CHAPTER 1

LITERATURE REVIEW

1.1 Effects of salinity in agriculture – An overview

Salinity of arable land is an increasing problem of many irrigated, arid and semi-arid areas of the world where rainfall is insufficient to leach salts from the root zone, and it is a significant factor in reducing crop productivity (Francois and Maas, 1994). Saline soils are defined by Ponnamperuma (1984) as those that contain sufficient salt in the root zone to impair the growth of crop plants. However, since salt injury depends on species, variety, growth stage, environmental factors, and nature of the salts, it is difficult to define saline soils precisely. The most widely accepted definition of a saline soil has been adopted from FAO (1997) as one that has an electrical conductivity of the saturation extract (EC_e) of 4 dS m^{-1} or more, and soils with EC_e's exceeding 15 dS m^{-1} are considered strongly saline. The common cations associated with salinity are Na^+, Ca^{2+} and Mg^{2+}, while the common anions are Cl^-, SO_4^{2-} and HCO_3^-. However, Na^+ and Cl^- ions are considered the most important, since Na^+ in particular causes deterioration of the physical structure of the soil and both Na^+ and Cl^- are toxic to plants (Dudley, 1994; Hasegawa *et al*., 2000). Soils were historically classified as saline, sodic, or saline-sodic based on the total concentration of salt and the ratio of Na^+ to Ca + Mg in the saturated extract of the soil (Dudley, 1994). However, this classification has been abandoned in favor of a management-oriented approach, and a soil that contains excessive salt is presently referred to as saline or salt-affected regardless of the specific nature of the problem.

Due to an increase in population, there is competition for fresh water among the municipal, industrial and agricultural sectors in several regions. The consequence has been a decreased allocation of freshwater to agriculture (Tilman *et al*., 2002). This phenomenon is expected to continue and to intensify in less developed, arid region countries that already have high population growth rates and suffer from serious environmental problems. For this reason there is increasing pressure to irrigate with water of certain salt content, like ground water, drainage water and treated wastewater (Table 1.1).

Table 1.1 Classification of water (Rhoades *et al*., 1992)

Type of water	EC (dS/m)	TDS[1] (g/l)	Water class
Drinking & Irrigation water	<0.7	<0.5	Non-saline
Irrigation water	0.7-2.0	0.5-1.5	Slightly saline
Primary drainage water & groundwater	2.0-10.0	1.5-7.0	Moderately saline
Secondary drainage water & groundwater	10.0-20.5	7.0-15.0	Highly saline
Very saline groundwater	20.0-45.0	15.0-35.0	Very highly saline
Seawater	>45.0	>35.0	Brine

[1] Total dissolved solutes

According to Carvajal *et al*. (1999); Yeo (1998); Grattan and Grieve (1999) the direct effects of salts on plant growth may be divided into three broad categories: (i) a reduction in the osmotic potential of the soil solution that reduces plant available water, (ii) a deterioration in the physical structure of the soil such that water permeability and soil aeration are diminished, and (iii) increase in the concentration of certain ions that have an inhibitory effect on plant metabolism (specific ion toxicity and mineral nutrient deficiencies). The relative contribution of osmotic effects and specific ion toxicities on yield are difficult to quantify. However, with most crops, Dasberg *et al*. (1991) reported that yield losses from osmotic stress could be significant before foliar injury is apparent.

1.2 Causes of salinity

1.2.1 Primary cause

Most of the saline-sodic soils are developed due to natural geological, hydrological and pedological processes. Some of the parent materials of these soils include intermediate igneous rocks such as phenolytes, basic igneous rocks such as basalts, undifferentiated volcanic rocks, sandstones, alluviam and lagoonal deposits (Wanjogu *et al*., 2001). Climatic factors and water management may accelerate salinization. In arid and semi-arid lands (ASAL) evapotranspiration plays a very important role in the pedogenesis of saline and sodic soils. Wanjogu *et al*. (2001) reported that most of the ASAL receive less than 500 mm of rainfall annually and this, coupled with an annual potential evapotranspiration of about 2000 mm leads to salinization. Another type of salinity occurs in coastal areas subject to tides and the main cause is intrusion of saline water into rivers (Cyrus *et al*., 1997) or acquifers (Howard and Mullings, 1996). Coastal rice crops in Asia, for instance, are frequently affected by exposure to seawater brought in by cyclones around the Indian Ocean (Sultana *et al*., 2001).

1.2.3 Secondary salinization

Secondary salt-affected soils are those that have been salinized by human-caused factors, mainly as a consequence of improper methods of irrigation. Poor quality water is often used for irrigation, so that eventually salt builds up in the soil unless the management of the irrigation system is such that salts are leached from the soil profile. Szabolcs (1992) estimated that 50% of all irrigated schemes are salt affected. Too few attempts have been made recently to assess the degree of human-induced secondary salinization and, according to Flowers and Yeo (1995) this makes it difficult to evaluate the importance of salinity to future agricultural productivity. Nevertheless, Ohara (1997) has reported increasing salinization with increasing irrigation since the 1950's, and in the Shanxi Province in China, more than one-third of the total area of irrigated land is salinized (Qiao, 1995). The land area under irrigation in Kenya is estimated to be about 84,000 ha (Ngigi, 2002) and according to Mugwanja *et al*., (1995), about 26,000 ha is considered salt degraded mainly due to poor irrigation management and poor drainage, especially in areas with a high ground water table. Anthropic salinization occurs in arid and semi-arid areas due to waterlogging brought about by improper irrigation (Ponnamperuma, 1984). Secondary salt-affected soils can also be caused by human activities other than irrigation and include, but are not limited to, the following:

(a) Deforestation is recognized as a major cause of salinization and alkalization of soils as a result of the effects of salt migration in both the upper and lower layers.

In Southeast India, for example, vast areas of former forestland became increasingly saline and alkaline a few years after the felling of the woods (Szabolcs, 1994). In Australia, a country where one-third of the soils are sodic and 5% saline (Fitzpatrick *et al*., 1994), there is serious risk of salinization if land with shallow unconfined acquifers containing water with more than 0.25% total soluble salt is cleared of trees (Bui *et al*., 1996).

(b) Accumulation of air-borne or water-borne salts in soils

Szabolcs (1994) has reported that chemical accumulation from industrial emissions may accumulate in the soil, and if the concentration is high enough, can result in salt accumulation in the upper layer of soil. Similarly, water with considerable salt concentration such as waste water from municipalities and sludge may contaminate the upper soil later causing salinization and/or alkanization (Bond, 1998; Bouwer, 2002).

(c) Salinization caused by contamination with chemicals

This kind of salinization more often occurs in modern intensive agricultural systems, particularly in greenhouses and intensive farming systems. In closed or semi closed systems (e.g. greenhouses) salts tend to accumulate if chemicals are not removed regularly, resulting in salinity or alkalinity. In countries with intensive agriculture such as Japan and the Netherlands, this type of salinization appears more frequently (Pessarakli, 1991).

