DISCUSSION

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Sodium chloride at 0.05, 0.075 and 0.1 M concentrations reduced only the rate of emergence of seeds. However, salt at 0.15 and 0.2 M concentrations brought about a significant reduction in the rate of emergence as well as the final percentage of germination of seeds. Thus, a delay and decrease in germination was observed with increasing concentrations of salt (Table 1). Similar effects of salinity have been observed in soybean (Abel and Mackenzie, 1964), sunflower (Karami, 1974), niger (Kachapur and Badanur, 1977), arhar and cowpea (Paliwal and Maliwal, 1973), peas (Kaur and Gupta, 1970), jowar and bajra (Abichandani and Bhatt, 1965), jowar, mung and tobacco (Maliwal, 1967), wheat and barley (Maliwal and Paliwal, 1967), wheat (Asana and Kale, 1965; Sarin and Narayanan, 1968) and rice (Kaddah, 1963; Pearson _et_ _al_., 1966; Dixit and Lal, 1971; Sanzo and Chibas, 1978 and Subhash, 1981). According to Novikov, the failure of seeds to germinate in saline solutions results from the fact that the osmotic pressure of the solution is higher than that of the cells and therefore, the seeds cannot absorb all the water required for their germination. Sergeev (1936) demonstrated a toxic effect of salt on germinating seeds and claimed that the effect results from the disturbances of enzymatic reaction and form a depression of growth. According to Sergeev therefore, the effect of salt on germination differs from that on imbibition. According to Long (1943) salt affects plants in two independent ways. Firstly, the high osmotic concentration of the substrate disturbs the water balance of the plants and secondly, the salts have a direct toxic effect on the protoplasm.

Salt at 0.1 M and above concentrations brought about a significant reduction in the growth of root system. Shoot system exhibited retardation of growth at all concentrations of salt tried. In general, the growth of root and shoot systems decreased progressively with increasing concentrations of salt (Table 2). Reduction in growth by higher concentrations of salt has been reported in tomato (Hayward and Long, 1943), beans (Lunin et al., 1961; Nieman and Bernstein, 1959 and Meiri and Poljakoff-Mayber, 1970), peas (Kaur and Gupta, 1970; Uprety and Sarin, 1973), arhar and cowpea (Paliwal and Maliwal, 1973), soybean (Abel and Mackenzie, 1974), cowpea and mung bean (Balsubramanian and Sinha, 1976), wheat (Bhardwaj and Rao, 1960) and rice (Williams, 1960; Kaddah, 1963; Pearson et al., 1966; Korkor and Abdel, 1974 and Subhash, 1981). Stunted growth of plants under high salinity level has also been reported by Strogonov (1962), Poljakoff-Mayber and Gale

(1975) and Joshi (1976). Solov'ev (1969) studied the effect of NaCl salinization on growth of pumpkin and noticed 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. Greenway and Munns (1980) are of opinion that reduction in growth by salinity is either by 'water deficit' or 'ion excess'. Reduction in growth of embryo axis of mung bean (Sheoran and Garg, 1978) and radish (Ramana and Ram Das, 1978) under salinity has been attributed to the inhibition of hydrolysis of reserve food materials from the cotyledons. It is believed that phytohormones are involved in the response of plants to salinity stress (Poljakoff-Mayber, 1982). An increase in the content of ABA and decrease in the endogenous level of cytokinin under salinity stress, resulting in inhibition of growth has been observed by Mizrahi et al. (1971). Inhibition of water uptake due to accumulation of ABA in the cotyledons of pea seeds when exposed to salinity and its transportation to the embryo epicotyl has been reported by Hasson and Poljakoff-Mayber (1980/'81). Thus, reduction in growth under the influence of salt may be either due to water deficit or ion excess or hormonal imbalance.

Effect of various chemicals on germination of seeds and growth of seedlings under the influence of salt

$CaCl_2$

Among different concentrations of calcium chloride tried, only at 0.1 M level, it could increase the percentage of germination of seeds under the influence of salt (Table 3). Maximum stimulation of growth of root and shoot systems under the influence of salt was also brought about by 0.1 M $CaCl_2$ (Table 4). Stimulation of germination of wheat seeds under saline condition by $CaCl_2$ has been reported by Chaudhuri and Wiebe (1968). A 25% reduction in the uptake of $^{22}Na^+$ by tissues pretreated with Ca^{++} has been shown by Levitt (1972). Ca^{++} ions have also been shown to decrease the increase in membrane permeability induced by Na^+ ions (Scarth and Lloyd, 1930). Thus, the stimulation of germination and growth of seedlings induced by Ca^{++} under the influence of salt may be due to a reduction in the uptake of Na^+.

