CHAPTER - I

Review of Literature ...

1. Salinity and Plant Metabolism :

The problem of salinity is becoming more and more severe in modern agriculture, because every year more and more land is becoming non-productive due to accumulation of salts. About 25% of the earth's surface (millions of hectares) is considered to be saline (Thorne and Peterson, 1954) to produce sufficient yields of crops. This problem is more serious particularly in arid and semiarid regions, because insufficient rainfall cannot transfer the salts from the soil level.

In Asia extensive saline lands are present (Thorne and Peterson, 1954). In India this problem is more severe because one third of the land is arid or semiarid and secondly the rainfall is scanty, seasonal and irregular. It is estimated that about 12 million hectares of land in our country has been affected by salinity and alkalinity (Sharma and Gupta, 1986). In Maharashtra State about 1.4 million hectares of the black cotton soil is saline besides the coastal saline soils.

Saline soils are those soils which have been adversely modified to interfere the growth and normal metabolic processes of a plant. Saline soils contain excessive concentration of soluble salts, principally NaCl, Na_2SO_4, Na_2CO_3 or Mg salts, amounting about more than 1%. The electrical conductivity of the saturation extract of such soil is 4 m S cm^{-1}. These

soils are neutral to pH, show good aeration and high permeability for water. The primary or naturally occuring saline soils are the result of the accumulation of weathering products which are not leached away due to insufficient rainfall. On the other hand secondary salinity results from human activity, due to improper management of irrigation, over application of fertilizers and unwise agricultural practices.

The principle causes of salinity are -

1) Evapotranspiration of pure water from irrigated soils and accumulation of dissolved salts in soils.
2) High salt concentration of rivers and streams because of returned drainage water.
3) Transfer and accumulation of dissolved salts to areas with inadequate drainage and
4) Coastal soils which receive salts from sea sprays.

In order to cope up with increasing demand of agricultural products, the utilization of saline soils is essential. Salt affected soils can be made productive by reclaimation and proper management. Plant rotation, tillage of soils, flushing and drainage of soils are principle means for reclaimation of saline soil. However, this requires special management practices and hence it is costly. Various researches have indicated that besides these means, development of crops tolerant to salinity is a strategy to meet this problem. It

is observed that some plants can grow satisfactorily with irrigation water containing 1% NaCl (Boyko, 1966). There is much more difference regarding salt tolerance capacity of the plants under natural conditions e.g. halophytes can grow well under saline conditions but glycophytes are unable to withstand saline conditions. Most of the crop plants are glycophytic in nature, but even some of them do possess some degree of salt tolerance capacity. The wild and cultivated species show wide variation in their salt tolerance capacity depending upon the soil structure, climate and their genetic make-up. Even physiological races are also desirable (Chapman, 1966). Hence crop selection is most important for reclaimation of saline or problem soils. Bernstein (1964), based on electrical conductivity of saturated soil extracts suggests that Bermuda grass, tall wheat-grass, barley, sugarbeet and cotton are tolerant; alfalfa, soybean, rice and tomato are medium tolerant while clover, bean and onion are sensitive. According to Strogonov (1964), maximum salt tolerance was exhibited by sugarbeet and that minimum by carrot. From the data collected by Maas and Hoffman (1977), it appears that each crop has a certain threshold for salinity, beyond which crop yield decreases linearly with increasing salinity. They examined the salt tolerance capacity of economically important species and found that barley, Bermuda grass, cotton and sugarbeet are tolerant, almond, apple, onion and beans are most sensitive while remaining species show intermediate tolerance.

The accumulated salts exert various harmful effects on the growth and development of plants, influencing even their existence. The changes include anatomical, morphological and metabolic changes. These may be considered as adaptive features or signs of damage caused by salinity. Because of accumulated salts in soil, the osmotic potential of soil solution changes, this in turn affects the environment of root and results into the disturbed equilibrium of dissolved ions. All such changes lead to reduction of growth causing stunting and subsequent loss in yields. Glycophytes which are sensitive to salinity show drastic changes under saline conditions, while halophytes show a better growth under saline conditions (Strogonov, 1964). According to Strogonov (1964), decrease in growth is due to change in state of protoplasm. Disturbed pattern of hormonal balance (Itai et al., 1968) also contributes to such effects. Besides salinity also causes several structural changes, like 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).