(d) Overgrazing

Szabolcs (1994) reported that this process occurs mainly in arid and semi arid regions, where the natural soil cover is poor and scarcely satisfies the fodder requirement of extensive animal husbandry. Because of overgrazing, the natural vegetation becomes sparse and progressive salinization develops, and sometimes the process ends up in desertification as the poor pasture diminishes.

1.3 Salinity effects on plants

According to Dubey (1997) and Yeo (1998) salt causes both ionic and osmotic effects on plants and most of the known responses of plants to salinity are linked to these effects.

The general response of plants to salinity is reduction in growth (Romero-Aranda *et al*., 2001; Ghoulam *et al*., 2002). The initial and primary effect of salinity, especially at low to moderate concentrations, is due to its osmotic effects (Munns and Termaat, 1986; Jacoby, 1994). Osmotic effects of salts on plants are a result of lowering of the soil water potential due to increasing solute concentration in the root zone. At very low soil water potentials, this condition interferes with the plant's ability to extract water from the soil and maintain turgor. Thus, in some species salt stress may resemble drought stress. However, at low or moderate salt concentrations (high soil water potentials), plants adjust osmotically (accumulate internal solutes) and maintain a potential for the influx of water (Guerrier, 1996; Ghoulam *et al*., 2002). Plant growth may be moderated under such conditions, but unlike drought stress, the plant is not water deficient (Shannon, 1984).

At high salinity, some specific symptoms of plant damage may be recognized, such as necrosis and leaf tip burn due to Na^+ or Cl^- ions (Wahome *et al*., 2001). High ionic concentrations may disturb membrane integrity and function, interfere with internal solute balance and nutrient uptake, causing nutritional deficiency symptoms similar to those that occur in the absence of salinity (Grattan and Grieve, 1999).

Sodium and chloride, usually the most prevalent ions in saline soils or water, account for most of the deleterious effects that can be related to specific ion toxicities (Levitt, 1980). The degree to which growth is reduced by salinity differs greatly with species and to a lesser extent with varieties (Bolarin *et al.*, 1991; Ghoulam *et al.*, 2002). The severity of salinity response is also mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon *et al*., 1994). Salt accumulation in leaves causes premature senescence, reducing the supply of assimilates to the growing regions and thus decreasing plant growth (Munns *et al.*, 1995). In more sensitive varieties salt accumulates faster, and because cells are unable to compartmentalize the salt in the vacuoles to the same high degree as tolerant varieties, leaves are expected to die sooner (Munns, 1993). Neumann (1997) considered that inhibition of leaf growth by salt decreases the volume of new leaf tissues into which excess salt can be accumulated and, combined with continuous salt accumulation it could lead to earlier build up of excess salt levels. Salt stress affects all the major processes such as growth, water relations, photosynthesis and mineral uptake.

1.3.1 Effects of salinity on plant growth

Several investigators have reported plant growth reduction as a result of salinity stress, e.g. in tomato (Romero-Aranda *et al*., 2001), cotton (Meloni *et al*., 2001) and sugar beet (Ghoulam *et al*., 2002). However, there are differences in tolerance to salinity among species and cultivars as well as among the different plant growth parameters recorded. For instance, Aziz and Khan (2001) found that the optimum growth of *Rhizophora mucronata* plants was obtained at 50% seawater and declined with further increases in salinity while in *Alhagi pseudoalhagi* (a leguminous plant), total plant weight increased at low salinity (50 mM NaCl) but decreased at high salinity (100 and 200 mM NaCl) (Kurban *et al.*, 1999). In sugar beet leaf area, fresh and dry mass of leaves and roots were dramatically reduced at 200 mM NaCl, but leaf number was less affected (Ghoulam *et al*., 2002). Fisarakis *et al.* (2001), working with sultana vines recorded a larger decrease in accumulation of dry matter in shoots than in roots, particularly at high NaCl concentration, indicating partitioning of photoassimilates in favor of roots. They proposed that the results may be due to a greater ability for osmotic adjustment under stress by the roots.

1.3.2 Effects of salinity on water relations

The main cause of reduction in plant growth may result from salinity effects on water status. According to Sohan *et al*. (1999) and Romero-Aranda *et al*. (2001) increase of salt in the root medium can lead to a decrease in leaf water potential and, hence, may affect many plant processes. Osmotic effects of salt on plants are as a result of a lowering of the soil water potential due to increasing solute concentration in the root zone. At very low soil water potentials, this condition interferes with plants' ability to extract water from the soil and maintain turgor (Sohan *et al*., 1999). Thus, in some aspects salt stress may resemble drought stress. However, at low or moderate salt concentrations (higher soil water potential), plants adjust osmotically (accumulate solutes) and maintain a potential gradient for the influx of water. Under such conditions Shannon (1984) reported that growth may be moderated, but unlike drought stress, the plant is not water deficient.

Several authors found that water potential and osmotic potential of plants became more negative with an increase in salinity, whereas turgor pressure increased (Meloni *et al*., 2001; Romero-Aranda *et al*., 2001; Gulzar *et al*., 2003). In the halophyte *Suaeda salsa*, Lu *et al*. (2002) found that leaf water potential and evaporation rate decreased significantly with increasing salt concentration. Ashraf (2001) reported similar decreases in leaf water potential with increasing salt concentration in all the six *Brassica* species studied. At 200 mM NaCl *B. campestris* and *B. carinata* maintained significantly higher leaf water potentials than the other species, and were, therefore, considered more tolerant to salt stress. With increasing salt concentration, water potential became more negative in sunflower (Sohan *et al*., 1999). According to these investigators, the results seem to stem from two factors: (1) under high salt concentration, plants sequester more NaCl in the leaf tissue than normally occurs. Increases in NaCl within the leaf tissue then result in lower osmotic potentials and more negative water potentials, and (2) the reduction in root hydraulic conductance reduces the amount of water flow from the roots to the upper portion of the canopy, causing water stress in the leaf tissue.

Salt treatment caused a significant decrease in relative water content (RWC) in sugar beet varieties (Ghoulam *et al*., 2002). According to Katerji *et al.* (1997), a decrease in RWC indicates a loss of turgor that result in limited water availability for cell extension processes.

1.3.3 Effects of salinity on leaf anatomy

Salinity has been reported to cause leaf anatomical changes in a number of plants. For instance, leaves of bean, cotton and *Atriplex* are reported to increase in epidermal thickness, mesophyll thickness, palisade cell length, palisade diameter, and spongy cell diameter with increasing salinity (Longstreth and Noble, 1979). In contrast both epidermal and mesophyll thickness and intercellular spaces decreased significantly in NaCl-treated leaves of the mangrove *Brugueira parviflora* (Parida *et al*., 2004). In leaves of spinach salinity was found to reduce intercellular spaces (Delfine *et al*., 1998) while in tomato plants, a reduction of stomatal density occurred (Romero-Aranda *et al*., 2001).