Succinic acid

Succinic acid tried at all concentrations significantly improved the germination of seeds under saline condition. The maximum stimulation of germination under the influence of salt was brought about by 5 mg/l succinic acid

(Table 5). Maximum stimulation of growth of root and shoot system under saline condition was brought about by succinic acid at 10 and 25 mg/l concentrations respectively (Table 6). Promotary effect of succinic acid on growth was more pronounced on the root system than the shoot system. As in the case of germination maximum stimulation of growth of root system was observed under the influence of 5 mg/l succinic acid. Stimulation of germination of seeds by succinic acid has been reported in cereals (Drozdov and Babuk, 1968), barley and lupin (Siuliauskas, 1967) and corn (Korolova, 1964; Maurina et al., 1969 and Gertsuskii, 1959) and cotton (Blagoveshchenshii and Rakhmanov, 1964). Succinic acid has also been shown to enhance the growth of cucumber (Artemova, 1968) and maize (Gertsuskii, 1959) and cereals (Drozdov and Babuk, 1968). Stimulation of germination of seeds and growth of seedlings of rice by succinic acid under saline condition has been reported by Subhash (1981).

Proline

Proline tried at all concentrations significantly enhanced the germination of seeds under the influence of salt. Maximum stimulation of germination of seeds and growth of root and shoot systems under the influence of salt was brought about by 0.02% proline (Tables 7 and 8). Stimulation of germination by proline has been reported in rice (Bal, 1976 and Subhash, 1981). Accumulation of proline in plants under stress condition has been reported by Chu et al. (1974) and Storey and Jones (1977). Accumulation of osmoticum like proline or glycinebetaine has been shown in plants during their adaptation to decreasing external water potential or atmospheric drought (Poljakoff-Mayber, 1982). A reduced rate of proline oxidation has been accounted for the accumulation of proline under stress condition (Stewart and Lee, 1974). Thus, proline lowers the internal water potential. Stimulation of germination of seeds and growth of seedlings by proline under saline condition may be due to increased uptake of water.

Polyethylene glycol (PEG, 6000)

Presowing soaking of seeds in PEG, 6000 for 12, 24 and 48 hours significantly increased the final percentage of germination of seeds under the influence of salt (Table 9). Among different durations of soaking tried maximum stimulation of germination was observed when seeds were given a presowing soaking treatment in PEG for a period of 24 hours. The final percentage of germination of seeds under normal condition was not significantly affected by PEG. However, soaking of seeds in PEG for 24 and 48 hours significantly enhanced the rate of emergence of seeds at the end of 48 hours of germination. Acceleration of germination of seeds by PEG under moisture stress has been reported by Khan (1977). The increased rate of germination of seeds observed under the influence of PEG in the present studies confirms results of Heydecker et al. (1973). It is highly interesting to note that PEG significantly enhanced the growth especially under saline condition (Table 10).

Kinetin

Kinetin tried at all concentrations significantly increased the rate of emergence and final percentage of germination of seeds under saline condition. Maximum stimulation of germination of seeds under the influence of salt was brought about by kinetin at a concentration of 20 mg/l (Table 11). As in the case of germination, the growth of root and shoot systems of seedlings under the influence of salt was also stimulated by kinetin at all concentrations tried (Table 12). However, significant increase in growth as a result of kinetin treatment could be observed only in the case of shoot system. Maximum stimulation of growth of shoot system under the influence of salt was brought about by 20 mg/l kinetin. Kinetin has been shown to alleviate the osmotically induced dormancy caused by NaCl in *Lycopersicum esculentum*, *Hordeum vulgare* and *Gossypium hirsutum* (Bozuck, 1981). Itai *et al*. (1968) observed a reduction in the level of cytokinins under conditions of moisture stress in sunflower, bean and tobacco. This suggests that one reason for metabolic disturbances at the cellular level under saline conditions may be due to the suboptimal level of cytokinins and the exogenously supplied kinetin may be helping the tissues in maintaining their optimum level. Kinetin is also reported to promote the uptake and incorporation of amino acids into proteins in NaCl-stressed tissue (Kahane and Poljakoff-Mayber, 1968). Thus, the stimulation of germination of seeds and growth of seedlings brought about by kinetin under saline condition may be due to the increased rate of synthesis of proteins.