Salinity affects almost each and every stage of plant life. Germination, the most important starting phase of plant's life, is adversely affected by salinity. Under medium or low

salinity levels, germination is either decreased or delayed while at high salt concentration the final germination percentage is decreased. The osmotic pressure of the seed cell sap is lower than that of the external salt solution. Hence seeds cannot absorb sufficient water necessary for germination (Novikov, 1936). Under high saline conditions the time and rate of germination are severely affected. High salt concentration inhibits germination due to toxic effects of Na^+ and Cl^- also. Salinity affects germination even in halophytes. However, the species and varietal differences in salt tolerance during germination have also been observed by Heerkloss et al. (1980). It is observed that the germination percentage, germination rate index, seedling length and dry weight of seedlings progressively decreased with increase in salt concentration and that the germination responses are different with different salts (Rizk et al., 1979). It is observed by Sung (1981) that NaCl stress inhibits the enzymatic and respiratory activity during germination, which results in growth reduction. Salinity causes stunting in plants and decrease in leaf area (Meiri and Poljakoff-Mayber, 1970). Increase in succulence due to NaCl salinity is observed by Strogonov (1962). The vascular tissue, cell size and number of cells are also decreased thereby reducing stem diameter.

The growth can be affected by 2 ways. (1) Osmotic effect and (2) Specific ion effect.

(1) Osmotic effect - due to reduction in osmotic potential below the cell potential and thereby decreasing water potential and water available to the plants, i.e. physiological drought. Schimper introduced this concept first in 1898. The high concentration of the soluble salts was thought to prevent plant water uptake. Most of the adverse effects of salinity are related to the disturbed water balance, resulting in decrease in transpiration and subsequent growth.

This view predominated till Slatyer (1961) demonstrated that most of the plants do adopt themselves osmotically in saline habitat. For osmotic adjustment the salts are accumulated within the plant tissue, which in turn alters the degree of hydration of the cytoplasm, resulting in growth reduction. Thus the plants can adopt osmotically under salt stress and maintain the waterflow from soil solution (high osmotic potential) into plant tissue. Bernstein (1961, 1963 and 1964) supported this view assuming that the increased osmotic potential of the cell sap in osmotically adapted plants is mainly responsible for inhibition of growth, instead of the reduction in water absorption. The degree of adaptation can be considered as a criterion of salt tolerance, which differs from species to species. Osmotic adjustment is mainly achieved by active or passive accumulation of salts or synthesis of organic solutes. Salt accumulation is achieved by release of K^+ ions from the binding sites within the cells. The accumulated

organic solutes are simple sugars, polyols, proline, glycine-betaine, keto acids etc. According to Strogonov et al. (1970) accumulated carbohydrates, organic acids, proline, anthocyanins, carotenoids, nucleic acids and proteins have protective properties. On the other hand substances like putrescine, amino acids like leucine, iso-leucine, phenyl-alanine, oxyproline, tyrosine, methionine etc. have toxic effects. Further, he suggests that the plant's survival under saline conditions depends upon the regulation of metabolic processes and quantitative ratio between protective and toxic intermediates of metabolism. Plants thus adapt to saline conditions but rarely show complete osmotic adjustment. According to Greenway (1973), large amount of energy is utilised for such adjustments and this is one of the important factors in growth reduction.

(2) Specific ion effect :- Increase in concentration of certain ions which has characteristic toxic effects on plants creates number of problems for plant growth. The specific ions like Na^+, Cl^-, SO_4^{2-}, HCO_3^-, NO_3^- etc. disturb the normal physiological and biochemical process of a plant by disrupting the basic features of the proteins. Extensive concentration of such ions hampers the nutrition requirement, compete with nutrient ions (mainly causing intracellular K^+ deficiency) and it may be toxic to various physiological process. In combination, ion comprises the total salt concentration which produces osmotic effects. NaCl salt sensitivity in many

glycophytes is mainly due to absorption and accumulation of high amounts of Cl^- and Na^+ or both. The accumulated ions in leaves of sensitive varieties like rice, soybean and wheat show various leaf injuries (Abel & Mackenzie, 1964; Sharma et al. 1984; Joshi et al., 1985). The difference in absorption and accumulation of Na^+ and Cl^- ions and the difference between degree of osmotic adjustment is related to the difference in salt tolerance capacity of plants (Sharma et al., 1984). Epstein (1972) suggested that halophytes and salt tolerant glycophytes have developed a mechanism for preferential uptake of K^+. Halophytes are able to compartmentalise Na^+ and Cl^- and thus the cytoplasm remains free from such ions. However, sufficient experimental evidences are not available to prove such compartmentalisation.

The response of the plants to various salts are different and they also differ with salt composition. Each of the ions have its own specific effects on the plants, the mechanism of which is not well understood. Further information regarding how these ions exert their adverse effects especially on membrane permeability and enzyme fractions is lacking. The response also differs with plants growth stage. Most of the crop plants like rice, barley, wheat, cotton and sugarbeet are sensitive during germination and early seedling growth. Strogonov (1964), suggested that the salt tolerance capacity of the plants changes with their stage of development. However,

there is no sharp difference between salt tolerance capacity of the tissues from the halophytes and those from glycophytes. It is possible that the difference in response shown by plants to specific ions is due to the properties and probably the genetic composition of plant itself and more pronounced at the tissue level e.g. sorghum tissue is resistant to NaCl but sensitive to Na_2SO_4. However, salt tolerance at tissue level can not be correlated with that of entire plant.