1.3.4 Effects of salinity on photosynthesis

Growth of plants is dependent on photosynthesis and, therefore, environmental stresses affecting growth also affect photosynthesis (Salisbury and Ross, 1992; Dubey, 1997; Taiz and Zeiger, 1998). Studies conducted by a number of authors with different plant species showed that photosynthetic capacity was suppressed by salinity (Dubey, 1997; Kao *et al*., 2001; Ashraf, 2001; Romero-Aranda *et al*., 2001). A positive association between photosynthetic rate and yield under saline conditions has been found in different crops such as *Gossypium hirsutum* (Pettigrew and Meredith, 1994) and *Asparagus officinalis* (Faville *et al*., 1999). Fisarakis *et al*. (2001) found that inhibition of vegetative growth in plants submitted to salinity was associated with a marked inhibition of photosynthesis. In contrast, there are many studies in which no or little association between growth and photosynthetic capacity is evident, as in *Triticum repens* (Rogers and Noble, 1992) and *Triticum aestivum* (Hawkins and Lewis, 1993).

The effect of salinity on photosynthetic rate depends on salt concentration and plant species. There is evidence that at low salt concentration salinity may stimulate photosynthesis. For instance, in *B. parviflora*, Parida *et al*. (2004) reported that photosynthetic rate increased at low salinity and decreased at high salinity, whereas stomatal conductance was unchanged at low salinity and decreased at high salinity.

Iyengar and Reddy (1996) attributed decreases in photosynthetic rate as a result of salinity to a number of factors:

(1) Dehydration of cell membranes which reduce their permeability to CO_2. High salt concentration in soil and water create high osmotic potential which reduces the

availability of water to plants. Decrease in water potential causes osmotic stress, which reversibly inactivates photosynthetic electron transport via shrinkage of intercellular space.

(2) Salt toxicity caused particularly by Na^+ and Cl^- ions. According to Banuls *et al*. (1990), Cl^- inhibits photosynthetic rate through its inhibition of NO_3-N uptake by the roots. Fisarakis *et al*. (2001) found that NO_3-N was significantly reduced in salt-stressed sultana vines and this reduction was correlated with photosynthetic reduction. The reduced NO_3-N uptake combined with osmotic stress may explain the inhibitory effect of salinity on photosynthesis.

(3) Reduction of CO_2 supply because of closure of stomata. The reduction in stomatal conductance results in restricted availability of CO_2 for carboxylation reactions (Brugnoli and Bjorkman, 1992). Iyengar and Reddy (1996) reported that stomatal closure minimizes loss of water by transpiration and this affects chloroplast light-harvesting and energy-conversion systems thus leading to alteration in chloroplast activity. Higher stomatal conductance in plants is known to increase CO_2 diffusion into the leaves and thereby favor higher photosynthetic rates. Higher net assimilation rates could in turn favor higher crop yields as was found by Radin *et al*. (1994) in Pima cotton (*Gossypium barbadense*). However, the results for photosynthetic rate and stomatal conductance presented by Ashraf (2001) for six *Brassica* species did not show any significant relationship. There are also reports of nonstomatal inhibition of photosynthesis under salt stress. Iyengar and Reddy (1996) reported that this nonstomatal inhibition is due to increased resistance to CO_2 diffusion in the liquid phase from the mesophyll wall to the site of CO_2 reduction in the chloroplast, and reduced efficiency of RUBPC-ase.

Other causes of reduced photosynthetic rates due to salinity have been identified by Iyengar and Reddy (1996) as: (4) enhanced senescence induced by salinity, (5) changes of enzyme activity induced by changes in cytoplasmic structure, and (6) negative feedback by reduced sink activity.

Although the rate of photosynthesis is reduced under salt stress, this is not the cause of reduction in the rate of cell expansion as suggested by several lines of evidence. According to Yeo *et al*. (1991) and Alarcõn *et al*. (1994) growth is reduced more rapidly and at lower concentrations of sodium in the leaf than is photosynthesis. This means that plants can withstand a certain loss in photosynthetic rate without any effect on growth. The relationship between photosynthesis and growth of plants under saline conditions is not well understood. Many changes take place in plants in order to enable them tolerate saline conditions and maintain photosynthetic activity. An understanding of the mechanisms by which salinity affects photosynthesis would aid the improvement of growth conditions and crop yield and would provide useful tools for future genetic engineering.

1.3.5 Effects of salinity on ion levels and nutrient content

High salt (NaCl) uptake competes with the uptake of other nutrient ions, such as K^+, Ca^{2+}, N, P resulting in nutritional disorders and eventually, reduced yield and quality (Grattan and Grieve, 1999). Increased NaCl concentration has been reported to induce increases in Na^+ and Cl^- and decreases in Ca^{2+}, K^+ and Mg^{2+} level in a number of plants (Perez-Afocea *et al*., 1996; Khan *et al*., 2000; Bayuelo-Jiménez *et al*., 2003). Ghoulam *et al*. (2002) observed an increase in Na^+ and Cl^- content in the leaves and roots of sugar beet with increasing NaCl concentration in the rooting medium. The K^+ content of the leaves decreased in response to NaCl, but that of roots was not affected by the salt treatment. A significant increase in Na^+ and Cl^- content in leaves, stem, and root of the mangrove (*B. parviflora*) has been reported without any significant alteration of the endogenous level of K^+ and Fe^{2+} in leaves (Parida *et al*., 2004). Decreases of Ca^{2+} and Mg^{2+} content of leaves have also been reported upon salt accumulation in this species.

Under salt stress conditions, the uptake of N by plants is generally affected. A number of studies have shown that salinity can reduce N accumulation in plants (Feigin *et al*., 1991; Pardossi *et al*., 1999; Silveira *et al*., 2001). An increase in Cl^- uptake and accumulation has been observed to be accompanied by a decrease in shoot NO_3^- concentration as in eggplant (Savvas and Lenz, 1996) and sultana vines (Fisarakis *et al*., 2001). Various authors have attributed this reduction to Cl^- antagonism of NO_3^- (Bar *et al*., 1997) while others attributed the response to salinity’s effect on reduced water uptake (Lea-Cox and

Syvertsen, 1993). The nitrate influx rate or the interaction between NO_3^- and Cl^- has been reported to be related to the salt tolerance of the species under investigation. Kafkafi *et al*. (1992) found that the more salt-tolerant tomato and melon cultivars had higher NO_3^- flux rates than the more sensitive cultivars.

The effect of salinity on P concentration has been reported by Grattan and Grieve (1994) to be highly dependent on plant species, plant developmental stage, composition and level of salinity, and the concentration of P in the substrate. In most cases, salinity decreased the concentration of P in plant tissue (Sonneveld and de Kreij, 1999; Kaya *et al*., 2001), but the results of some studies indicate salinity either increased or had no effect on P uptake (Ansari, 1990). The reduction in P availability in saline soils was suggested by Sharpley *et al*. (1992) to be a result of ionic strength effects that reduce the activity of phosphate, the tight control of P concentrations by sorption processes and by the low solubility of Ca-P minerals.

Salinity stress has stimulatory as well as inhibitory effects on the uptake of some micronutrients by plants. For a detailed review on this subject refer to (Vîllora *et al*., 1997; Grattan and Grieve, 1999). According to these authors nutrient imbalances may result from the effect of salinity on nutrient availability, competitive uptake, transport or partitioning within the plant, or may be caused by physiological inactivation of a given nutrient resulting in an increase in the plant's internal requirement for that essential element.

