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Effect of salt stress on osmolyte accumulation in two groundnut cultivars (*Arachis hypogaea* L.) with contrasting salt tolerance

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The effects of salt stress on the level of osmolyte accumulation in two different cultivars (K-134 and JL-24) of groundnut seedlings were studied. Seeds were grown at different concentrations of NaCl stress: 50, 100 and 150 mM and their respective controls (0.0% NaCl) for nine days. Salt stress resulted in a significant modification in the level of osmolyte accumulation in the two cultivars of groundnut. The accumulation level of osmolytes such as proline, glycine betaine, soluble sugars, free amino acids and polyamines were increased significantly in both cultivars with increasing stress severity and duration when compared with their controls. However, the percent increase of osmolyte accumulation was higher in cv. K-134 and lower in cv. JL-24. The present study indicated that cv. K-134 is salt tolerant than cv. JL-24 based on osmolyte accumulation and growth parameters. The osmolyte accumulation in relation to the salt tolerance of these cultivars was discussed.

Key words: Osmolytes, salt tolerance, groundnut, cultivar.

INTRODUCTION

Soil salinity is one of the most significant agricultural problems in arid and semi-arid regions in different parts of the world. Nearly 20 to 40% of the world's cultivated area and half of the world's irrigated land are affected by salinity (Rhodes and Loveday, 1990; Flowers, 2004). The economic prosperity of a nation like India, where a majority of population is primarily dependent on agriculture depends on crop productivity. Soil salinity creates extremely unfavorable conditions for plant growth and development. In response to various environmental stresses such as salt and drought stresses, plants have developed different physiological and biochemical mechanisms to adapt or to tolerate stress (Bartels and Salamini, 2001; Rahnama and Ebrahimzadeh, 2004; Faical et al., 2009). An understanding of the mechanism of plant salt tolerance will lead to effective means to breed or gene-tically engineer salt tolerant crops. Indeed, osmolyte accumulation in plant cells results in decrease of the cell osmotic potential and thus in maintenance of water absorption and cell turgor pressure (Blum et al., 1983). A number of investigators (Xiong and Zhu, 2002; Rahnama and Ebrahimzadeh, 2004) have reported that the osmotic adjustment in plants subjected to salt stress occurs by the accumulation of high concentrations of osmotically active compounds known as osmolytes such as proline, glycine betaine, soluble sugars, free amino acids, polyamines, etc, in order to lower the osmotic potential.

Osmoprotectants serve to raise osmotic pressure in the cytoplasm and can also stabilize proteins and membranes when salt levels or temperatures are unfavorable,

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therefore osmoprotectants can play an important role in the adaptation of cells to various adverse environmental conditions (McNeil et al., 1999; Jagesh et al., 2010).

Proline accumulation was found to be an early response to salt stress, which acts as an osmotic protectant and increased accumulation shows greater tolerance to salt and drought stress (Fedina et al., 2002). Increased levels of proline contribute to the turgor maintenance of cells and its accumulation is considered as a stress indicator in several plant species under salt stress conditions (Giridarakumar et al., 2003; Jagesh et al., 2010). Glycine betaine is one of the quaternary ammonium compounds and is regarded as an effective compatible solute that accumulates in the chloroplasts of certain plants when exposed to environmental stresses, such as drought and salinity. It could play a major role in maintaining intracellular osmotic equilibrium during stress conditions (Subbarao et al., 2001; Giridarakumar et al., 2003). The accumulation of soluble sugars in plants has been responsible for salinity or drought stress and act as osmoprotectants (Murakeozy et al., 2003). According to Cram (1976), sugars contribute up to 50% of the total osmotic potential in glycophytes subjected to the saline conditions. Further, the amino acid accumulation associated with stress may actually be a part of an adaptive process contributing to osmotic adjustment and increased level of free amino acids together with organic acid and ammonium compounds serve as compatible cytoplasmic solutes to maintain the osmotic balance under stress conditions (Dubey, 1994).