GA_3

GA_3 tried at all concentrations significantly enhanced the rate of emergence and the final percentage of germination of seeds under the influence of salt (Table 13). Treatment of seeds with 10 and 15 mg/l GA_3 resulted in almost a 4-fold increase in the final percentage of germination of seeds under the influence of salt as compared to that of the control (salt alone). GA_3 has been shown to increase the germination of seeds of *Spergularia media* under saline conditions (Ungar and Binnet , 1975). Boucoud and Ungar (1976) have reported the alleviation of dormancy induced by osmotic stress in seeds by GA_3. Studies with *Hordeum jabatum* by Benner also indicate that GA_3 is stimulatory to germination under salinity stress. Similar results have also been observed in rice seed germination by Subhash (1981). The growth of root and shoot systems under the influence of salt was also enhanced by 10 mg/l GA_3 (Table 14). Promotion of growth by GA_3 under saline conditions has been reported in beans (Nieman and Bernstein, 1959) and pea (Uprety and Sarin, 1973). Since GA_3 was found capable of reversing the toxic effects of salt on germination of seeds and growth of seedlings to a great extent further studies were carried out to understand the mechanism by which GA_3 stimulates the germination of seeds and growth of seedlings under saline condition.

The decrease in the dry weight of endosperms observed during germination of seeds clearly suggests the mobilization of endosperm reserves to the growing axes. Only a slight reduction in the dry weight of endosperms could be observed during germination of seeds under the influence of salt as compared to that of the control (Table 15). This clearly shows the inhibition of mobilization of food materials from the endosperm to the growing axis by the salt. High concentrations of NaCl are known to inhibit the hydrolysis of stored food materials in the storage organs (Ramana and Ram Das, 1978; Sheoran and Garg, 1978 and Gururaj Rao *et al.*, 1980). Endosperms of seeds treated with 10 mg/l GA_3 showed a lesser dry weight under the influence of salt as compared to that of the control (salt alone). This may be due to the slightly enhanced mobilization of the food materials from the endosperm by GA_3. Endosperms of seeds treated with GA_3 alone showed a rapid loss in its dry weight as compared to the pure control. Enhanced rate of mobilization of food materials from cotyledons under the influence of GA_3 has also been reported in cotton (Dave, 1976), cucumber (Halvey <u>et</u> <u>al</u>., 1964), and rice (Hayashi, 1940) seeds during germination.

During germination of seeds, the starch content of endosperms decreased steadily (Fig. 1) with a parallel increase in the levels of soluble and reducing sugars (Figs. 2 and 3). Treatment of seeds with GA_3 markedly stimulated this process. NaCl at a concentration of 0.2 M adversely affected the depletion of starch during germination of seeds. The low levels of soluble and reducing sugars observed in the endosperms of seeds during germination under saline condition gives further support to the said observation. The decrease in the starch content of endosperm and the parallel increase in the levels of soluble and reducing sugars observed during germination are found due to increased activity of amylases, maltase and invertase (Figs. 8 to 12). The breakdown of the endosperm starch during germination of rice has been shown mainly due to an increase in the activity of α-amylase and maltase (Manners, 1974). However, in the present studies, the activity of β-amylase was found more than that of α-amylase. The increase in the activity of β-amylase observed during germination of seeds may be due to its *de novo* synthesis during the early phase of germination and the activation of the preformed enzyme associated with the starch granules of the endosperm during the later stages of germination (Okamoto and Akazava, 1980). A poor rate of depletion of starch and low levels of soluble and reducing sugars observed during germination of seeds under saline condition are due to highly reduced activity of amylases, maltase and invertase. The rapid rate of depletion of starch and the marked increase in the levels of soluble and reducing sugars observed during germination of GA$_3$-treated seeds have been found due to the stimulated activity of amylase, maltase and invertase. GA$_3$ enhanced the rate of depletion of starch along with an increase in the levels of soluble and reducing sugars during germination of seeds under saline condition by slightly stimulating the activity of amylases, maltase and invertase. A reduction in the activity of amylases as well as the content of reducing sugars under the influence of salt in the endosperms of wheat during germination has been reported by Sarin and Narayanan (1968). They found that treatment with GA$_3$ increased both amylase activity and reducing sugar concentration except seedling growth. A decrease in the activity of amylase during germination of seeds under the influence of salt and its enhancement by GA_3 has also been reported in wheat (Ansari et al., 1977). A rapid depletion of starch and the corresponding accumulation of reducing sugars in the treated seeds suggest an enhanced amylolytic activity as a result of GA_3 treatment (Paleg et al., 1962; Roswell and Goad, 1964 and Ingle and Hageman, 1965). A marked increase in the activity of maltase in endosperms of barley during germination has been reported under the influence of GA_3 (Briggs, 1963). Treatment of seeds with GA_3 resulting in an increase in the activity of invertase during germination of cotton has been reported (Prathapasenan, 1970).