1.4 Salt tolerance

Plant salt stress resistance has been defined by Shannon and Grieve (1999) as the inherent ability of plants to withstand the effects of high salt concentrations in the root zone or on the leaves without a significant adverse effect. Sacher and Staples (1984) have defined salinity tolerance as the ability of a plant to grow and complete its life cycle on a substrate that contains high concentrations of soluble salt. In this habitat a plant has to meet two requirements: osmotic adaptation and the acquisition of the mineral elements needed for growth and functional metabolism. Levitt (1980) and Shannon *et al*. (1994)

have classified plants into halophytes and glycophytes depending on their sensitivity to salinity. Halophytes are plants that can grow in the presence of high concentrations of salt, even higher than that of seawater (ca. 500mM) and have a competitive advantage over non-halophytes in this environment. Glycophytes on the other hand are plants that are sensitive to relatively low salt concentrations. Almost all major crop species as well as most wild species are glycophytes. Although individual responses to high salinity may differ, several lines of evidence suggest that all plants use the same general salt tolerance regulatory mechanisms, and that the differences between halophytic and glycophytic species are of a quantitative rather than qualitative nature (Greenway and Munns, 1980; Zhu, 2001). Plant sensitivity to salt levels in the soil is also highly depended on environmental factors (Shannon *et al*, 1994), plant species, cultivars within a species (Greenway and Munns, 1980; Ashraf, 2002), as well as the stage of plant development (Vicente *et al*., 2004).

1.4.1 Interactions between salinity and environmental factors

The ability of plants to tolerate salinity depends on the interaction between salinity and environmental factors such as soil, water, and climatic conditions (Shannon *et al.*, 1994). For instance, many crops are less tolerant to salinity when grown under hot and dry conditions than under cool and humid conditions (Maas and Hoffman, 1977). Under hot and dry conditions yield will decrease more rapidly with increasing salinity compared to yield reduction under cool and humid conditions. This is mainly due to decreased ion accumulation and/or improved plant water relations in the latter conditions (Salim, 1989). Hence, a basic understanding of these interactions is necessary for an accurate assessment of salt tolerance.

1.4.2 Differences in salinity resistance among plant genotypes

Crop yield decreases markedly with increase in salt concentration, but the threshold concentration and rate of yield decrease vary with the species. There are marked interspecific differences in crop tolerance for salinity and within a species ecotypes exist that can tolerate much higher salt concentrations than normal populations (Hester *et al*., 2001). The genus *Phaseolus*, for instance, includes important cultivated as well as wild species with diverse ecological adaptations. Cluster analysis by Bayuelo-Jiménez *et al*. (2002) revealed substantial intraspecific and interspecific variation in salinity tolerance. Wild species were generally found to be more salt tolerant than the cultivated species, and many tolerant accessions originated in arid, coastal, or saline areas. Such inter-and intra-crop diversity suggests that field trials should be conducted to identify local crops that are adapted to saline conditions (Shannon, 1997).

1.4.3 Influence of growth stage on salinity resistance

The response of plants to salinity varies with growth stage at which salinization is initiated. However, information about the salt tolerance of crops at different stages of growth is limited. It has been demonstrated that the reaction to salt stress varies with the stage of plant development and that a given cultivar may be tolerant at one stage and sensitive at another (Vicente *et al*., 2004). The available data generally agree that the early seedling stage of growth is the most salt sensitive for most crops (Maas and Poss, 1989; Vicente *et al*., 2004). It is during this stage of growth of cereal crops that leaf and spikelet primodia are initiated and tiller buds are formed (Maas and Grieve, 1990). Consequently, high soil salinity during this stage can severely affect final seed yield. Significant and non-significant associations between tolerance at the germination stage and adult plant growth and development have been indicated (Lovato *et al*., 1994; Bayuelo- Jiménez *et al*., 2002). Although salt stress delays germination and emergence, most crops are capable of germinating at higher salinity levels than they would normally tolerate at the vegetative or reproductive stages of growth (Maas and Grieve, 1990). However, this high tolerance at germination stage is of little benefit when plants are less tolerant during later growth stages.

It has been argued that selection for salinity tolerance at germination, seedling stage or early vegetative growth may not produce tolerant adult plants (Kingsbury and Epstein, 1984). In contrast, the performance of seedlings under saline conditions has been considered highly predictive of the response of adult plants to salinity (Azhar and McNeilly, 1987). Ashraf *et al*. (1986) evaluated seedlings of barley, wheat, and seven forage grass species, and demonstrated considerable tolerance of salinity at the adult stage. Similarly, in studies conducted by Bayuelo- Jiménez *et al*. (2002), five accessions of *Phaseolus filiformis* previously identified as the most tolerant at germination and early seedling growth, were also tolerant during the vegetative growth stage when exposed to 180 mM NaCl. The tolerance observed in this species, however, may or may not be expressed during reproduction. Nevertheless, tolerance observed at germination, early seedling, and vegetative growth stages is of great importance because salinity tolerance at every stage of growth is of value in determining the ultimate tolerance and performance of the species (Shannon, 1984).

1.5 Mechanisms of salt stress resistance

A variety of mechanisms contribute to salt tolerance (Gorham, 1995). Resistance is the ability of plants to adapt to salinity. It can be achieved by the ability of growing cells of a plant to avoid high ion concentrations or the ability of cells to cope with high ion concentrations (Greenway and Munns, 1980). Levitt (1980) characterized these mechanisms as avoidance and tolerance, and has used the term salt resistance to refer to a combination of tolerance and avoidance strategies. Examples of salt avoidance mechanisms include delayed germination or maturity until favorable conditions prevail; the exclusion of salt at the root zone or preferential root growth into nonsaline areas; compartmentalization of salt into and secretion from specialized organelles such as salt glands and salt hairs; or storage in older leaves (Hasegawa *et al*., 1986). These tolerance mechanisms are discussed under separate headings.

1.5.1 Selective accumulation or exclusion of ions

Both glycophytes and halophytes cannot tolerate large amounts of salt in the cytoplasm and therefore under saline conditions they either restrict the excess salts in the vacuole or compartmentalize the ions in different tissues to facilitate their metabolic functions (Iyengar and Reddy, 1996; Zhu, 2003).

In general, exclusion mechanisms are effective at low to moderate levels of salinity, whereas ion accumulation is the primary mechanism used by halophytes at high salt levels, presumably in conjunction with the capacity to compartmentalize ions in the vacuole (Jeschke, 1984). Glycophytes limit sodium uptake, or partition sodium in older tissues, such as leaves, that serve as storage compartments which are eventually abscised (Cheeseman, 1988). Apse *et al*. (1999) reported that removal of sodium from the cytoplasm or compartmentalization in the vacuoles is done by a salt-inducible enzyme Na^+/H^+ antiporter.