Due to high salt concentration, the degree of dissociation of nutrients in the soil is lowered. Similarly, their uptake is also decreased due to high osmotic potential and reduction in root growth. Further, the absorbed NaCl competes with nutrient ions like K^+, Ca^{2+}, Fe^{2+}, Mn^{2+} etc. This results in decrease in uptake of such essential ions. Ultimately there is accumulation of Na^+, Cl^-, SO_4^{2-} etc. in the plant tissue while intake of K^+ and Ca^{2+} is reduced. High concentration of Na^+ loosens the protoplast and increases the membrane permeability. In fact at low concentration of salt, Na^+ enhances uptake of K^+ and Ca^{2+}, but at higher concentrations it inhibits K^+ uptake in many plants (Singh, 1967; Nimbalkar and Joshi, 1975; Kulkarni, 1975; Karadge and Chavan, 1979; Chavan and Karadge, 1980). Thus it appears that K^+ and Ca^{2+} have some important role in salt tolerance of plants. Some mangrove species, some rice cultivars and Eleusine show increase in K^+ uptake even at the high Na^+ concentration (Mishra, 1967; Hegde,

1972; Joshi, 1975; Chavan, 1980; Kotmire and Bhosale, 1980). It is suggested that Ca^{2+} prevents the uptake of Na^{+} and enhances that of K^{+} (Waisel, 1962). This is supported by the observations that addition of Ca^{2+} to the salt affected soils improves the growth and development of the plants. Na/K ratio is quite high in saline soils than the normal ones. The sensitive plants fail in preferential K^{+} uptake from such saline soils, while the salt tolerant ones can preferentially absorb K^{+} from such soils (Epstein, 1972).

According to Zukovskaya (1962) phosphorous content is decreased due to chloride and sulphate salinity. Even salinity influences intracellular phosphate concentration. Nitrogen uptake in barley plant is also affected by salinity (Helal et al., 1975). But halophytes, even under such conditions do not show any significant difference in their nitrogen uptake. The rate of accumulation of different elements in different plant parts is also altered by salinity. Na^{+} accumulates more in sensitive line of bread wheat (Kingsbury and Epstein, 1986). Wieneke and Lauchli (1980) observed that, more Na^{+} accumulates in the leaves of sensitive cultivar of soybean. While in Sesbania, a salt tolerant plant, Na^{+} and Cl^{-} accumulates more in leaf rachis than leaflets suggesting its adaptive feature (Chavan and Karadge, 1986). Regarding the microelements, recent work of Maas et al. (1972) suggested no much difference in their uptake under normal and saline conditions. Ion uptake is

usually accompanied with increase in respiration to cope with the increasing demand for energy necessary for osmotic adjustments. Thus there is no clear picture regarding the exact nature of mineral nutrition under saline habitat. Further the interaction between osmotic effect, specific ion toxicity and mineral uptake makes the ideas more complicated.

It is reported by number of workers like Gale et al., (1967), Gale and Poljakoff-Mayber (1970), Lapina and Popov (1970), Udovenko et al. (1971), Hoffman and Phene (1971) and Lapina & Bikmukhametova (1972), that rate of photosynthesis is reduced under saline conditions. However, halophytes show enhancement in photosynthesis at low salt concentrations (Gale and Poljakoff-Mayber, 1970). But in many others, as salt concentration increases, the rate of photosynthesis decreases, though the percent reduction varies from species to species and from variety to variety.

The reduction in photosynthesis may be due to (1) Low diffusion of CO_2 into the chloroplasts, (2) Alteration in structure and function of chloroplasts, (3) Changes in light and dark reactions of photosynthesis and (4) Effects on transport of assimilated products and intermediate compounds. Due to water imbalance the stomata remain closed inspite of high turgor pressure. This interferes with CO_2 diffusion into the chloroplasts and this in turn may reduce photosynthesis.

Chloroplasts are severely affected by salts. The number and size of chloroplasts is decreased due to salinity. After prolonged exposure of chloroplasts to saline conditions, swelling within granal loculi and frets and accumulation of lipid-droplets is observed by Poljakoff-Mayber (1975). Thus the fine structure of chloroplast is affected by salt. It is suggested that the binding forces between pigment-protein-lipid-complex of chloroplast are affected by salt (Strogonov et al., 1970). However, in resistant plants chlorophylls are tightly bound to the chloroplastic stroma and the complex is more stable even though swelling of chloroplasts is also observed in halophytes like Atriplex. Strogonov et al. (1970), have recorded shrinkage of chloroplasts due to salinity. Dehydration by NaCl causes reduction in Hill reaction and phbtophosphorylation (Santarius and Renate, 1967). According to Strack et al. (1975), the transport of photosynthates and pattern of ^{14}C distribution is also altered due to salinity.