Furthermore, the increase in total polyamine content in plants has been shown to occur in a variety of plant species in response to salt stress (Pedro et al., 2004). Polyamines are cationic molecules, positively charged under intracellular pH, which are essential for plant growth and differentiation. It has been reported that polyamines in plants protect plasma membrane under salinity stress and thus enhance salt tolerance (Mansour and Al-Mutawa, 1999).

Groundnut (*Arachis hypogaea* L.) is an important oilseed cash crop for all tropical and sub-tropical regions of the world. Anantapur, a district in Andhra Pradesh, India, occupies the first place in groundnut cultivation with 1.88 million hectares and the production of about 1.2 million tones (www.icrisat.org). Information is lacking regarding the relative levels of salt tolerance among the existing groundnut cultivars. Hence, the present study was aimed to make comparative analysis of tolerance potentials based on osmolyte accumulation in two different groundnut cultivars differing in salt tolerance. Further, these lines are used in improving programmes; it seems to be effective and economic improvement.

MATERIALS AND METHODS

Seeds of groundnut (*A. hypogaea* L.) cultivars namely (cv. K-134 and cv. JL-24) were procured from Acharya N.G.Ranga Regional research station, Kadiri, Andhra Pradesh, India. Seeds were surface sterilized with 0.1% (w/v) mercuric chloride for 5 min and thoroughly rinsed with distilled water and allowed to germinate in Petri dishes of 15 cm diameter lined with filter papers. Distilled water alone served as control, while for stress treatment, NaCl solution was added at concentrations of 50, 100 and 150 mM and kept in dark at 24±1°C under aseptic conditions for three days. After three days the Petri dishes were transferred to a programmed plant growth chamber under controlled conditions (light/dark regime of 16/8 h at 24±1°C, relative humidity of 60-65%, photon flux density 350 µmol m^{-2} s^{-1}) up to nine days. After nine days total seedlings were used for experimental analysis.

Free proline content was extracted from both control and stressed groundnut seedlings in 3% aqueous sulfosalicylic acid and estimated by Bates et al. (1973) using ninhydrin reagent. Quarternary ammonium compounds were extracted and measured as glycine betaine from 0.5 g of dried material, according to Grieve and Grattan (1983). Soluble sugars were extracted following the method of Highkin and Frankel (1962) from 0.5 g of dried tissues in 80% ethanol by heating at 80°C for 30 min and centrifuged, then supernatant was used for the estimation of total soluble sugars following Nelson (1944) method as modified by Somogyi (1952). The oxidation of sugars by copper was monitored at 500 nm by formation of colorimetric complex of arsenic and molybdenum salts, using UV-visible spectrophotometer (helios-α thermospectronic).

The extraction and estimation of amino acids were determined spectrophotometrically according to Moore and Stein (1948). The extraction and estimation of total polyamine content was done according to Seiler and Wiechmann (1967). 0.5 g of plant material was homogenized with 5 ml of 5% perchloric acid ($HClO_4$), centrifuged at 12000 rpm for 20 min. Supernatant fraction was dansylated, and then benzene extract was separated on TLC plates coated with silica gel by using chloroform : triethylamine (25:2 v/v) as solvent system. Polyamines were identified with the help of authentic samples. The spots were eluted with ethyl acetate and quantified using a UV-visible spectrophotometer (Thermo- Spectronic, USA).

RESULTS

Seedling growth

The growth of the seedlings (shoot and root length) was measured in both control and stressed conditions on day 9 (Figure 1). The total seedling growth was decreased in both cultivars during salt stress. However, the inhibition of seedling growth was found to relatively less in cv. K-134 than cv. JL-24 during severe stress treatment (150 mM NaCl).