Inclusion of ions in the cytoplasm can lead to osmotic adjustment that is generally accepted as an important adaptation to salinity (Guerrier, 1996). The decrease of leaf osmotic potential would compensate the salt-induced lowering of water potential, helping to maintain turgor pressure and cell functions under adverse water conditions. Under salt stress, sugar beet accumulated more inorganic ions in the leaves (Ghoulam *et al*., 2002). Such varieties are qualified as “includers” (Yeo, 1983). Similar results were reported in rice (Lutts *et al*., 1996a) and in sorghum (Colmer *et al*., 1996). The tomato cultivar ‘Daniela’ responded to salinity by decreasing leaf osmotic potential more than ‘Moneymaker’ did and, in this sense, it was considered more adaptable to salty conditions than ‘Moneymaker’ (Romero-Aranda *et al.,* 2001). This accumulation of salt ions could play an important role in osmotic adjustment in stressed plants if they were efficiently compartmentalized. The ability to regulate salt concentration through compartmentalization is an important aspect to salt tolerance.

1.5.2 Synthesis of compatible solutes

The presence of salt in the growth media often results in accumulation of low-molecular-mass compounds, termed compatible solutes, which do not interfere with the normal biochemical reactions (Hasegawa *et al*., 2000; Zhifang and Loescher, 2003). These compatible solutes include mainly proline and glycine betaine (Ghoulam *et al*., 2002, Girija *et al*., 2002; Khan *et al*, 2000; Wang and Nii, 2000).). It has been reported that proline levels increase significantly in leaves of rice (Lutts *et al*., 1996b) and in sugar beet (Ghoulam *et al*., 2002). The increase in proline content in sugar beet was positively correlated to the level of salt tolerance. The proposed functions of proline under stress conditions include osmotic adjustment, protection of enzymes and membranes, as well as acting as a reservoir of energy and nitrogen for utilization during exposure to salinity (Bandurska, 1993; Perez-Alfocea *et al*., 1993a).

Exposure to saline stress results in accumulation of nitrogen-containing compounds (NCC) such as amino acids, amides, proteins, polyamines and their accumulation is frequently correlated with plant salt tolerance (Mansour, 2000). For instance, glycine betaine content has been observed to increase in green gram (Sudhakar *et al*., 1993); in amaranth (Wang and Nii, 2000) and in peanut (Girija *et al*., 2002). According to Sakamoto *et al.* (1998), subcellular compartmentation of glycine betaine biosynthesis in rice is important for increased salt tolerance. These compounds have been reported to function in osmotic adjustment, protection of cellular macromolecules, storage of nitrogen, maintenance of cellular pH, detoxification of the cells and scavenging of free radicals.

Other compatible solutes that accumulate in plants under salt stress include: (a) carbohydrates such as sugars (glucose, fructose, sucrose, fructans) and starch (Parida *et al*., 2002; Kerepesi and Galiba, 2000), and their major functions have been reported to be osmotic adjustment, carbon storage, and radicle scavenging, (b) Polyols are reported to make up a considerable percentage of compatible solutes and serve as scavengers of stress-induced oxygen radicals and are also involved in osmotic adjustment and osmoprotection (Bohnert *et al*., 1995).

According to Greenway and Munns (1980), salt sensitivity in non-halophytes may result from either (i) inability of osmoregulation, which may result from either an insufficient uptake of salt ions or a lack of synthesis of organic solutes being used as osmotica, or (ii) injury caused by inorganic ions which are absorbed by the cell and are not compartmentalized.

1.5.3 Control of ion uptake by roots and transport into leaves

Plants regulate ionic balance to maintain normal metabolism. For example, uptake and translocation of toxic ions such as Na^+ and Cl^- are restricted, and uptake of metabolically required ions such as K^+ is maintained or increased. They do this by regulating the expression and activity of K^+ and Na^+ transporters and of H^+ pumps that generate the driving force for transport (Zhu *et al*., 1993). It is well documented that a greater degree of salt tolerance in plants is associated with a more efficient system for the selective uptake of K^+ over Na^+ (Noble and Rogers, 1992; Ashraf and O'Leary, 1996). It has been reported that a salt tolerant barley variety maintained a cytosolic Na 10 times lower than a more sensitive variety (Carden *et al*., 2003). The tomato cultivar 'Radja' seems to possess a high ability to select and translocate the major nutrients (K^+, Ca^{2+}, Mg^{2+} and NO_3^-) to young leaves under moderate salinity (Perez-Alfocea *et al*., 1996). At high salinity, however, this did not occur for NO_3^-. Thus, decreases in shoot growth observed in this genotype at high salinity could be explained not only by the great amount of toxic ions accumulated in the leaves but also by decrease of NO_3^- in young leaves. Nitrate selectivity over Cl^- in shoot has been correlated with salt tolerance in tomato cultivars (Perez-Alfocea *et al*., 1993a).

The use of plant ionic status to identify salt tolerance has been shown to be applicable (Ashraf and Khanum, 1997), and its relationship with salt tolerance is considered strong enough to be exploited as a selection tool in the breeding of salt tolerant cultivars (Omielon *et al*., 1991).

1.5.4 Changes in photosynthetic pathway under salinity

The reduction in photosynthetic rates in plants under salt stress is mainly due to the reduction in water potential. The main aim of salt tolerance is, therefore, to increase water use efficiency under salinity. To this effect, some plants such as the facultative halophyte (*Mesembryanthemum crystallinum*) shift their C3 mode of photosynthesis to CAM (Cushman *et al*., 1989). This change allows the plant to reduce water loss by opening stomata at night, thus decreasing transpiratory water loss. In salt-tolerant plant species such as *Atriplex lentiformis*, there was a shift from the C3 to the C4 pathway in response to salinity (Zhu and Meinzer, 1999).

1.5.5 Induction of antioxidative enzymes by salinity

All environmental or man-made stresses have been reported to lead to the production of reactive oxygen species (ROS) that cause oxidative damage (Smirnoff, 1993; Schwanz *et al*., 1996). Plants possess efficient systems for scavenging active oxygen species that protect them from destructive oxidative reactions (Foyer *et al*., 1994). As part of this system, antioxidative enzymes are key elements in the defense mechanisms. Garratt *et al*. (2002) has listed some of these enzymes as catalase (CAT), glutathione reductase (GR), superoxide dismutase (SOD) and glutatione-S-transferase (GST). Superoxide dismutase, for example, metabolizes oxygen (O_2) radicals to hydrogen peroxide (H_2O_2), thus protecting cells from damage. Catalase, ascorbate peroxidase, and a variety of peroxidases catalyze the subsequent breakdown of H_2O_2 to water and oxygen (Chang *et al*., 1984; Garratt *et al*., 2002). Plants with high levels of antioxidants have been reported to have greater resistance to this oxidative damage (Spychalla and Desborough, 1990).