Contradictory results regarding the chlorophyll content of a plant under saline conditions are available. According to Garter and Myers (1963), Matukhin (1963), Galaktionov (1963 a,b), Hoffmann (1964), Udovenko (1964) and Sivtsev (1973) the chlorophyll content of the plants, decreases with salinity. On the other hand, Shakhov (1956), Pokrovskaya, (1958) and Siegel and Bjarsch (1962) reported an increase in chlorophyll content under saline habitat. Garter and Myers (1963) found

that Na_2SO_4, $MgCl_2$ and NaCl in equal cationic concentrations affected chlorophyll and carotenoids equally. The individual ion has different effects on chlorophyll synthesis. The decrease in chlorophyll content is more pronounced in sensitive plants.

Chlorophyll a and b contents decrease at the beginning and then increases even above the control level, (Sivtsev, 1973). According to Strogonov (1974), salinity causes accumulation of chlorophyll a and b. Though, both chlorophylls a and b accumulate, chlorophyll 'a' accumulates faster while chlorophyll 'b' remains more or less stable. The chlorophyll a/b ratio is thus altered. It appears, therefore, that accumulation of chlorophylls depends upon the specific nature of plant metabolism, developmental stage and salt tolerance of the species.

Fragmentory and contradictory reports regarding the effect of salinity on biosynthesis and accumulation of carotenoids are available. It is reported by Koverga (1959), and Garter and Myers (1963) that salinity lowers the carotenoids while Shakhov (1956) and Siegel and Bjarsch (1962) reported that salinity increases carotenoid content. Strogonov (1974), suggests that the rate of synthesis of carotenoids depends upon the salinizing agent. Thus salt also causes marked changes in carotenoid content of a plant depending upon its salt tolerance, salt concentration and properties of salinizing agent.

Both RuBP case and PEP case, the photosynthetic enzymes, are adversely affected by salinity even at the low levels of salt. Even PEP case from salt tolerant Atriplex is more severely affected than that from salt sensitive Zea mays (Osmond and Greenway, 1972). Activity of malate dehydrogenase is enhanced which is accompanied by decreased activity of PEP case and RuBP case in Pennisetum typhoides, a C_4 plant (Sankhla and Huber, 1974). Opposite to this stimulation of PEP case from a C_3 halophyte, Cakile maritima and seagrasses is observed by Beer et al. (1975). They have recorded an inhibition of RuBP case in seagrasses. Increased activity of RuBP case in salt tolerant sugarbeet is reported by Heuer and Plaut (1982). It is suggested by them that salinity may induce conformational changes in the enzyme structure. Thus the results reported so far are variable with species, age of leaf, salt concentration and properties of salt and much more information is essential to get clear picture of the effect of salt on photosynthetic enzymes.

Salinity is known to alter the photosynthetic CO_2 fixation pathways in plants. Joshi et al. (1962), while studying the effect of NaCl on dark CO_2 fixation in marine plants, observed a shift of label from organic acids to amino acids. This may be due to activation of transaminases and inhibition of malate dehydrogenase under saline conditions. The marine algae and mangroves follow a modified C_4 pathway (Patil, 1967; Gowda, 1971;

Joshi and Karekar, 1973; Joshi et al. 1974; Joshi and Shitole, 1977). According to Shomer-Ilan and Waisel (1973) presence of NaCl in the medium influences the balance between PEP case and RuBP case in the leaves of Aeluropus litoralis, causing a shift in their carbon fixation pathway. Thus the balance between C_4 and C_3 pathway is disturbed. A shift from C_3 to C_4 pathway or intermediate is recorded by Ghevade and Joshi (1980) in sea grass Halophila becarii; Karadge and Chavan (1981), in groundnut and Hegde and Patil (1982) in Parthenium hysterophorus. In these plants more label is observed in C_4 products i.e. malate and/or aspartate. A shift from C_3 to CAM is reported by Winter and Luttge (1976) in Mesembryanthemum crystallinum which can tolerate high levels of salt. Increased C_4 activity has been reported by Joshi and Karadge (1979) in Portulaca oleracea grown under saline conditions and suggested the tendency of this plant towards CAM (Karadge and Joshi, 1983). Stimulation of dark $^{14}CO_2$ fixation and CAM activity due to NaCl salinity have been reported by Karmarkar and Joshi (1969) and Kulkarni (1975) in Bryophyllum pinnatum. Stimulation of organic acid synthesis is observed by them in this CAM plant. Thus salinity affects the basic nature of CO_2 fixation. The response given by plants are various and differs from plant to plant and depends upon the age of a plant and the type of salinity.

