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Introduction

Different types of edaphic factors affect plant growth. Salinity is one of the most important stress factors which limits the growth and development of plants by altering their morphological, physiological and biochemical attributes, according to United States Department of Agriculture Salinity Laboratory (Mudgal *et al.*, 2010).

Salinity refers to the total concentration of all salts in the water or the soil. Soil sodicity represents the relative preponderance of exchangeable sodium compared to other exchangeable cations like calcium, magnesium, potassium, hydrogen and aluminium. Sodium Absorption Ratio (SAR) is a measure of dissolved sodium in water compared to the amounts of dissolved calcium and magnesium (Van De Graaff and Patterson, 2001).

According to FAO land and plant nutrition management, the total world area under salinity status is 397×10^6 hectare and almost in 1500×10^6 hectare of dry land agriculture about 2% are salt affected (Dajic, 2006).

Salinization of soil is a serious problem and is increasing steadily in many parts of the world, in particular in arid and semiarid areas (Giri *et al.*, 2003). Saline soils occupy 7% of the earth's land surface (Ruiz-Lozano *et al.*, 2001) and increased salinization of arable land will result up to 50% land loss by the middle of the 21st century (Wang *et al.*, 2003). At present, out of 1.5 billion hectares of cultivated land around the world, about 77 million hectares (5 %) is affected by excess salt content (Sheng *et al.*, 2008). High levels of salinity in soils is mainly due to the soluble salts in irrigation water and fertilizers used in agriculture (Al-Karaki, 2000), low precipitation and high temperature in these regions and over-exploitation of available water resources (e.g. ground water) (Cantrell and Linderman, 2001; Al-Karaki, 2006; Mouk and Ishii, 2006). The significance of soil salinity for agricultural yield is enormous (Tester and Davenport, 2003) as it affects the establishment, growth and development of plants leading to huge losses in productivity (Giri *et al.*, 2003; Mathur *et al.*, 2007). Saline environments affect the plant growth in different ways such as a decrease in water uptake, an accumulation

of ions to toxic levels and a reduction of nutrient availability. In some extensive reviews concerning strategies of overcoming the salinity problem, two primary lines of action were emphasized: reclamation of salt-affected soils by chemical amendments and alternatively, the saline soils can be used to grow salt-tolerant plants (Ashraf and McNeilly, 2004 and Tunçturk *et al.*, 2011).

Salinity is an ever increasing problem especially in areas where lands are irrigated with water containing salts. Worldwide about hundred million hectares of arable land is affected by salinity which accounts for about six to seven percentage of the total arable land (Munns and James, 2003). Salinity adversely affects plant growth at all stages and at seedling and reproductive stages in particular, dramatically reducing the crop yield (Munns *et al.*, 2002).

To tackle the detrimental effects of salinity, scientists are also in the process of engineering plants genetically using different genes (Zhang and Blumwald, 2001; Sanan-Mishra *et al.*, 2005; Tang *et al.*, 2005; Wu *et al.*, 2005; Wei-Feng *et al.*, 2008). Excess sodium in soil is a widespread and common stress in natural and agricultural ecosystems (FAO, 2008). High salt concentrations decrease the osmotic potential of the soil solution, thereby lowering the availability of water to plants. Therefore, salinity causes dehydration stress, at least in non acclimated plants. Furthermore, sodium competes with other cations, thereby affecting plant nutrition and ion homeostasis (Zhu, 2003; Munns and Tester, 2008; Shabala and Cuin, 2008). Since most plant species have only very limited capacities to cope with excess sodium, productivity on saline soil is strongly diminished and plant growth may even become impossible.

Globally, salinity is an important environmental problem, because the total area of salt-affected soils, including saline and sodic soils of currently about 831 million hectare, is continually increasing (Martinez-Beltran and Manzur, 2005).

Only 10% of the world's 13 billion hectare is farmed. Apart from urban areas much of the remaining 11.5 billion hectare are lands too hostile for any sort of agriculture. Moreover almost all the land that is farmable is under conditions sub-optimal, often to a considerable degree, for plant growth. Along side losses due

to pests and diseases, a further 70% of yield potential has been calculated to be lost to unfavourable physiochemical environments, even in developed agricultures. It is acknowledged that, in order to feed the eight billion mouths we expect by 2030, we will need to double world food production yet again. One component of that achievement will be the breeding of new varieties of food crops that will both improve yields on land presently being farmed on sub-optimal soils and extend our productive agriculture into lands which are currently barren (Gale, 2003).