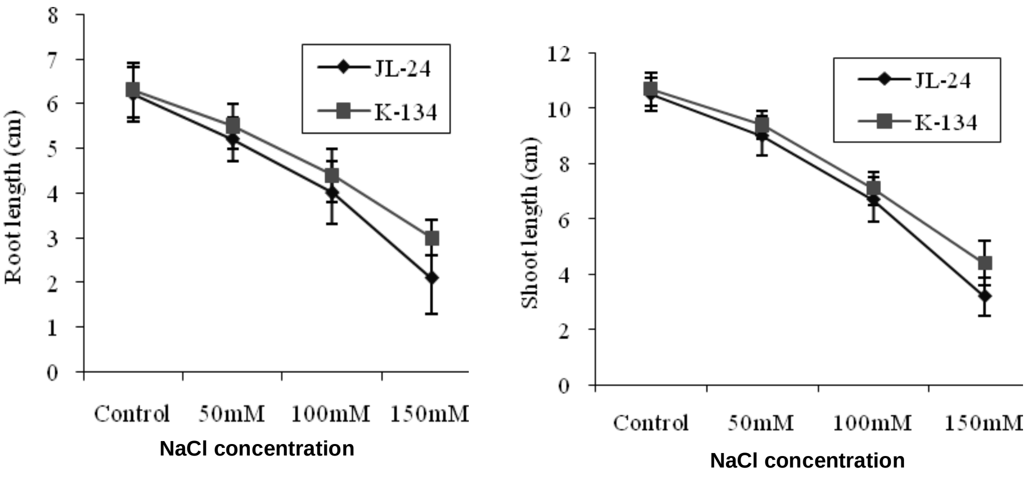
Free proline content

Free proline content was estimated in control and NaCl stressed seedlings of two groundnut cultivars and data are presented in Figure 2. The free proline content was significantly increased in stressed plants over control plants. Nevertheless, a significant difference was found in free proline accumulation between the cultivars by about 3.0 and 2.6 fold in cv. K-134 and cv.JL-24 respectively at 150 mM NaCl stress when compared to their respective controls.

However, the percent increase was comparatively more in cv. K-134 than in cv. JL-24.

Glycine betaine

The level of glycine betaine content was significantly increa-



a

b

Figure 1. Root length and shoot length in two cultivars of groundnut under control and NaCl stress. (a) Root length (cm); (b) shoot length (cm). Values are mean of five replications. Vertical bars indicate ±S.D.

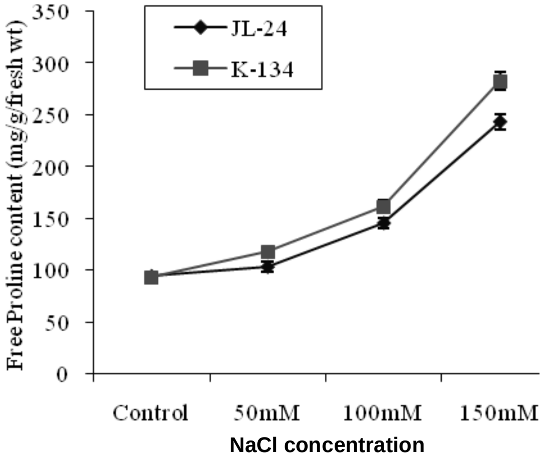


Figure 2. Free proline content in two cultivars of groundnut under control and NaCl stress. Values are mean of five replications. Vertical bars indicate ±S.D.

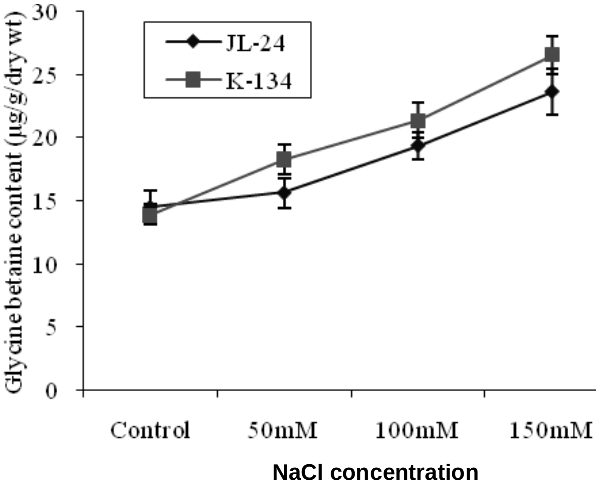


Figure 3. Levels of quaternary ammonium compounds (glycine betaine equivalents) in two cultivars of groundnut under control and NaCl stress. Values are mean from five replications. Vertical bars indicate ±S.D.

