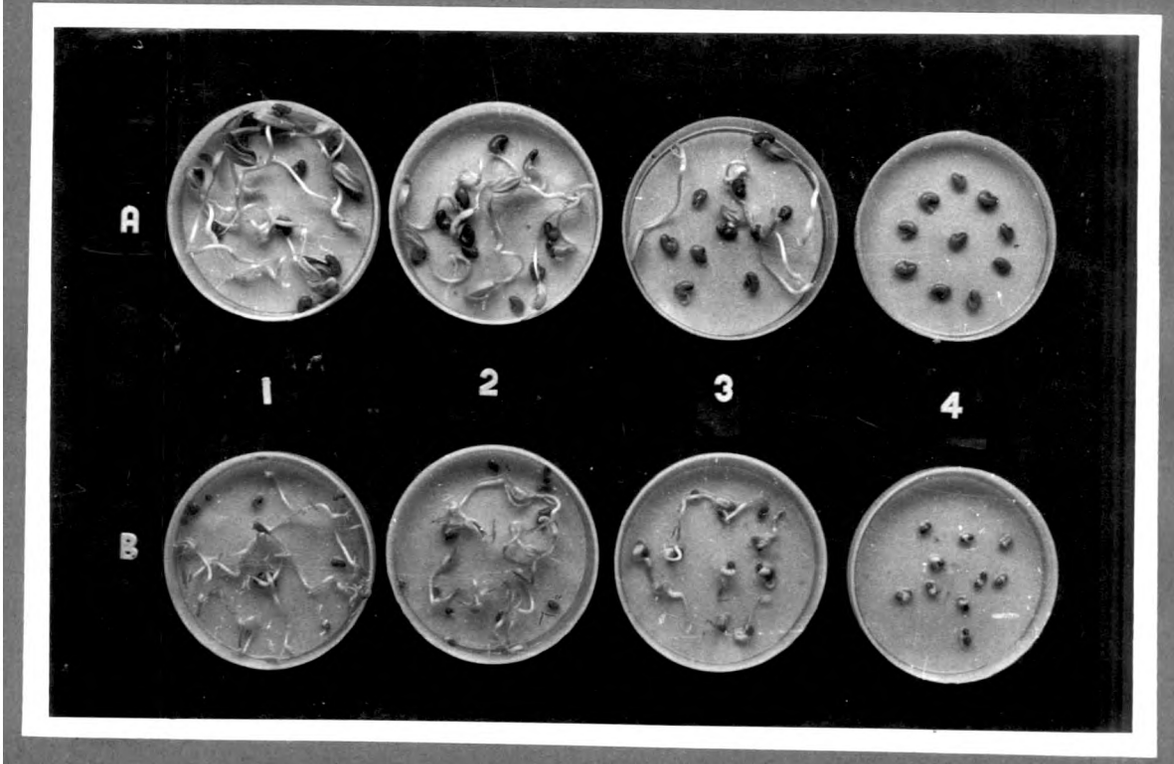


FIG : 2-b

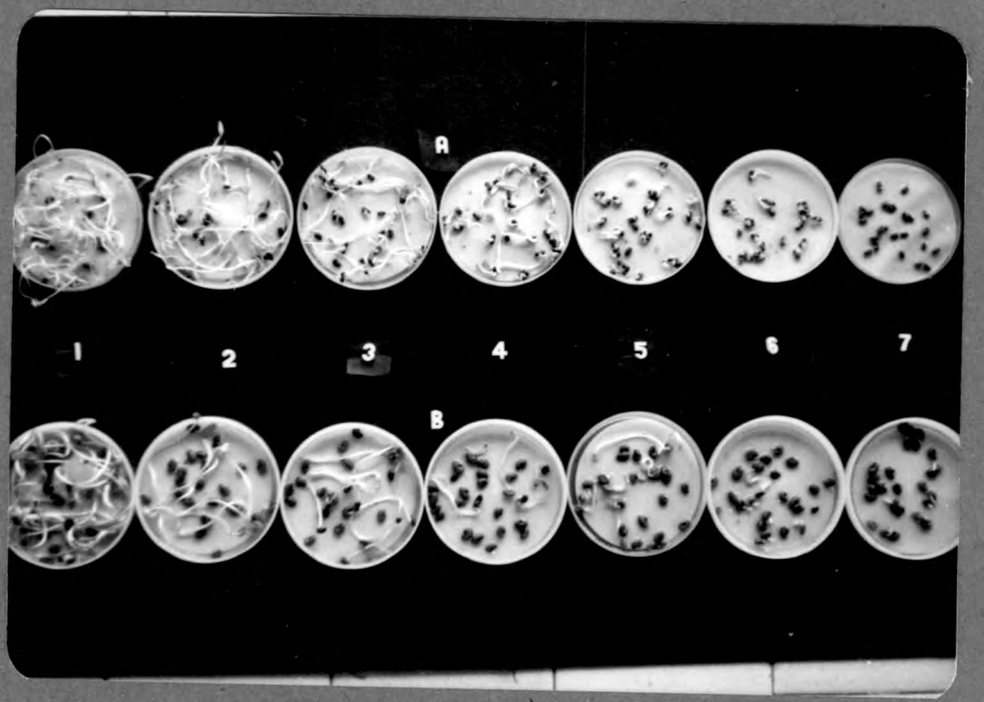


FIG: 3-a

FIG: 3-b

In countries like South-Western U.S.A., it is grown for pasture fooder and green manure. The green vine provides a palatable and nutritious pasture and hay for dairy cattle. Because of its mat-like spreading growth habit, the crop constitutes an excellent means of controlling soil erosion. The data collected at the dry Farming Research Station, Solapur, Maharashtra, have indicated that 'Matki' (P.aconitifolius) is very efficient and ideal crop for checking soil erosion.

