INTRODUCTION

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Botanists and agriculturists, from age long observations on natural plant populations and on behaviour of agricultural crops, have learned to estimate the range of change in the external environmental conditions that a certain plant, or a population of plants, can cope with. These conditions are broadly defined as "normal". It is expected that under these conditions a plant will be able to germinate, grow and reach a reasonable size, and be able to flower and produce fruits and seeds. If however, something happens contrary to expectations e.g. germination is delayed, the stand is uneven, growth is stunted, or there is little or no yield, something is considered to be wrong in the environment and the plants are considered to be under "stress" (Poljakoff-Mayber, 1982). Stress apparently interferes with the normal biochemical and physiological processes in the seed or in the plant, so that some abnormality develops which expresses itself externally as noted above (Levitt, 1972).

Development of conditions of stress in nature is a normal event which occurs periodically; heat waves, drought, mineral deficiency, disease epidemics and salinity in the soil have been known to man for many centuries.

In India about 7 million hectares (ha) of land is estimated to be afflicted by the problem of soil salinity or alkalinity mainly in the Indo-Gangetic plains, arid areas of Rajasthan and Gujarat, black cotton soil region and coastal tracts. The area under this problem has been increasing in extent greatly with expansion of irrigation networks. If proper soil and water management practices along with introduction of proper irrigation are not adopted, the problem of soil salinity is likely to increase day-by-day (Yadav, 1981). Approximately 3,04,582 ha of land in Gujarat lie under uncultivable conditions due to salinity. In modern times the development of medical sciences and social care have resulted in a tremendous growth of population, accompanied by problem of food supply. With the development of soil science and the extended use of modern agricultural technology, man must meet the challange of increasing food production and of using for agriculture, areas and sources of water formerly considered unsuitable for the purpose. Much has been achieved due to the development of various agrotechnical practices. Attempts are also being made to develop salt tolerant varieties of various crops. To succeed in this it is necessary to understand what is the actual damage caused to plants by salinity and what are the mechanisms by which individual plants, certain cultivars or plant communities withstand the damage of salinity and develop normally. These problems are nowadays under thorough investigation from both the agricultural and physiological points of view. As sodium chloride is the most common salt present in saline soils, most research on salinity has been done using NaCl. Both sodium and chloride are readily taken up by the root system of both halophytes and glycophytes. In most of the halophytes the absorbed sodium is transported to the leaves while in many glycophytes it is retained in the roots or in the stems (Jacoby, 1964, 1965).

The problem of soil salinity and the work on salt-induced damage as well as the responses of plants to saline environment have been reviewed from many standpoints, these are by Bernstein and Hayward (1958), Nieman (1962), Strogonov (1962), Rains (1972), Waisal (1972), Poljakoff-Mayber and Gale (1975), Jennings (1976), Flowers _et_ _al_. (1977), Ungar (1978), Greenway and Munns (1980) and Poljakoff-Mayber (1982). Bernstein and Hayward (1958) discussed the varying tolerance of different plant species and the effect of salt on the water balance of the plant. Strogonov (1962) has reviewed the research carried out on the effect of salinity on plants, mainly in the U.S.S.R. for the period 1920-1960. He emphasized the importance of combined anatomical and physiological studies for a better understanding of effect of salinity on plants. Bernstein and Hayward (1958) and Asana and Kale (1965) observed the adverse effects of salinity on the plant first expressed during the germination phenophase. A reduction in germination under saline condition was observed in soybean by Abel and Mackenzie (1964), sunflower by Karami (1974) and wheat by Singh and Dastane (1970). Delay and decrease in germination ~~was~~ were observed in jowar, mung and tobacco by Maliwal (1967) and wheat and barley by Maliwal and Paliwal (1967). Kaddah (1963) found both a delay in germination of rice seeds by salt and that young rice seedlings were highly sensitive to salt. Pearson _et al_. (1966) studied the relative salt tolerance of rice during germination and early seedling development, and noted that rice is least tolerant to salinity during seedling stage, and that all varieties are not equally salt tolerant. Varietal tolerance of germinating paddy seeds to different salt concentrations was also studied by Rao _et al_. (1973).