Garratt *et al*. (2002) and Mittova *et al*. (2002; 2003) reported increased activities of the antioxidative enzymes in plants under salt stress. They found a correlation between these enzyme levels and salt tolerance. Many changes have been detected in the activities of antioxidant enzymes in plants exposed to salinity. The activity of antioxidant enzymes was reported to increase under saline conditions in shoot cultures of rice (Fadzilla *et al*., 1997), wheat (Meneguzzo and Navarilzzo, 1999) and pea (Hernandez *et al*., 1999), but decreased in wheat roots (Meneguzzo and Navarilzzo, 1999) or was unaffected as in the case of SOD in cucumber (Lechno *et al*., 1997). The differences in these results may be due to the fact that salinity effects depend on a number of factors, for example, salt type, concentration, plant genotype, growth stage and environmental conditions (Shannon *et al*., 1994). The mechanism by which salinity affects the antioxidant responses is not yet clear. Meneguzzo and Navarilzzo (1999), however, proposed that it might be either via (i) the effect of Cl^- toxicity on photosystem II or (ii) the change in membrane integrity caused by a high Na^+ to Ca^{2+} ratio.

The results from these studies give the possibility with which to investigate the biochemical mechanisms, in particular the role of antioxidants, underlying salt tolerance. An understanding of such mechanisms in certain plant species is essential in evaluating the potential gene flow as a means for amending and introducing salt tolerance into crop species.

1.5.6 Induction of plant hormones by salinity

The levels of plant hormones such as ABA and cytokinins increase with high salt concentration (Aldesuquy, 1998; Vaidyanathan *et al*., 1999). Abscisic acid is responsible for the alteration of salt-stress-induced genes, and these genes are predicted to play an important role in the mechanism of salt tolerance in rice (Gupta *et al.*, 1998). The inhibitory effect of NaCl on photosynthesis, growth and translocation of assimilates has been found to be alleviated by ABA (Popova *et al*., 1995). Although the nature of ABA receptor(s) remains unknown Leung and Giraudat (1998) pointed out that there is substantial evidence of the involvement of ABA in reversible protein phosphorylation and modification of cytosolic calcium levels and pH. Chen *et al*. (2001) reported that the increase of Ca^{2+} uptake is associated with the rise of ABA under salt stress and thus contributes to membrane integrity maintenance, which enables plants to regulate uptake and transport under high levels of external salinity in the longer term. ABA has been reported to reduce ethylene release and leaf abscission under salt stress in citrus probably by decreasing the accumulation of toxic Cl^- ions in leaves (Gomezcadenas *et al.*, 2002).

Other plant hormones found to accumulate in the presence of salt include jasmonates. Higher levels of jasmonates were found to accumulate in salt-tolerant tomato cultivars compared to the salt-sensitive ones (Hilda *et al*., 2003). Jasmonates have been reported to have important roles in salt tolerance. They are generally considered to mediate signaling, such as defense responses, flowering, and senescence (Hilda *et al*., 2003). However, factors involved in the jasmonate signal-transduction pathway remain unclear.

1.6 Managing salinity in agricultural production

Saline lands can be converted to more productive croplands by preventing the influx of salt water through proper farm management practices, correcting soil toxicities and nutrient deficiencies, and leaching the salts out of the root zone. The reclamation costs can be reduced by growing salt-tolerant cultivars. These practices are discussed below.

1.6.1 Farm management practices

Salinity can be restricted by changed farm management practices. Munns *et al*. (2002) proposes that irrigated agriculture could be sustained by better irrigation practices such as adoption of partial root zone drying methodology, and drip or micro-jet irrigation to optimize use of water. They suggested that salinity could also be contained by reducing the amount of water passing beyond the roots by re-introducing deep rooted perennial plants that continue to grow and use water during the seasons that do not support annual crop plants. This may restore the balance between rainfall and water use, thus preventing rising water tables and the movement of salt to the soil surface. Deep-rooted perennial lucerne (*Medicago sativa*) has been found to lower the water table sufficiently to allow subsequent cropping (Ridley *et al*., 2001). Such practices will rely on plants that have a high degree of salt tolerance. Salt tolerance in crops will also allow the more effective use of poor quality irrigation water. Niknam and McComb (2000) suggested that trees could be planted to take up some of the excess salt since they have high water use and can lower water tables to reduce salt discharge into streams and prevent secondary salinization of the surrounding areas. However, it has not been proven to what extend the tree planting would assist in preventing salt stress in neighboring fields.

1.6.2 Amelioration through fertilization

Salinity causes nutrient imbalances, mainly resulting in lower concentrations of the macro-elements (N, P, K and Ca) in plant tissues. Hence, the most direct way to recover the normal nutrient concentrations within the plant would be by raising their concentrations in the root zone by higher fertilizer dosages. Many studies have shown that salt stress can be alleviated by an increased supply of calcium to the growth medium (Rausch *et al*., 1996; Ebert *et al*., 2002; Kaya *et al*., 2002). Depending on the concentration ratio, sodium and calcium can replace each other from the plasma membrane, and calcium might reduce salt toxicity (Rausch *et al*., 1996). Song and Fujiyama (1996) found that tomato plants grown in saline medium with supplemental Ca^{2+} accumulated 40% less Na^+ and 60% more K^+ than salinized plants without such supplement.

Increased Na^+ in the growth medium generally decreases the K^+ content, suggesting an antagonism between Na^+ and K^+ (Adams and Ho, 1995). Addition of K^+ to the nutrient solution has been found to raise K^+ concentrations in the leaves and ameliorate salinity stress effects (Lopez and Satti, 1996; Kaya *et al*., 2001). The effect of salinity on P in plants depends on P concentration in the nutrient solution. At high P concentrations, leaf injury has been interpreted as P toxicity induced by salinity (Awad *et al*., 1990). However, at low P concentrations in the root medium, salinity was reported to inhibit P uptake by roots and translocation to the shoot (Martínez *et al*., 1996). At low P concentration in the root medium, supplementary P applied to the saline growth medium enhanced the capacity of tomato plant to regulate Na^+, Cl^- and K^+ distribution, and improved plant growth (Awad, *et al*., 1990; Kaya *et al*., 2001). Under salt stress conditions, the uptake of N by plants is generally affected, and application of supplementary N has been found to ameliorate the deleterious effects of salinity (Gómez *et al*., 1996).

The approach of raising fertilizer dosages may work for irrigation with water at low salt concentrations. When water of high salinity is applied, however, the concentration of antagonistic ions required is so high that it causes a marked increase in the osmotic pressure of the soil solution, compounding the stress imposed by the salinity ions (Feigin, 1985). Furthermore, Grattan and Maas (1988) reported that in some species a very high concentration of nutrients, e.g. P, could interact negatively with salinity ions, resulting in severe toxic effects.

1.6.3 Leaching

Leaching soils to remove soluble salts is the most effective method known to reclaim saline soils. This requires good permeability of the soil and good quality irrigation water. Removal of salts by leaching reduces salt hazard for plants but might cause permeability to decrease and pH to increase resulting in decomposition of roots as soil is changed from saline-sodic to sodic (Dregne, 1976). Although the best long-term solution to salinization is to provide adequate drainage, this process is expensive. Hence, many irrigation schemes, particularly in developing countries lack, adequate drainage (Toenniessen, 1984).