Soil salinity is one of the most serious forms of land degradation affecting approximately ten percentage of the total land surface of the globe. The problem occurs in varying intensities in more than one hundred and twenty countries and this is prominently witnessed in the arid and semiarid regions (Pan *et al.*, 2010).

The physiological responses of plants for survival in stressed environment are based on their ability to express the preexisting defence programme or adaptation in which plants adjust to the stress (Senthil *et al.*, 2005).

Higher plants are sessile and therefore cannot escape from abiotic stress factors. They are continuously exposed to different abiotic stress factors without any protection. Variations do exist in tolerance mechanisms among plants (Mensah *et al.*, 2006).

In the present economic status and agricultural climate, with food surpluses in developed countries, the focus of the private sector will continue to be protection from disease and improvements in aspects of quality. Abiotic stress is a significant factor for production in developed countries and it is unlikely that genetic solutions will have to be actively sought by commercial breeding companies. If the problem is to be tackled at all, abiotic stress tolerance mechanisms and their genetic application in the crops of the developing world will have to be addressed by the public sector working in the developing world. In arid and semiarid regions, water availability is a major limitation to crop production and use of alternative water resources such as saline water and treated sewage effluent is increasing. The use of saline water and effluent which is of high salinity is mostly for field crops. The challenge to modern agriculture to increase food and fiber production demands

worldwide attention. Irrigation for example, allows the use of arid land to produce plants or to enhance plant production in regions with the shortage of water. However irrigated farming invariably involves soil salinisation, which becomes evident to the farmer as a loss of soil fertility. The tolerance range differs from species to species or even among cultivars. There is no general definition for high or low salinity stress levels (Heuer *et al.*, 2005).

The development of salt tolerant plants depends on the basis of physiological and biochemical markers recommended and may provide mechanistic understanding of tolerance. Hence many metabolic changes are known to occur in plants subjected to salt stress. Physiological parameters such as ionic relations have been suggested for use as tolerance indicators since they can be related to salt tolerance mechanisms (El-Baz *et al.*, 2003).

Removal of water due to evapotranspiration leads to seed concentration of salts in soils. Salinity occurs through both natural and human induced processes resulting in the accumulation of dissolved salts in the soil water and excess of sodium ions in the rhizosphere. Sodicy is a secondary consequence of salinity typical for clay soils where exchangeable sodium is bound to the negative charges of clay. Besides the naturally formed saline and sodic soils, the occurrence of so called secondary salt affected soils is becoming ever more visible due to application of different agricultural practices mainly irrigation (Rao *et al.*, 2006).

All plants are subjected to a multitude of stresses throughout their life cycle. The two major environmental factors that currently reduce plant productivity are drought and salinity and these two stresses create similar reactions in plants by inducing water deficit. Environmental stress salinity remains as one of the world's oldest and the most serious environmental problems, especially in arid and semiarid areas where poor crop establishment is the major limiting factor in crop production (Khan *et al.*, 2001).

The plant growth is either depressed or entirely prevented due to excessive build up of salinity and / or alkalinity in the soil. Salinity and sodicity problems are common in arid and semiarid regions where rainfall is insufficient to leach salts and

excess sodium ions out of the rhizosphere. In addition, these areas often take high evaporation rates, which can encourage an increase in salt concentration at the soil surface. Salt affected soils such as saline, saline sodic and sodic usually have low biological activity because of both osmotic and ionic effects of salts and also due to limitation of carbonaceous substrates (Yokoi *et al.*, 2002).

Salt injury involves both osmotic effects and specific ion effects. During osmotic adjustment, solutes in the rooting zone generate a low osmotic potential that lowers the soil water potential. The general water balance of plants is thus affected, because leaves need to develop even lower water potential to maintain a downhill gradient of water potential between the soil and the leaves. This effect of dissolved solutes is similar to that of a soil water deficit. Most plants can adjust osmotically when growing in saline soils and in this way prevent loss of turgor, which would slow the extension growth of cells, while generating a lower water potential. Other effects of salts on plants are ionic effect due to toxicities of specific salts and nutritional imbalances. Some elements such as sodium, chlorine and boron have specific effects on plants. Plants sensitive to these elements may be affected at relatively low salt levels if the soil contains enough of the toxic element. Because many salts are also plant nutrients, high salt levels in the soil can upset the nutrient balance in the plant and interfere with the uptake of some nutrients. The role of many plant growth regulating chemicals in alleviating the adverse effects of stresses and imparting adaptation has been reviewed (Larkindale and Huang, 2004).