Salinity is known to affect many aspects of the metabolism of plants and to induce changes in their anatomy and morphology. These changes are often considered to be adaptations which increase the chances of the plant to endure the stress imposed by salinity; alternately, they may be considered to be signs of damage and disruption of the normal equilibrium of life processes (Poljakoff-Mayber, 1975). Salinity has been shown to affect the time and rate of germination, the size of plants, branching and leaf size, and overall plant anatomy (Poljakoff-Mayber, 1975). Morphologically most typical symptom of saline injury to the plant is stunted growth (Nieman, 1962). Solov'ev (1969) studied the effect of NaCl salinization on the growth of pumpkin in relation to water stress and mineral element supply, and found that the main cause of growth inhibition was due to the difficulty in mineral uptake by the shoots, due to competition with the salinizing ions absorbed by the plants. Waisal (1972) reviewed the various effects of salinity on plants, including the morphological and anatomical changes occurring in response to salinity, or typical for halophytes. Salt tolerance in the halophyte Suaeda maritima and the effect of salinity has been studied by Yeo and Flowers (1980). Abnormal germination of seeds and growth of seedlings of jowar were observed under the influence of NaCl and Na_2CO_3 by Lall and Sakhare (1970). Ungar (1978) concluded that all vascular plant species investigated display both delay in time of germination and reduction in the total number of seeds germinated as salt stress is increased beyond the optimal levels for seed germination. This delay in germination and final reduction in total number of seeds germinated may occur in salt concentrations as low as 0.1% NaCl for the least tolerant species or not until concentrations above 1.0% NaCl are reached in the case of halophytes. Korkor and Abdel (1974) studied effects of total salinity as well as the specific ion NaCl, $CaCl_2$ and Na_2SO_4 on the yield of rice crop and found that increase in salinity depressed both vegetative growth and grain yield of rice. The growth and yield of rice depressed steadily with increase in concentration of salt and osmotic pressure (Williams, 1960). The reduction in the rate of swelling of seeds and formation of shoots in wheat as a result of an increase in the osmotic potential have been observed by Udovenko and Alekseeva (1973). They also observed inhibition of growth of seedlings during further salinization, which was probably due to the accumulation of salt in the seedlings. Greenway (1973) is of opinion that the energy expenditure during osmotic adjustment to salinity, is one of the main factors in reducing growth. The effects of NaCl on higher plants have been reviewed by Jennings (1976). Recently Poljakoff-Mayber (1982) has published an article on the biochemical and physiological responses of higher plants to salinity stress. Salinity may induce an internal imbalance between various nutritionally obligatory ions as manifested by deficiency signs. Metabolically these effects may be considered as toxic in nature.

The recognition of the importance of turgor for extension of growth resulted in a thorough investigation of osmotic adjustment of plants under salinity and water stress (Flowers et al., 1977; Hellebust, 1977 and Greenway and Munns, 1980). Any reduction in turgor due to salinity, even if temporary is liable to induce growth inhibition (Hoffman et al., 1980). The water potential of leaves at zero turgor differs in plants differing in tolerance (Johnson, 1978) as does the change of growth extension per unit in turgor (Bunce, 1977). The degree of osmotic adjustment and the rate of growth were functions of the proportion of the root system exposed to saline conditions (Kirham *et al.*, 1969). Osmotic adaptation allows, therefore, maintenance of turgor so that a decrease in the water potential of the tissue is not necessarily accompanied by a decrease in the relative water content (Lawlor, 1969; Mayer and Boyer, 1972; Acevedo *et al.*, 1980). The transport of the absorbed ions is also of great importance. This is important for the osmotic adaptation of the shoot (Greenway and Munns, 1980) but it is apparently equally important for regulation of the ionic content in the successive layers of a tissue such as the root cortex. In addition to osmoregulation and transport the Na/Ca ratio is regarded by Hyder and Greenway (1965) and Greenway and Munns (1980) as being of great importance. This ratio is apparently an important factor in regulating membrane permeability and thus ion uptake, translocation and compartmentation. Osmotic adaptation under salinity is usually achieved by ion uptake from the external solution or by internal synthesis of organic solutes. It is usually the halophytes which maintain the gradient for water uptake by ion accumulation (Flowers *et al.*, 1977 and Erdei and Kuiper, 1979). The inability of some glycophytes to adapt osmotically under saline conditions is sometimes ascribed to their inability to accumulate enough ions from the external surroundings (Greenway and Munns, 1980). A high concentration of sodium and potassium strongly inhibits most enzymatic reactions (Greenway, 1973). However, as enzymatic reactions do continue in the cytoplasm, it was hypothesized that compartmentation of ions inside the cell will prevent the exposure of the enzymes to their toxic effect. According to Jeschke and Stelter (1976), most of the sodium is apparently located in the vacuole, which constitutes over 80% of the cell volume, while potassium is more abundant in the cytoplasm. However, osmoregulation in the cytoplasm is believed to be achieved mainly by increase in the concentration of organic solutes, especially proline and glycinebetaine (Stewart and Lee, 1974; Chu et al., 1976 and Flowers and Hall, 1978).