Efficiency of respiration is decreased due to salinity (Bhardwaj and Rao, 1960; Sarin, 1961; Boyer, 1965). At high

salt concentrations respiration is reduced especially in sensitive plants which may result in failure of plants to maintain themselves in saline habitat. However, an increase in respiration due to saline environment is reported by Nieman (1962), and Livne and Levin (1967). Increased respiration may provide energy for osmotic adjustment and maintenance. Increase in respiration may be due to activation of ion transport system Na - K ATPase and direct effect of Na on respiratory chain (Gordon and Bichurina, 1973). Increase in respiration is considered to be an adaptive feature and it is found that this increase is more pronounced in the tolerant species (Udovenko et al,,1972). However, the response given by various plants are different. Increase in respiration is responsible for decrease in photosynthesis which in turn results in decrease in overall growth (Hoffman and Phene, 1971).

Salinity affects normal conformational properties of protein and affects the composition of the cell cytoplasm. This in turn affects the nature and function of enzymes. The in vitro preparation of enzymes either from glycophytes or halophytes do not differ much in their response to salt (Greenway and Osmond, 1972; Flowers, 1972-a,b). However, Weimberg (1970) did not find any significant difference in different enzyme systems in pea seedlings. Porath and Poljakoff-Mayber (1964, 1968) reported increased activity of enzymes of pentose phosphate pathway. An induction of new

isoenzyme of malate dehydrogenase is recorded in pea, grown in in saline media (Hassan-Porath and Poljakoff-Mayber, 1969). The activity of Na-K-ATPase which is related to ion transport and membrane properties, is increased due to salinity. Activity of enzymes like catalase, peroxidase, protease, chlorophyllase etc. is also increased.

The salt injuries occur usually through the influence of salt on the nitrogen metabolism of a plant. The amino acids and other soluble nitrogenous compounds play an important role in salt tolerance mechanism of a plant. Salinity influences nitrate uptake by inhibiting it (Rush and Epstein, 1976; Aslam et al., 1984; Bottacin et al., 1985). On the other hand Smith et al. (1980) recorded an increase in nitrate uptake in rye grass under saline conditions. Decrease in the activity of NR (nitrate reductase) is recorded by Heimer (1973); Sharma & Garg (1983); Safaralliev et al. (1984) and Bottacin et al. (1985). Opposite to this Dias and Costa (1983) reported an increase in NR activity, while Aslam et al. (1984) found no significant difference in in vivo NR activity. Salinity influences basic structure of NR by dissociating the flavoprotein with molybdenum. Salinity also affects the amino acid synthesizing enzymes. Increase in the activity of glutamate dehydrogenase is observed by Tur & Skazhenik(1980),Sharma and Garg (1985) and Bottacin et al. (1985). Activity of glutamate synthatase is reduced in sensitive glycophyte, Phaseolus,while remains stable in a halophyte

(Billard et al., 1982). Increase in the activity of amino transferases is recorded by Sharma and Garg (1985). Bottacin et al. (1985) suggested that the high activity of glutamate dehydrogenase and glutamine synthatase in salt tolerant Pennisetum cultivars is related to its salt tolerance capacity. High glutamine synthatase activity recorded by Ericson and Stewart (1984) suggests its role in proline synthesis. Thus due to salinity normal amino acid metabolism is disturbed. Due to saline conditions plant accumulates ammonia, amides, free amino acids like lysine, proline, leucine, glutamine, aspartate, phenylanine, glutamate, alanine, tyrosine, valine etc. This indicates incomplete utilization of nitrogen and further influence on protein synthesis. Accumulation of each amino acids may also have toxic effects on plants. Some of the amino acids like phenylalanine are toxic or they may synthesize toxic substances like cadaverin (Strogonov, 1964). Further, Strogonov (1964) suggests that the actual toxic substances vary from species to species and their accumulation depends upon the salt tolerance mechanism of the plant. Accumulation of proline and quaternary ammonium compounds has been observed by a number of workers, which is considered to be an adaptive feature in salt tolerance.

The rate of accumulation of non-protein nitrogen decreases sharply with increasing salinity level, while total nitrogen content increases except at the high salt concentration. The

balance between soluble amino acids and proteins is changed by saline habitat. Salt treatment results in decrease in protein content of plant tissue (Nieman, 1965; Huber et al., 1977; Pessarakli and Tucker, 1985; Reddy and Vora, 1985-a). According to Longstreth et al. (1984) the level of soluble proteins remains constant. On the other hand stimulation of protein synthesis is reported by Singh and Vijay Kumar (1975); Helal et al. (1975), Kumar et al. (1982) and Parihar and Baijal (1983). However, this stimulation depends on the degree of salinization. Increase in protein content is observed at the low levels of salinity, but protein synthesis is inhibited sharply at the higher doses of salinity. It is suggested that the protein synthesis is stimulated in the nucleus, while it is inhibited in the cytoplasm. Salinity disturbs the nucleic acid metabolism also.