sed in both cultivars at all stress regimes. However, the rate of increase was significantly different in both cultivars (Figure 3). The rate of increase in glycine betaine content was found to be higher in cv. K-134 than cv. JL-24 at severe stress level (150 mM NaCl).

Soluble sugars

Total soluble sugar content was increased with increasing severity of stress in both cultivars of groundnut (Figure 4). However, the degree of increase in soluble sugar content was dependent on species tolerant potential and stress severity. There was, by about 3.3 fold increase in cv. K-134, 2.5 fold increase in cv. JL-24 at 150 mM NaCl stress when compared with their respective controls.

Total amino acids

The pool sizes of amino acid levels were increased significantly in both cultivars at all stress regimes (Figure 5). However, the degree of increase in free amino acid contents was more in cv. K-134 than cv. JL-24. Therefore, the accumulation of total amino acids showed increase by

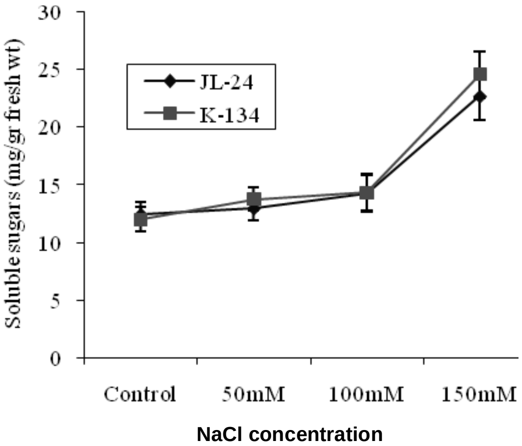


Figure 4. Levels of soluble sugars in two cultivars of groundnut under control and NaCl stress. Values are mean of five replications. Vertical bars indicate ±S.D.

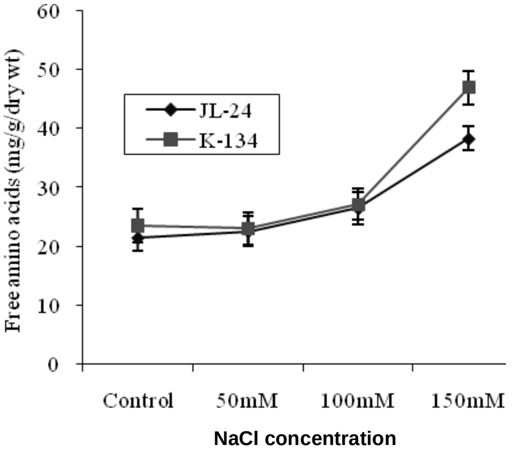


Figure 5. Total amino acid content in two cultivars of groundnut under control and NaCl stress. Values are mean of five replications. Vertical bars indicate ±S.D.

about 2.1 fold in cv. K-134, 1.8 fold in cv. JL-24 at severe stress (150 mM NaCl).

Total polyamines

Total polyamine content was increased significantly with severity of stress in all cultivars (Figure 6). Nevertheless, a difference in the accumulation of total polyaminecontent was observed between the cultivars. However, the percent increase was found to be higher in cv. K-134 than in cv. JL-24.