Salinity diverts the metabolism, at least of exogenously supplied glucose from the usual glycolytic pathway to pentose phosphate pathway (Poljakoff-Mayber, 1982). This was considered as salinity damage. Some investigators believe that levels of several key enzymes are lowered in salt damaged plants than their control plants (Hasson-Porath and Poljakoff-Mayber, 1969; Khan and Begum, 1972; Plaut, 1974). Others have reported either increase (El-Fouly and Jung, 1972; Arad and Richmond, 1973) or no differences in activity of enzymes (Greenway and Osmond, 1972 and Weimberg, 1970 and 1975). Salinity caused inhibition of RNase activity in cotyledons and roots; but increase in embryo axis and leaves of mung beans (Sheoran and Garg, 1978). The stimulatory effect of NaCl on ATPase of ~~*Beta* and~~ *Salicornia* has been reported by Flowers (1972). Nieman (1962) measured O_2 consumption of leaf section of twelve crops, and reported stimulation effects of NaCl. Boyer (1965) measured CO_2 evolution by infrared gas analysis and found that whenever cotton plants were exposed to NaCl, respiration always decreased. Hasson-Porath and Poljakoff-Mayber (1964) found progressive inhibition of respiration by increasing salt concentration. The pentose phosphate pathway enzymes were found more sensitive than glycolytic enzymes to salinity and most of them were stimulated by exposure of roots to salinity (Poljakoff-Mayber, 1982).

Salinity has been shown to interfere with the uptake of inorganic ^{15}N into young barley plants (Hellal *et al.*, 1975), whereas the incorporation of ^{15}N into protein was not affected or was even stimulated. On the other hand, salinity was shown to inhibit the uptake of externally supplied amino acids and their incorporation into protein (Kahane and Poljakoff-Mayber, 1968).

Phytohormones are involved in the response of plants to salinity stress (Gale, 1975). The content of gibberellins, IAA and ethylene changes on exposure to salinity but the most marked changes occur in the content of two hormones - cytokinin and ABA (Mizrahi et al., 1970, 1971). Cytokinins are synthesized in the roots and are transported upwards. Their content decreases in the xylem exudate as the level of external salinity increases. ABA is synthesized, apparently, in the leaves and is transported downwards. ABA content, especially of the shoot, rises very quickly on exposure to salinity. There may be gradation of cytokinin/ABA ratios along the plant axis that changes under effect of salinity, but there are no experimental data which would indicate the importance of such a change. Under salinity, when adaptation takes place, the osmotic potential of the tissue is lowered and ABA content remains high even when the gradient for water influx is restored (Wright, 1978). Ungar and Boucaud (1975) and Boucaud and Ungar (1976) found that the NaCl concentration of 0.85 M strongly reduced the endogenous cytokinin concentration of Suaeda. Hasson and Poljakoff-Mayber (1980) found that in cotyledons of pea germinated at 192 mM NaCl, free ABA accumulated to much higher concentration than the bound form. In the cotyledons of control seeds germinated in Hoagland's solution, the situation was reversed while the total content was much lower than in cotyledons exposed to salinity. In such peas, germinated in 192 mM NaCl, the increase in ABA content of the cotyledons was accompanied by increased osmolarity and increase in proline content.