2. Salt Tolerance Studies in Millets :

The term millet refers to small seeded cereal and forage grasses used for food, feed and forage. They are especially cultivated in tropical and subtropical regions of the world. They are considered as hardy cereals and usually possess drought and temperature tolerance. They have low seed requirement and are able to produce more grain even under stress conditions. They possess good nutritive value, especially protein quality is better than other cereals. They are rich in Ca^{2+}, Mg^{2+}, P^{5+}, Fe^{2+}, K^{+} etc. They are also rich in fat content. Millets have

a wide range of adaptation and use. They can fit in almost every situation like high temperature and low humidities, low temperature and high humidity, infertile soils, varying soil elevations, flooding or limited moisture conditions and needs even far less ideal conditions. They have relatively few insect pests and diseases. They possess general tolerance to adverse conditions and economy of water use. Even though they posses such adaptive features they have received very little attention by the scientific community as regards their improvements and physiological aspects leading to general tolerance especially salt tolerance.

Very few reports are available describing the effect of salinity on growth and metabolism of millets. Recently, Chavan (1980) has studied the effect of NaCl salinity on growth and mineral nutrition of finger millet (Eleusine coracana). According to him growth of this plant is not affected by lower salinity levels while higher salt regimes delay the panicle emergence and grain filling and leaves become halosucculent. Under saline conditions Na^+ accumulates in stem while that Cl^- in the leaves. Ca^{2+} uptake is decreased while Fe^{2+} uptake increases under saline conditions. Carbohydrates, total nitrogen and proline accumulate in the leaves suggesting the plant's capacity of osmotic adjustment. Grain composition is also altered by salinity. It was observed that the activity of enzymes peroxidase, catalase, acid phosphatase and NR is enhanced

by salinity while amylase was slightly inhibited. PEPcase shows marked inhibition only at high salt concentrations indicating its salt tolerance. The rate of photosynthesis is enhanced at low levels of salt while it decreases at the higher salt levels. ^{14}C is incorporated more in amino acids fraction under saline conditions which can be regarded as an adaptive feature of Eleusine. The response given to Na_2SO_4 salinity is however, slightly different. Sharma et al. (1983), have suggested that Eleusine can be cultivated under saline conditions.

Comparatively more work is done on Pennisetum typhoides. Singh and Chandra (1980) suggest that varietal difference regarding salt tolerance exists in Pennisetum. This view is supported by Saxena and Kolarkar (1982). Yadav and Gupta (1984) classified it among tolerant crops. According to Reddy and Vora (1985-b) germination is not much affected by different types of salinity except it is delayed. Further, they suggest that accumulation of high amount of proline during stress conditions is an adaptive feature of this millet. However, Chandra and Chauhan (1985) suggest a negative correlation between proline accumulation and salinity. Due to salinity protein and RNA content is decreased accompanied by an increase in activity of protease and RNAase, resulting in accumulation of amino acids (Reddy and Vora, 1985 a). Salinity decreases chlorophyll a, b and total chlorophylls, carotenoids, reducing sugars, starch and Hill reaction activity and increases chlorophyllase and invertase

activity (Reddy and Vora, 1986). Bottacin et al. (1985) have studied the effect of NaCl salinity on nitrogen absorption and assimilation in salt resistant and sensitive genotypes of Pennisetum. According to them more Na^+ and Cl^- accumulate in sensitive cultivars, however, K^+ uptake is inhibited in both the cultivars. In all ecotypes nitrogen absorption is negatively affected by NaCl. However, NO_3^- uptake is inhibited in sensitive variety more than NH_3^+ uptake. In vitro NR activity decreases due to salinity. GDH/GS ratio is found to be slightly decreased in tolerant variety and increased in sensitive one. High GDH/GS ratio in sensitive variety indicates that nitrogen is assimilated by GDH as in glycophytes, while low GDH/GS ratio in tolerant variety is suggestive of GS-GOGAT pathway of nitrogen assimilation as in halophytes.

Very little information is available regarding the salt tolerance of other millets like Panicum and Setaria, except Manga & Saxena (1981) report about germination of seeds of these two millet crops under saline conditions. According to them with increasing salinity level, germination percentage and root and shoot growth is decreased.

From the above literature it is clear that millets are very rarely studied for their physiology of salt tolerance especially Setaria is not at all studied. Hence in the present investigation, an attempt has been made to study the physiology

of salt tolerance in two cultivars of Setaria italica differing in salt tolerance at their seedling stage. The effect of salinity on the germination, growth and nitrogen metabolism of S.italica cultivars SIC-1 and CO-5 has been studied.

Setaria italica is known as Italian fox-tailed millet, cultivated as a food grain crop all over India. It forms staple food in our country as well as other developing countries. The crop can be grown in wide range of soil and climatic conditions. It is mostly grown under rainfed and occassionally under irrigated conditions. It responds well to manure and fertilizers applied. Usually it is sown as a mixed crop.