DISCUSSION

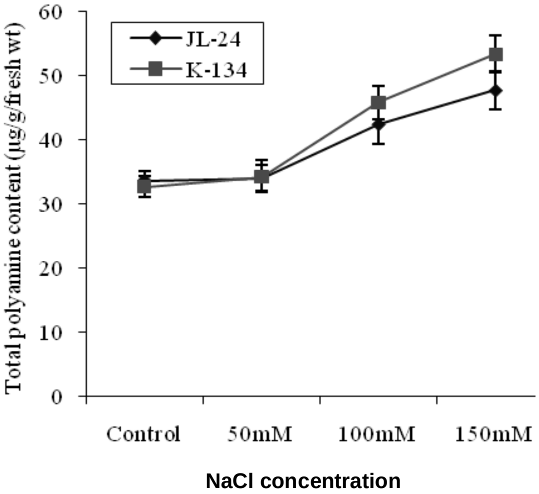


Figure 6. Total polyamine content in two cultivars of groundnut under control and NaCl stress. Values are mean of five replications. Vertical bars indicate ±S.D.

There is a general agreement that the whole plant growth responses to salinity is multigenic and that a better knowledge of the underlying physiology is required in order to understand why some plant species and varieties are more salt resistant than others. This is a complex task since plant growth responses to salinity can vary with degree and duration of stress, plant organ, variety or species and developmental stage (Neumann, 1997). It has been shown that the stress caused by salts present in the soil alters water status and brings about initial growth reduction of the plant (Yeo, 1998; Fatemeh et al., 2010). One of the classic manifestations of salt stress in many plant species is marked reduction in plant height, due to the osmotic effects of the salt outside the roots which distinguishes a salt-susceptible plant from a more tolerant one (Munns et al., 1995). Similarly, in the present study, we recorded reduced growth during stress conditions and degree of reduction in seedling growth was dependent on intensity or severity of stress. It revealed that the 150 mM NaCl stress treatment has caused significant reduction in both cultivars, but more pronounced reduction was found in cv. JL-24 than cv. K-134 (Figure 1). Several investigators (Mishra et al., 1996; Fatemeh et al., 2010) reported reduced growth in different plant species under salt stress. This reduced growth under salinity stress has been ascribed either to osmotic or ionic effects; inhibition of cell division and cell elongation process associated with the growth of the seedling and decrease in plastic extensi-bility of the growing cell walls.

The accumulation of compatible solutes may help to maintain the relatively high water content obligatory for plant growth and cellular functions. Osmotic and oxidative stress induced by salinity could be reduced by the production and accumulation of compatible solutes. Osmoprotectants and their accumulation play a key mechanism in the plants for increasing yield of crops subjected to stress conditions. The levels of osmoprotectants increased during exposure to stresses such as salinity, water deficit, and low temperature (McNeil, 1999). The frequently observed metabolites with an osmolyte function are proline, glycine betaine, soluble sugars, free amino acids and polyamines.

The accumulation of proline is an early response to salt stress (Fedina et al., 2002). Several investigators (Delauney and Verma, 1993; Kavikishor et al., 1995) have demonstrated that the positive correlation was found between the accumulation of proline and osmoprotective role at the whole plant level and cell cultures. Convincing evidence is still lacking as to whether accumulation of proline can provide any biochemical adaptation for plants during stress. Giridarakumar et al. (2003) in mulberry, Veeranagamallaiah et al. (2007) in foxtail millet and Fatemeh et al. (2010) in potato demonstrated the differences in proline accumulation and a positive correlation between magnitude of free proline accumulation during salt and water stress. Similarly, in the present study, we have noticed a positive correlation between salt stress and free proline accumulation between the two groundnut cultivars (Figure 2), however a greater accumulation rate of free proline content (3.7 fold) was found in salt tolerant cv. K-134, where as the salt sensitive cv. JL-24 showed lesser accumulation rate (2.0 fold). The result obtained in this study further strongly supports Fatemeh et al. (2010) who reported the increased accumulation of in tolerant potato variety. Free proline accumulation and salt tolerance has been suggested as an index for determining salt tolerance potentials between the cultivars (Sudhakar et al., 1993; Giridarakumar et al., 2003; Veeranagamallaiah et al., 2007). In contrary, very few reports for instance Lutts et al. (1999) reported that salt sensitive cultivars accumulated significantly higher levels of proline accumulation compared to the tolerant ones.