In order to avoid stress during germination, we have to time the sowing of crops in order to avoid inclement conditions; we can treat the seeds with chemicals to widen the range of field conditions under which they can germinate successfully (Heydecker, 1973). It has long been known that the Ca^{++} antagonizes the injurious effects of Na^{+}. Wheat grains pretreated with water germinated only to the extent of 8% at 1% NaCl while grains pretreated with 1% $CaCl_2$ showed 90% germination (Chaudhuri and Wiebe, 1968). The pretreatment of seeds with calcium resulted in a 25% reduction in their ^{22}Na uptake. Bean plants (Phaseolus vulgaris) suffered no damage upto one week at 50 mM NaCl, if the Ca concentration was 1 mM higher (La Haye and Epstein, 1969). Ali Qadar et al. (1980) found that the treatment of rice seeds with $Ca(NO_3)_2$, $CaCl_2$, KCl, KNO_3 and ascorbic acid significantly increased the yield.

Succinic acid has been shown to affect growth and development of plants (Artemova, 1968 and Maurina et al., 1969). Cereal seeds treated with succinic acid showed enhanced rate of germination (Blagoveshchenshii and Rakhmanov, 1964; Gertsuskii, 1959 and Maurina et al., 1969). An increase in dry matter production under the influence of succinic acid has been observed in potato (Shevchenko, 1967) and corn (Koroleva, 1964).

Accumulation of proline following salt stress has been reported (Chu et al., 1976 b; Storey and Jones, 1977 and

Goas, 1965 and 1967). The balance between soluble amino acids and protein is changed by salinization (Strogonov, 1962) and is reminiscent of the events occurring during drought (Vaadia et al., 1961). Treatment of rice seeds with 0.02% proline increased their germination under saline conditions (Bal, 1976).

Treatment of seeds with polyethylene glycol (PEG) improved the germination of seeds under stress conditions (Heydecker, 1973).

Growth hormones have been reported to protect plants against salt injury. Interrelationships between exogenous growth regulator treatments and alleviation of dormancy induced by salt stress have been demonstrated by several researchers with both glycophytes and halophytes (Ungar, 1978). Soaking of wheat seeds in IAA overcame the depression of root growth by a solution of Na_2SO_4 (Sarin, 1962). A cent per cent increase in yield was observed when wheat plants growing in saline soil were given foliar spray with 5 ppm IAA (Sarin and Rao, 1961). Kinetin is reported to promote the uptake and incorporation of amino acids into proteins in NaCl stressed tissue (Kahane and Poljakoff-Mayber, 1968), although inhibited the same in non-stressed and Na_2SO_4 stressed pea roots. Ramesh Babu and Sunil Kumar (1979) have reported that kinetin and benzyl-adenine were effective in promoting wheat seed germination and seedling survival under osmotically stressed condition by soil salt solution upto 3.5%. They have also found the promoting effect of kinetin and GA_3 in Cicer, Cajanus and Phaseolus species.

Gibberellins have been successfully employed in overcoming growth suppression in plants resulting from diverse factors. Khan and Tao (1977) reported that gibberellin was able to overcome an osmotic inhibition in the germination of lettuce seeds. Nieman and Bernstein (1959) reported the interactive effects of GA_3 and salinity in increasing stem growth of beans. However, Levitt (1972) reported that GA_3 is not generally able to overcome salt stress induced retardation of growth.

The stimulatory effect of applied GA_3 on the germination of both dormant and non-dormant seeds has been widely reported (Lang, 1965; Stokes, 1965 and Villiers, 1972). In recent years, GAs have been implicated in several biochemical reactions and shown to play an important role in physiological processes of diverse nature. These include breaking of dormancy, regulation of germination process, control of synthesis and release of various hydrolysing enzymes, etc.

Application of GA stimulates protein synthesis in shoot and root meristems and in the developing vascular tissues of charlock seeds (Edwards, 1976). Increase in total RNA content and enhanced DNA-dependent nuclear RNA synthesis by GA_3 in isolated pea and sugar beet nuclei have been reported by