Setaria belongs to monocot family - Graminae. It is an annual herb, upto 5 feet tall. The stem is hollow, errect, usually branched, tufted rooting towards base. Leaves are simple, narrow, alternate, linear, tapering towards tip and show parallel venation. Flowers are in terminal dense panicles. It is usually sown in June-July as a Kharif crop while irrigated crop is sown in February-March. The crop flowers in about 50-60 days and matures in about 80-100 days; when the ears are dry, crop is harvested, either by cutting the whole plant or ears separately. Threshing is easy and grains have to be dehusked before use. The straw can be fed to cattle. The yield of rainfed crop ranges from 500-1000 Kg hectares^{-1} while that of irrigated crop ranges from 1000-1600 Kg hectare^{-1}.

Setaria italica is rich in protein and fat content, It is rich in vitamin thiamine. Mg^{2+}, P^{5+}, Fe^{2+} & K^{+} contents are high in grains of this crop as compared to other millets. Protein quality is as good as or even better than that of other cereals. It contains 63-70% total carbohydrates (free sugars - 0.46 - 0.69%, Starch 56.0 - 61.0%, Cellulose, 0.7 to 1.8% and Pentosans 5.5 to 7.2%). It is also rich in nitrogen.

Raghavendra (1978) and Raghavendra and Das (1975, 1977, 1978 a,b,c) have done extensive work on photosynthesis in S.italica. They have partially purified PEP case and found that OAA competetively inhibits enzyme activity indicating allosteric nature of the PEPcase from this species. According to them the leaves fix most of the radioactivity into C_4 acid malate and/or aspartate indicating that the CO_2 is initially fixed through PEPcase. This species has been classified by them as NADP-ME C_4 plant. Setaria possesses high activity of PEPcase and Pyruvate Pi-dikinase and shows low levels of photo-respiratory enzymes. This plant shows active cyclic photophos-phorylation, high chlorophyll a to b ratio and higher content of P_{700} indicating its enrichment in PS-I. They further suggested that about 35-52% chlorophylls are located in bundle sheath chloroplasts and they are rich in photochemical activi-ties. The bundle sheath chloroplasts possess high activity of PS-II. According to Apel and Peisker (1978) the CO_2 compensa-tion point of this species is only insignificantly influenced by high oxygen concentration.

Setaria is considered as a drought tolerant and can be grown on alkaline clay soils also. Though Setaria is not studied for nutritive values and physiology of drought tolerance, it may have unrealised potential for grain production. Very little attention has been given for its improvement, relative importance as a food crop and for increasing food production especially in the areas where rainfall is scanty.

3. Nitrogen Metabolism :

Since in the present investigation more emphasis is given on the nitrogen metabolism of Setaria cultivars under saline conditions, a brief idea of basic nature of nitrogen metabolism in plants has been given in the following few pages.

Most of the higher plants prefer NO_3^- as a nitrogen source. Assimilation of NO_3^- is a continuous process beginning with uptake of NO_3^- by the roots. The NO_3^- absorbed must be reduced to ammonia which is then assimilated into organic compounds mostly amino acids and then proteins.

Reduction of NO_3^- to NH_4^+ takes place both in the leaves as well as in roots which is achieved in two stages involving enzymes nitrate reductase and nitrite reductase. Nitrate reductase - NR (EC 1.6.6.2) catalyses reduction of NO_3^- to NO_2^-. The enzyme was originally isolated from Neurospora (Nason and Evans, 1953). The enzyme from higher plants shows a specific

requirement for NADH (Beevers and Hageman, 1969). For reduction catalysed by NADPH, NADPH is 1st converted into NADH and actually NADH donates electrons. $FMNH_2$ and $FADH_2$ can also act as electron donors. Essentiality of molybdenum for NO_3^- reduction has been demonstrated (Aparicio et al., 1971; Notton and Hewitt, 1971). The enzyme is present both in the leaves as well as in roots. In roots it is present in the cytoplasm while in the leaves it is present either in cytoplasm or loosely attached with outer membrane of chloroplast. In the leaves of C_4 plants nitrate reduction occurs in mesophyll cells (Moore and Black, 1979; Losada et al., 1981).