1.6.4 Uses of salt stress tolerant plants

Some areas have naturally occurring salinity and salt-tolerant crop plants may provide a better or perhaps the only means of utilizing these resources for food production. Salinity can possibly also be managed through biologically manipulating the plants (Shannon, 1984). Identification of plant genotypes with tolerance to salt, and incorporation of desirable traits into economically useful crop plants, may reduce the effects of salinity on productivity. Developing crop plants tolerant to salinity has the potential of making an important contribution to food production in many countries. This will permit the use of low quality water and thereby reduce some of the demand for higher quality water. Great effort is, therefore, being directed toward the development of salt-tolerant crop genotypes through the use of plant-breeding strategies involving the introgression of the genetic background from salt-tolerant wild species into cultivated plants (Shannon, 1984; Pitman and Laüchli, 2002). However, it should be borne in mind that there is also the risk that the availability of salt tolerant genotypes will result in less effort to reclaim saline areas or to prevent salinization. In the longer term this will be counter productive.

1.7 The Amaranth

Amaranth is native to South and Central America where its cultivation by the Aztecs dates back 5000 to 7000 years ago (Kauffman and Weber, 1990; Stallknecht and Schulz-Schaeffer, 1993). Amaranth was both an important food crop for the Aztecs, and an important item in their religious ceremonies (Myers, 1996). Currently, amaranths are widely grown as a green leafy vegetable or as grain crop in many parts of sub-tropical and tropical Asia, Africa and Central America. According to Feine *et al*. (1979) this is probably due to the ability of these plants to adapt readily to new environments and extremely broad climates, as well as their competitive ability that permits culture with minimum crop management.

Amaranth comes in different forms. Some species have colored leaves, stems and flowers of purple, orange, red and gold (National Research Council [NRC], 1984). Several ornamental forms of this species are widely grown all over the world. Growth habits vary from prostrate to erect and branched to unbranched while leaf and stem colors range from red to green, with a multitude of intermediates; and seed colors range from black to white (NRC, 1984; Kochhar, 1986). This group of plants belongs to the family *Amaranthaceae*, contains about 800 species and is divided into grain and vegetable types (Allemann *et al*., 1996).

1.7.1 Grain Amaranth

Amaranth is one of the few non-grasses with the potential of becoming a cereal-like grain crop. The seedheads resemble those of sorghum (Figure 1.1) and the individual seed is extremely tiny and barely bigger than a mustard seed (0.9 to 1.7 mm in diameter). Seeds occur in massive numbers, sometimes more than 50,000 to a plant, and are cream colored, golden or pink (NRC, 1984). The three principal species considered for grain production are: *Amaranthus hypochondriacus*, *A. cruentus* and *A. caudatus*, which are distinguished by morphological characteristics of the inflorescence and florets (NRC, 1984). *Amaranthus cruentus* is thought to be the most adaptable of all amaranth species and flowers under a wide range of day lengths than the others. It is also often grown as a vegetable and an ornamental (Figure 1.1), while *A. hypochondriacus* has excellent seed quality and shows the greatest potential for use as a food ingredient (NRC, 1984).

Figure 1.1 *Amaranthus cruentus* in flower

1.7.2 Vegetable Amaranth

There is no distinct separation between grain and vegetable amaranth types. In general, grain forms have light seeds and vegetable forms have dark seeds. However, NRC (1984) and Kochhar (1986) have listed amaranth grown for vegetables as: (a) *Amaranthus tricolor* L. (Syn. *A. oleraceus* L., *A. gangeticus* L.) which is grown mainly in East Asia and is probably the best developed of the vegetable amaranth species. The plants are succulent, low growing, and compact, with growth habits much like spinach. Some ornamentals with very beautiful foliage also belong to this species, (b) *A. dubius* is a weedy species used as a green vegetable in West Africa and the Caribean. Its seeds are extremely small, and it has distinctive dark-green, broad ridged leaves, (c) *A. hybridus* is a weedy species commonly used as a leafy vegetable throughout tropical areas. Its size and color vary greatly. Red-stemmed varieties are usually planted as ornamentals while green varieties are commonly used as vegetables, and (d) *A. lividus* is well adapted to temperate climates. It has a number of weedy forms with either red or green leaves.

1.7.3 Weedy species

Only a limited number of all the amaranth species are used as crops. The majority of the others are considered weedy species. The main weedy types are *A. viridis, A. spinosus. A. retroflexus,* and *A. hybridus* (NRC, 1984). *Amaranthus retroflexus* (pigweed) is considered one of the world's worst weeds. The difference between the weedy species and the cultivated types is the fact that the former tend to be indeterminate and robust in growth habit.

1.7.4 Utilization and nutritional importance

1.7.4.1 Vegetable amaranth

The leaves, petioles and young tips of all *Amaranthus* species (including the weedy types) are edible and several species are already widely used as salads and as potherbs (boiled greens) (Grubben, 1976; NRC, 1984; Larkcom, 1991). As a vegetable, Amaranth is nutritionally more valuable than most spring and summer vegetables. According to Allemann *et al*. (1996), 100g portion of amaranth provides the same amount of vitamins as 600g of swiss chard or 280g of cabbage. It also provides more energy, protein, minerals and vitamins (especially vitamin A and C) than other leafy vegetables (Larkcom, 1991; Food Gardens Foundations, 1994; Allemann *et al.*, 1996). In terms of mineral content, notably iron and calcium, amaranth greens rank particularly well when measured against other potherbs (Makus, 1984; Makus, 1990; Table 1.2). High levels of the nutritionally valuable amino acids, lysine and methionine, have been found in 13 amaranth species (Saunders and Becker, 1983). Vegetable amaranths are also an important source of vitamins especially vitamin A, the lack of which results in a serious nutritional deficiency in the tropics, and leads to blindness and even death in thousands of children each year (NRC, 1984; Okigbo, 1990).

Table 1.2 Nutrient content of selected raw vegetable leaves (per 100g of edible portion) (Saunders and Becker, 1983)

Component	**Amaranth**	**Spinach**	**Basella**	**Chard**
Dry matter (g)	13.1	9.3	6.9	8.9
Food energy (cal)	36	26	19	25
Protein (g)	3.5	3.2	1.8	2.4
Fat (g)	0.5	0.3	0.3	0.3
Carbohydrates				
Total (g)	6.5	4.3	3.4	4.6
Fiber (g)	1.3	0.6	0.7	0.8
Ash (g)	2.6	1.5	1.4	1.6
Calcium (mg)	267	93	109	88
Phosphorus (mg)	67	51	52	39
Iron (mg)	3.9	3.1	1.2	3.2
Sodium (mg)	----	71	----	14.7
Potassium (mg)	411	470	----	550
Vitamin A (IU)	6,100	8,100	8,000	6,500
Vitamin C (mg)	80	51	102	32
Riboflavin (mg)	0.16	0.2	----	0.17
Niacin (mg)	1.4	0.6	0.5	0.5
Thiamin (mg)	0.08	0.1	0.05	0.06

1.7.4.2 Grain Amaranth

According to the NRC (1984) amaranth seed can be used in several ways, for example, as cereals or as an ingredient in confection. When heated, the tiny amaranth grains pop and taste like a nutty-flavored popcorn. The seeds can be milled to produce a light-colored flour suitable for biscuits, bread, cakes and other baked goods. However, since amaranth grain is known to contain little functional gluten, it must be blended with wheat flour to make yeast leavened baked goods “rise” (NRC, 1984).