Jacobsen (1977). Jarvis et al. (1968 b) reported that GA treatment enhanced the DNA availability for transcription in hazel seeds. GA_3 stimulates de novo synthesis and release of hydrolyzing enzymes including α-amylase, ribonuclease and protease in aleurones (Jones, 1973) and other enzymes involved in intermediary metabolism in cotyledons. GA_3 is known to regulate the hydrolysis of stored food materials and the mobilization during germination of seeds. Ho and Varner (1974) showed that mRNA specific for α-amylase was relatively stable and was synthesized in response to GA_3. Inhibition of GA -enhanced synthesis of α-amylase in barley aleurone by ABA was observed by Chrispeels and Varner (1967 b). The hormonal role of GA in biogenesis of α-amylase and its isozymes have been reviewed by Machaiah and Vakil (1979). GA_3 has also been shown to induce de novo synthesis and release of laminarinase (β-1,3 glucanase), α-glucosidase and diastase activities in germinating barley (Pollard and Nelson, 1971). Induction of lipase (Black and Altschul, 1965) and invertase (Edelman and Hall, 1964) by GA_3 has been reported. Enhanced activities of amylases, invertase and isocitratase during germination of cotton under the influence of GA_3 have been reported by Prathapasenan (1971). Influence of GA_3 on the activities of β-amylase, β-1,6,dextranase, maltase, invertase and glucosidase has been reported by various authors (Briggs, 1963;

Curtis and Cantlon, 1965 and Paleg, 1960 a and b). Studies on the early responses of aleurone layers to GA_3 have shown that α-amylase is not the only enzyme to be secreted. Within 5 hours of the addition of GA to isolated aleurone layers of barley there is increased secretion into the surrounding medium of several hydrolytic enzymes, including ATPase, phytase, β-glucosidase and phosphomonoesterase (Mann, 1975). Stimulation of phytase in barley by GA_3 has been reported by Srivastava (1964) and Kathju <u>et al</u>. (1971). Increased activities of phytase and ATPase during germination of cotton seeds under the influence of GA_3 have been observed by Dave (1976). GA_3 stimulates extensive proliferation of cellular membranes during early germination (Evins, 1971). GA_3 is shown to enhance the synthesis of marker enzymes of glyoxysomal and mitochondrial membranes in germinating almond seeds (Hawker and Bungey, 1976). Recently, studies with dwarf maize mutant by electrone microprobe analysis, have indicated that GA_3 selectively changes the permeability of aleurone plasmalemma and thereby regulates the influx of monovalent (Na^+, K^+, Cl^-) and divalent (Mg^{++}) ions (Neumann and Janossy, 1977). Thus, GA_3 alters the micro-environment in different cell compartments. It has been found that tissue whose growth was augmented by gibberellin treatment also manifested an increase in respiratory rate (Kato, 1956; Weller <u>et</u> <u>al</u>., 1957; Halevy, 1964). Increased rate of respiration of cotton seedlings treated with GA_3 has also been reported by Dave (1976). Thus, GA_3 regulates a number of vastly different rate-limiting steps in plant metabolism.

GA has been successfully employed in overcoming growth suppression in plants resulting from such diverse factors as genetic deficiencies, virus infection or restrictive light and temperature conditions. Khan et al. (1957) have reported that GA was able to overcome an osmotic inhibition in the germination of lettuce seed. GA_3 was found to overcome salt stress induced dormancy in several taxa of Spergularia, Suaeda and Salicornia (Ungar and Binet, 1975; Boucaud and Ungar, 1976 and Ungar, 1977). Kawasaki and Takada (1978) have reported that GA and NaCl sinergistically enhanced hypocotyl elongation and kinetin and IAA were found to be ineffective in promoting hypocotyl elongation. Studies with Hordeum jabatum by Benner also indicate that GA_3 is stimulatory to germination under salinity stress, and that GA_3 apparently alleviates a portion of the dormancy induced by low water potential. GA_3 overcomes the inhibitory effects of high osmotic pressure (e.g. rice and lettuce seeds), of high salt concentration and of water imposed dormancy (e.g. in barley grains) (Thomas, 1973). The stimulation activity of various hydrolases from endosperms of paddy seeds treated with GA_3 under the toxic level of salt has been reported by Subhash (1981).

In agriculture, ready germination of seeds and the establishment of healthy seedlings are two most important features as the final yield producing value much depends on the said processes. Poor yield of crops under saline condition is often due to unsatisfactory stand of crop resulting from poor germination of seeds. Paddy, (Oryza sativa L.) variety GR-3 was found to be sensitive to salt as evidenced by a reduction in percentage of germination of seeds and growth of seedlings. The present studies were, therefore, taken up with a view to find~~ing~~ out various factors responsible for the failure of paddy seeds to germinate under saline condition and also to evolve some chemical treatments which would render the seeds readily germinable under the said condition .

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