Genus Crotalaria :

It is a large genus comprising nearly 470 species scattered all over the world. About 80 species are found in India. C.juncea and some other species are important plants which have been tried as a source of fibre or as green manure. Many of the species are reported to be useful for fodder, e.g. C.alata, C.anagyroides, C.burhia, C.ferruginea, C.juncea, C.medicaginea, C.sericea and C.striata but some are toxic to cattle. A few of the species are medicinal. The roots of C.albida and C.trifoliastrum are used as purgative. The leaves of C.verrucosa are used for scabies and impetigo.

ii) Crotalaria juncea (L.) :

Crotalaria juncea (L) is commonly called 'sunn hemp'. It is probably a native of India but it has never been found in a

truely wild state. It is distributed in tropical and sub-tropical regions of the globe. It is mainly cultivated either for the fibre or as a green manure crop in rotation with grain or cash crops. This Asiatic species is second in importance to jute as a source of bast fibre. At present sunn hemp is grown as a commercial fibre crop in India and to a lesser extent in Sri Lanka. India grows about 325 ha of sunn hemp per year with an annual production of 80000 - 100000 t of which 20 to 30 percent is exported mainly to the United Kingdom, Belgium and United States. Though grown in nearly all parts of India, Uttar Pradesh alone contributes about 40 percent of the total output. It is also cultivated in Bihar, Andhra Pradesh, Tamil Nadu and Madhya Pradesh.

Crotalaria juncea is an annual erect herb (Fig.1) about 1-3 m in height with a strong tap root system. Root nodules are freely produced and are much branched and lobed. All the vegetative parts of the plant are covered with short downy hairs. The leaves are simple, small, subsessile, lanceolate, stipulate and alternately placed. Petiole is slender. The flowers are small, bright yellow borne in terminal or axillary racemes of 6 to 10 flowers. The fruits are inflated hairy pods with pointed beak and grooved along the upper surface. The pods are tough skinned about 1 to 1.5 inch long and contain 10-15

10 - 15 kidney shaped dark grey to black seeds that become loose at maturity and produce a rattling sound.

The sunn hemp grows well in tropical and subtropical climates. It is a hardy and drought resistant crop, growing on poor soils. For fibres, light loamy and well drained soils are preferred. Waterlogging is harmful. The crop needs a moderate but well distributed rainfall of at least 50-75 cm during the growing phase. It is cultivated mostly as a 'kharif' crop i.e. sown in June and harvested before October. In some places it is sown as a 'rabi' crop in September or October and harvested in February or March.

Crotalaria juncea is mainly cultivated for its fibres as a green manure. For fibre production, the crop is sown thickly on a well prepared soil. No further cultural practices are needed after sowing. The plants are usually cut or pulled at the pod formation stage, generally after three to three and a half months. The fibres are obtained from stems after subjecting the retting process. The average yields are about 300 to 900 Kg of dry fibres per hectare and 8.0 percent of the weight of the dried stem. The dried fibres are then twisted and sometimes folded and made into bundles or hanks, before being marketed.

The quality of fibres required for various articles is judged by its length, fineness, colour uniformity and the extent of associated extraneous matter. Sunn hemp is a bast fibre very light in colour ranging from white or grey to yellow and fibre strands are generally 1.2 to 1.5 m long, lustrous and fairly resistant to microaganisms and moisture. The individual fibre cells are cylindrical or oval and chiefly composed of cellulose with a a layer of lignin around each fibre cell. The bast fibres having good pulping characteristics can be used in manufacturing papers. The high cellulose (60-80%) and low ash contents have made the fibres more suitable for high grade tissue paper and cigaratte papers and other high quality papers.

Sunn hemp is essentially a cordage fibre and is used in the manufacture of ropes, twiners, cords and marine cordage. It is also used in the manufacture of sail-cloth, canvas, matting, sacking and rope and soles of shoes and sandals, etc. In ship building the fibres are employed for closing the seams (Marine oakum) and for similar purposes. The fibres being resistant to deterioration in water, sunn hemp is suitable for making fishing nets. Attempts are being made to improve its fibres - the promising commercial product of textile.

As a green manure, it is excellent, as it provides abundance of root nodules. The nitrogen fixed by legume is

available to a large extent to the succeeding crop. Hence inclusion of a legume in general or C.juncea as green manure in particular will save use of chemical nitrogen fertilizers. By employing C.juncea as green manure nitrogen economy can be achieved, (Bharadwaj et al., 1981). Good crop of Sunn hemp yields about 18,000 to 28,000 lb of organic matter per acre, when compared with the incorporation of farm yard manure, and hence beneficial. For green manure, the crop is ploughed in after two to two and half months when the plants begin to flower. The land development practices including ploughing plus levelling followed by summer fallowing and green manuring with C.juncea almost completely eliminate the nematodes (Lal et al., 1983). Green manuring with C.juncea has better effect on the levels of exchangeable 'Ca' and water (Bavaskar and Zende, 1973). Its organic economic and marketing prospects have been studied by Wood and Angus (1974). Although there is a great demand for sunn hemp from outside markets, there has been no marked expansion in the area under its cultivation.

The preliminary studies with the above legumes, indicated that the species respond differently to the salinity conditions, particularly during germination. P.aconitifolius showed a wide range of salinity tolerance as compared to that

shown by C. juncea. It was thought worthwhile, therefore, to examine some of the biochemical and physiological events in these two species for the mechanism of salt tolerance during germination.