Amaranth produce a high protein seed compared to other non-legume grain crops. Its valuable source of protein exceeds that of wheat or any other cereal grain, including that of lysine, which is normally low in grains (NRC, 1984; Pedersen *et al*., 1987). Saunders and Becker (1983) reported that amaranth protein itself is low in leucine. However, this amino acid is found in excess in conventional plant protein sources, hence, it is a nutritional complement to conventional cereals. As a result, amaranth grain has been incorporated into a range of human food products, which are primarily targeted at health conscious consumers (Pedersen *et al.*, 1987; Breene, 1991).

Amaranth grain consists of approximately 5 to 9% oil, which is generally higher than in other cereals. The lipid fraction of amaranth grain is similar to that of other cereals. Detailed studies and a review on amaranth grain oil have been published (Lyon and Becker, 1987; Becker, 1989). NRC (1984) reported that unprocessed amaranth grain can be used as an animal feed, particularly for poultry.

1.7.5 Physiology of the amaranth

Evidence indicates that amaranths are adapted to many environments and tolerate adversity because they use an especially efficient type of photosynthesis (Wang *et al*., 1992; 1993). Amaranth is one of the few C4 species that are not grasses. The C4 pathway is a modification of the normal (C3) photosynthetic process that makes efficient use of the CO_2 available in the air by concentrating it in the chloroplasts of specialized cells surrounding the leaf vascular bundles (Kanai and Edwards, 1999). In C3 species the primary carbon fixation enzyme is ribulose-1,5-biphosphate carboxilase/oxygenase (RuBPC-ase) and the first product of carboxylation is a three carbon molecule, 3-phosphoglyceric acid (3-PGA), hence the name C3. In C4 plants, the initial fixation of atmospheric carbon dioxide produces three C4 acids (malate, aspartate, oxaloacetate), hence the name C4 (Leegood, 1999). This is accomplished by the enzyme phosphoenolpyruvate carboxylase (PEPC-ase). There are a number of advantages associated with the C4 photosynthetic pathway.

The photorespiratory loss of CO_2, the basic unit for carbohydrate production, is suppressed in C4 plants. According to Gardner *et al*. (1994) and Leegood (1999), the C4 species are believed to have little or no photorespiration because movement of the four-carbon acids into the vascular sheath cells concentrates CO_2 in these cells, which would favor the RuBP carboxylase reaction over RuBP oxygenase. Consequently, plants that use the C4 pathway can convert a higher ratio of atmospheric carbon to plant sugars than those that possess the classical C3 pathway. In C4 species photosynthesis can operate at low intercellular concentrations of CO_2, and hence, lower stomatal conductance. This means that the C4 plants can restrict water loss to a minimum, and yet photosynthesize at rates equivalent to those of C3 plants (Leegood, 1999). Thus, water use efficiency of C4 plants is roughly double that of C3 plants.

One of the physiological features noted of C4 plants is their high rate of photosynthesis at full sunlight under tropical conditions (Hatch, 1992). Photosynthesis in C3 species at light saturation is often limited by the quantity of RuBPC-ase and by the capacity for regeneration of the acceptor RuBP molecule (Evans and Farquhar, 1991). The maximum rate of CO_2 assimilation that both of these limitations can support is lowered by oxygenation of RuBP and the resulting photorespiratory evolution of CO_2 (Leegood and Edwards, 1996). C4 species avoid most of this loss by concentrating CO_2 at the site of Rubisco in the bundle sheath (Leegood, 1999). This allows C4 species to attain potentially higher photosynthesis in full sunlight. C3 species reach light saturation at much lower light intensities than that of full sunlight.

As a result of CO_2 concentration at the site of Rubisco in C4 species, the requirement for nitrogen in photosynthesis is lower than in C3 species (Hocking and Meyer, 1991). The C4 pathway is particularly efficient at high temperature, in bright sunlight, and under dry conditions. Plants that use it tend to require less water than C3 carbon-fixation plants. For these reasons amaranth may be a promising crop for hot and dry areas which are also more often saline.

1.7.6 Salinity studies in amaranth

Relatively few salinity stress studies have been conducted with amaranth. Murata *et al*. (1992) and Brownell and Bielig (1996) reported that the plant family *Amaranthaceae*, to which amaranth belongs, is one of the three families of higher plants which are natrophilic, i.e., having a sodium requirement for growth. Sodium ions have been found to stimulate the regeneration of phosphoenolpyruvate in mesophyll chloroplasts of *A. tricolor* (Murata *et al*., 1992). However, excess amounts of sodium, usually in conjunction with chloride, can reduce plant growth through many physiological and biochemical processes (Match *et al*., 1986; Volkmar *et al*., 1998; Hasegawa *et al*., 2000; Kashem *et al*., 2000a, b). *Amaranthus tricolor* has been reported to germinate at concentrations of 250 mM NaCl (EC ~ 25 dS m^{-1}) (Macler *et al*., 1990), and in sand culture greenhouse experiments, *A. tricolor* has been judged ‘relatively salt-tolerant’ when grown with nutrient solutions containing 0 to 60 meq./L NaCl (EC ~ 0 to 6 dS m^{-1}) (Shimose *et al*., 1991). Salt tolerance has also been reported for *A. tricolor* by Wang *et al*. (1999) and Wang and Nii (2000) who suggested that amaranth may be of value as a crop for salt affected regions.

In field-grown *Amaranthus* spp., Gaikwad and Chavan (1995; 1999) found that increasing the salinity of the irrigation water from 4 to 6 dS m^{-1} reduced plant carbohydrates, soluble and total oxalates, and nitrates at both vegetative and flowering stages of growth. Increasing the salinity of irrigation water to 16 dS m^{-1} resulted in decreased leaf transpiration, diffusive resistance to CO_2, and more negative osmotic potential in field-grown *A. caudatus*, *A. hypochondriacus*, and *A. paniculatus* (Gaikwad and Chavan, 1998). The effect of salinity on accumulation of glycine betaine (GB) in *A. tricolor* was investigated by Wang and Nii (2000). These authors found that GB increased several days after salt stress, supporting the notion that the compound is active in the process of osmotic adaptation to salinity in *Amaranthus*. The results were interpreted as evidence that the level of GB in *Amaranthus* leaves is roughly regulated by the salt concentration in the root zone (Wang and Nii (2000).

In a more recent study Makus (2003) investigated the effect of salinity and nitrogen level on agronomic performance of *A. tricolor* and found that supplemental N improved yield and leaf greenness in response to higher soil salinity. It is evident that differences in the tolerance of amaranth genotypes to salinity, particularly at different growth stages, have not been sufficiently researched. Although salinity is prevalent in arid areas the response of amaranth to the interactive effect of salinity and water stress has not yet been documented. With increasing salinity an integrated approach in dealing with the problem may be required. One approach will be to come up with ways to ameliorate salinity stress effects. However, little has been reported concerning amelioration of salinity stress in amaranth.