Isozyme pattern of amylases

Plates I to V clearly show the changes in the isozyme pattern of α-amylase during germination of seeds. The extract of the endosperms of dry seeds on electrophoresis did not show the presence of any band with α-amylase activity. This clearly suggests that the enzyme is not present in the dry seed in the preformed condition. The increase in the activity of α-amylase observed during germination of seeds (Fig. 9) may be due to synthesis of increased number of isozymes of α-amylase de novo. The *de novo* synthesis of α-amylase during germination of barley has been reported by Jacobsen *et al*. (1970). The decreased activity of α-amylase observed during germination of seeds under the influence of salt is probably due to the synthesis of lesser number of isozymes as compared to that of control. Similar reduction in the number of isozymes of amylase under the influence of high salt concentrations has been reported in *Vigna radiata* by Sheoran (1980). The additional band of α-amylase (No. 7) observed at the end of 120 hours of germination on the gels of GA_3 and salt plus GA_3 treated seeds clearly indicates the ability of GA_3 to induce the isozymes of α-amylase. GA_3 has been shown to induce the isozymes of α-amylase in barley (Jacobsen *et al*., 1970).

The location of 2 bands with β-amylase activity on the gel following the electrophoresis of the extract of endosperms of dry seeds clearly suggests the presence of preformed enzyme (Plate VI). The increase in the activity of β-amylase observed during germination of seeds (Fig. 10) has been found due to the synthesis of increased number of isoenzymes (Plates VII to XI). The low activity of β-amylase observed during germination of seeds under the influence of salt has been found due to the inhibition of the synthesis of isozymes (bands - No. 7 and 10) by salt (Plates X and XI). Similar inhibition of isozyme production by salt has been shown in *Vigna radiata* by Sheoran (1980). It is interesting to note that treatment of seeds with GA_3 results in the induction of isozymes of β-amylase (bands - No. 5 and 8) during germination (Plates VII, IX and X). The inhibitory effect of salt on the isozyme production of β-amylase (band No. 7) has been found nullified by treating the seeds with GA_3 (Plate X). The increased activity of β-amylase observed at the end of 120 hours of germination may be due to the increase in the intensity of bands (Plate XI). The development of a number of bands of isozymes of β-amylase has been reported in rice by Matsui _et_ _al_. (1975).

The total nitrogen and protein contents of endosperms decreased steadily during germination of seeds (Figs. 5 and 4). A highly reduced rate of depletion of total nitrogen and protein was observed during germination of seeds under the influence of salt. However, treatment of seeds with GA_3 enhanced the rate of depletion of total nitrogen and protein during their germination under the influence of salt. Treatment of seeds with GA_3 alone resulted in a rapid depletion of total nitrogen and protein contents of endosperms during germination. The depletion of protein observed during germination has been found due to an increase in the activity of protease (Fig. 13). The low rate of depletion of protein observed during germination under the influence of salt has been found due to a highly reduced activity of protease as compared to control. A depletion of protein observed during germination of GA_3-treated seeds was due to a highly stimulated activity of protease. Inhibition of mobilization of proteins and reduced activity of protease in cotyledons of seeds of *Vigna sinensis* under the influence of salt have been reported by Prisco and Gustavo (1976). Salinity has been shown to reduce the activity of protease during germination of mung seeds (Sheoran and Garg, 1978). Enhanced mobilization of reserve nitrogen from the cotyledons of lettuce seeds to the growing axis under the influence of GA_3 has been reported by Rai and Laloraya (1965). GA_3 has been shown to stimulate the activity of protease during germination of cotton (Dave, 1976), maize (Harvey and Oaks, 1974) and paddy (Goyal and Baijal, 1979). An increase in protease activity during germination of seeds of *Medicago sativa* under the influence of GA_3 has been observed by Cohen *et al*. (1969). According to them the increase in protease activity during germination could be due to *de novo* synthesis of protease rather than its activation as GA_3 fails to activate the enzyme *in vitro*. Marcus (1971) suggested that a primary site of GA_3 action is in inducing proteolysis, thereby making different amino acids available for the synthesis of broad range of enzymes.