Nitrate reductase is a complex, oligomeric enzyme having molecular weight 197 to 460 K daltons (KD) and is composed of a variable number of apparently identical sub-units. FAD, cytochrome b-557 and Mo are ubiquitous prosthetic groups. The enzyme consists of two subunits. One flavin and second flavoprotein with Mo. Flavin component accepts electrons first, then transferred to FAD and flavoprotein components and Mo is essential for this transfer. Finally electrons are accepted by NO_3^- which itself gets reduced to NO_2^-. The reaction is summarised as

$$NAD(P)H \longrightarrow \{FAD \longrightarrow \text{Cyt b-557} - Mo\} \longrightarrow NO_3^- \longrightarrow NO_2^-$$

The NO_2^- thus produced is further reduced to NH_3^+ and this reduction is catalysed by another important enzyme system,

nitrite reductase (Ec 1.6.6.4). In the leaves this enzyme is present in chloroplast probably in thylakoids, while in roots it is present in proplastids. NiR presents a marked specificity for ferridoxin as electron donor (Vega et al., 1980). Flavodoxin can substitute ferridoxin. The molecular weight of ferridoxin NiR is between 60-70 KD. Reduced ferridoxin which is a result of noncyclic photophosphorylation provides reducing power. The enzyme possesses iron porphyrin prosthetic group, 'siroheme'. Iron-sulphur centre of this enzyme plays an important role in NO_2^- reduction. Electrons from reduced ferridoxin are transferred to iron sulphur centre first and then to siroheme and finally electrons are accepted by NO_2^- which is reduced to NH_4.

Ferridoxin reduced — {(4 Fe-4S) → Siroheme} → NO_2^- → NH_4^+

Nitrite, the substrate of the enzyme is bound to the siroheme centre of NiR.

In roots the enzyme is associated with proplastids which are rich in enzymes of pentose phosphate pathway, which in turn can produce NADPH. But NADPH transfers the electrons via some intermediate to NO_2^-. However, such intermediate is not demonstrated.

The end product of nitrate reduction i.e. NH_4^+ is further incorporated into organic compounds. The three important enzyme systems involved in NH_4^+ assimilation are glutamate dehydrogenase (GDH), glutamine synthatase (GS) and glutamate synthatase (GOGAT).

Glutamate dehydrogenase (EC 1.4.1.3) catalyses reductive amination of ∝- ketoglutarate. It is localised in mitochondria of leaves and roots. It requires a divalent metal ion and utilises either NADH or NADPH as electron donor.

$$\text{∝- Ketoglutarate} + NH_3 + NAD(P)H \rightleftharpoons \text{Glutamate} + NAD(P) + H_2O$$

Kinetic study has revealed that it has low affinity for NH_4^+ and concentration of NH_4^+ in cells is much more lower than Km. Further the mitochondrial location suggests its degradative role under normal conditions.

Glutamine synthatase (EC 6.3.1.2) catalyses formation of glutamine from glutamate. It is present in chloroplast and cytoplasm. It requires a divalent metal ion like Mg^{2+} or Mn^{2+}. It consists of 8 monomers arranged in a two parallel sets. Energy required for synthesis is obtained by clevage of ATP.

$$\text{Glutamate} + NH + ATP \underset{Mg/Mn}{\overset{GS}{\rightleftharpoons}} \text{Glutamine} + ADP + i.P. + H_2O$$

Glutamate synthatase (GOGAT) catalyses reductive transfer of an amide - amino group from glutamine to ∝- ketoglutarate to produce two glutamate molecules. The enzyme is present in chloroplasts. The reducing power is supplied by reduced ferridoxin or NAD(P)H. It shows feed back regulation. The reaction catalysed is -

cc- ketoglutarate + glutamine + NAD(P)H/Ferridoxin reduced

⇣ ⇡ GOGAT

2 glutamate + NAD(P)/Oxd. ferridoxin.

Thus glutamine plays central role in NH_4^+ assimilation which occurs via GS-GOGAT pathway.

The bound nitrogen of glutamate and glutamine is further utilised for biosynthesis of aminoacids which are building blocks of proteins. Aminotransferases catalyse such reactions by transferring amino group of a donor amino acid to a keto acid. The enzymes show multisubstrate specificity. Pyridoxal phosphate is an essential co-factor for such transfer. These enzymes are located in cytoplasm, chloroplasts and microbodies. The reactions catalysed can be shown as

i) Amino acid (A) + Amino transferase - Pyridoxal phosphate

⇣ ⇡

2-oxo acid (A) + Aminotransferase - pyridoxime phosphate.

ii) 2-oxo acid (B) + Aminotransferase - pyridoxime phosphate

⇣ ⇡

Amino acid (B) + Aminotransferase - pyridoxal phosphate.

Thus, amino acid A + Oxo acid B $\xrightleftharpoons{\text{amino transferase}}$ Oxo acid A + amino acid B

Glutamate oxaloacetate transaminase - GOT (EC 2.6.1.1) catalyses reaction -

oc - ketoglutarate + aspartate $\xrightleftharpoons{GOT}$ glutamate + oxaloacetate

Alanine aminotransferase - AAT (EC 2.6.1.2) catalyses reaction

oc - ketoglutarate + Alanine $\xrightleftharpoons{AAT}$ glutamate + pyruvate.