Glycine betaine is regarded as an effective compatible solute that accumulates in the chloroplast of plants, when exposed to environmental stresses (Sawahel, 2004). Here, we reported on a positive correlation between glycine betaine accumulation and salt stress and also observed a genotypic variation in glycine betaine accumulation in two groundnut cultivars (Figure 3). An increased accumulation of glycine betaine content was noticed in tolerant cv. K-134 than cv. JL-24. Parallel to these results, an increase in glycine betaine content with increasing salt stress was found in green gram and mulberry (Sudhakar et al., 1993; Giridarakumar et al., 2003).

Several investigators have noticed that accumulation of glycine betaine under salt stress was found to be high in salt tolerant species (Jagendorf and Takabe, 2001). Besides osmoregulation glycine betaine stabilizes the oxygen evolving activity of photosystem-II protein complexes at high concentration of NaCl. The major role of glycine betaine might be to protect membranes and macromolecules from damaging effects of stress (Sawahel, 2004).

Soluble sugars have been specified as potential osmoregulators (Raggi, 1994). Elevated sugar levels relative to control in salt stressed plants may contribute to the turgor maintenance (Sacher and Staples, 1985). In the present study, the amount of elevated soluble sugars was relatively higher in tolerant cv. K-134 and lesser in salt sensitive cv. JL-24 at severe stress treatments (Figure 4). In analogy, several investigators noticed that soluble sugar levels were increased with increased level of salt stress, (Dubey and Singh, 1999; Murakeozy et al., 2003). Furthermore, Jouve et al. (2004) observed a higher accumulation of soluble sugars in aspen at 150 mM NaCl stress.

The changes in accumulation of free amino acid content induced by salt stress have an important role, since these relations were obtained several times in a relationship with stress tolerance by Livia et al. (2002) in cereal plants. Survival and growth of plants in saline environments is the result of adaptive processes such as ion transport and compartmentation of osmotic solute, synthesis and their accumulation lead to the osmotic adjustment and protein turnover for cellular repair (Munns and Termaat, 1986). In the present study, we have noticed the existence of variation in the accumulation of amino acid levels among the cultivars studied; the extent of increase was greater in the tolerant cv. K-134 than salt sensitive cv. JL-24 (Figure 5). Similar results were obtained by Livia et al. (2002) in cereal plants and Ramanjulu and Sudhakar (1997) in mulberry. Varietal variations in the magnitude of accumulation of amino acids have been taken as an index for determining the salt tolerant potentials of many crops (Madhusudan et al., 2002). Improved levels of free amino acids together with organic acids and quaternary ammonium compounds serve as compatible cytoplasmic solutes to maintain the osmotic balance under stress conditions (Dubey, 1994).

Polyamines are known to be involved in various cellular processes (Rajam et al., 1998) and they are ubiquitous aliphatic amines that are implicated in many aspects of plant growth and developments in a wide range, and play an important role in stabilizing the plasma membrane under salt stress condition (Galston and Sawhney, 1995). Effect of salt stress on polyamine metabolism is not always clear, since differences in polyamine accumulation in response to salt stress have been reported among and within the species (Pedro et al., 2004). Here, we noticed a positive correlation between salt tolerance and accumulation of higher levels of polyamines and exhibited genotypic variation (Figure 5). However, a greater accumulation was found in tolerant cv. K-134 than salt sensitive cv. JL-24. Similarly, Chattopadhaya et al. (2002) and Fatemeh et al. (2010) noticed a higher accumulation of polyamine content with varying levels in seven different plant species under salinity stress.

From this study, it is clear that cv. K-134 shows better salt tolerant nature as compared to cv. JL-24 based on above results through physiological and biochemical marker traits. Interesting features found through these results must be related to salt stress response and should be considered as general salt stress reaction markers for groundnut. Moreover, further independent analysis of molecular level may help in understanding the salt tolerant potentials of groundnut cultivars for breading programmes in future.

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