The decrease in the phytin content of endosperms observed during germination of seeds was due to its hydrolysis and is evidenced by the activity of phytase (Figs. 6 and 14). A very low activity of phytase was responsible for the low rate of depletion of phytin observed during germination of seeds under saline conditions. Stimulated activity of phytase by GA_3 led to the rapid depletion of phytin. Stimulated activity of phytase under the influence of GA_3 has been reported in seeds of cotton (Dave, 1976) and barley (Srivastava, 1964) during germination.

ATPase

The detection of the activity of ATPase in the endosperms of dry seeds clearly suggests the presence of the preformed enzyme. The enzyme activity increased during germination and registered its peak at the end of 24 and 96 hours (Fig. 15). The increase in the enzyme activity observed at the end of 24 hours of germination may be due to the activation of the preformed enzyme as a result of the hydration of tissue (Lai and Thompson, 1972). The increase in the enzyme activity during the later stages of germination (from 48 to 96 hours) may be due to its de novo synthesis (Brown et al., 1968, 1964 and Longo, 1968). The enhanced activity of the enzyme observed at the end of 24 hours of germination of seeds under saline condition may be due to activation of the preformed enzyme by hydration of tissues as well as by NaCl. Stimulation of the activity of ATPase by salinity has been reported in <u>Salicornia</u> by Flowers (1972 b). The low activity of ATPase observed under the influence of salt from 48 hours onwards may be due to inhibitory effect of ions. Inhibition of ATPase activity by salinity was observed in <u>Suaeda</u> (Flowers, 1972 a). GA_3 has been found to stimulate the activity of ATPase during germination of seeds under normal and saline conditions. Stimulation of ATPase by GA_3 has also been reported in cotton (Dave, 1976).

A steady increase in the rate of respiration was observed during germination of seeds (except initial 24 hours) (Fig. 16). The observed increase in the rate of respiration was found due to an increase in the activity of pyruvic-, α-ketoglutaric-, succinic- and glucose-6-phosphate dehydrogenases. The increased rate of respiration observed in GA_3-treated seeds has been found due to the enhanced activity of dehydrogenases. Under the influence of salt, seeds exhibited a marked increase in their rate of respiration at the end of 24 hours of germination as compared to that of control. This increase in respiration was found due to an increase in the activity of pyruvic-, α-ketoglutaric and succinic dehydrogenases. The glucose-6-phosphate dehydrogenase showed a reduction in its activity under the influence of salt at the end of 24 hours of germination as compared to that of the control. However, the O_2 uptake of seeds under the influence of salt decreased from 24 hours onwards. This decrease has been found due to a reduction in the activity of pyruvic-, α-ketoglutaric- and glucose-6-phosphate dehydrogenases. Similar pattern of respiration under the influence of salt has been reported in wheat and gram by Bhardwaj and Rao (1960). The increased rate of respiration of seeds under the influence of salt and GA_3 was found due to an increase in the activity of SDH till the end of 72 hours of germination as compared to the control (salt alone). When the activity of dehydrogenases of TCA cycle decreased during the later stage of germination, the glucose-6-phosphate dehydrogenase showed a sharp increase. This may be taken as an indication of a shift of the respiratory pathway from TCA cycle to pentose phosphate pathway. Inhibition of respiration and activity of dehydrogenase by salt has been reported in _Suaeda maritima_ and _Pisum sativum_ (Flowers, 1972). The inhibition of respiration by salinity has also been reported in wheat and gram (Sarin, 1961, Kalir and Poljakoff-Mayber, 1976), radish (Ramana and Ram Das, 1978) and nonresistant variety of rice (Aleshein and Krasnikova, 1978). Stimulation of respiration and the activity of dehydrogenases by GA_3 has been reported in cotton (Dave, 1976) and sorghum (Kamalavalli, 1970) respectively.

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