Brassinosteroids are a novel group of plant hormones that regulate cell division, cell expansion, reproductive development, retard leaf abscission and enhance resistance to stress through stabilizing membranes and DNA (Sheela and Gehlot, 2000). Salicylic acid has been shown to be essential for the plant protection against oxidative stress generated by environmental stresses. Therefore, it is directly involved in the defense mechanisms developed by the plants under stress conditions, particularly salt and osmotic stresses (Mikolajczyk *et al.*, 2000).

Legumes are important not only as a nutritious food and fodder but also for their contribution in increasing soil fertility in symbiotic association with Rhizobium.

However production of legume crop species is limited by salt stress. In plants the adverse effects of salinity are mediated through detrimental effects on nitrogen fixation, which in turn limits further growth and metabolism of plants (Garg and Singla, 2007).

Being leguminous with high content of edible oil and quality patterns, groundnut is suitable for industrial and home scale processing of nutritious food products. Groundnut (*Arachis hypogaea* L.) is an unmatched rich source of edible oil (42-52%). Groundnut is an important crop grown in an area of about 26 million hectares in more than 100 countries around the world (FAO, 2003) under different agro climatic conditions for its nutritious oil rich kernel. India, China, Indonesia, USA, Senegal, Nigeria, Brazil and Argentina are the major groundnut producing countries (FAO, 2003). Groundnut is one of the major oilseed crops in the world, but the area and production of this crop is fluctuating between 6.0 - 8.5 million hectare and 6.0 - 9.5 million tones respectively mainly due to climatic variations and biotic and abiotic stresses (Singh and Basu, 2004).

Groundnut is an important commodity in many developing countries particularly in India where the nitrogen rich crop residues are also used as fodder. The production of groundnut in India needs to be increased from the current 8 million to 14 million by 2020 to meet the increasing demand of oil and confectionery industry (Girdhar, 2004). This is partially achieved by growing groundnut in lands considered so far as unsuitable for agriculture like rice in fallow land affected by salinity. Little is known about the salinity tolerance of groundnut and a few attempts have been made to breed salinity tolerant groundnut varieties (Vadez *et al.*, 2005).

Saline and sodic soils limit groundnut cultivation as it is grouped under sensitive crop to soil sodicity and soil salinity and could be grown with water having electrical conductivity up to 3 dsm^{-1} , although enough genotypic variation exists for its tolerance (Singh *et al.*, 2007). Thus developing management practices and saline tolerant varieties to alleviate salinity stress are of utmost importance to bring more area under groundnut cultivation to increase production. But there is hardly any study on inside field screening of large number of groundnut genotypes till

maturity to identify few tolerant ones. Recently an attempt has been made for developing screening protocol (Vadez *et. al.*, 2005).

Both Na⁺ and Cl⁻ ions disrupt the basic feature of protein, enzyme as well as plasma membrane. The physiology and biochemistry of salt tolerance mechanism in groundnut is poorly understood, but the characters associated with salt tolerance could be identified and their systematic investigation could lead to the exploration of the mechanism of salt tolerance. Saline soil which causes reductions in yield is one of the important abiotic constraints to groundnut production. Pulses in general are sensitive and have inadequate control over ion uptake which leads to high internal salt concentration and results in plant injury. Tremendous variability exists regarding salt tolerance among different species/cultivars in all pulses (Chauhan and Singh, 2000).

Consequently there is differential reduction in growth and yield when grown in salt affected soils. Information on salt tolerance of local groundnut varieties is scanty. A better understanding of the mechanisms, by which, plants respond to salinity / sodicity stress may help in developing more tolerant varieties. Besides this, development of amelioration technology may pave the way for improving growth and yield of crop plants, particularly sensitive species, growing under the hostile environments.

The objectives of the present study are therefore

- To screen the groundnut varieties for tolerance to salinity stress by *in vitro* method
- To understand the physiological, biochemical and molecular mechanisms of salt tolerance in groundnut
- To evaluate the efficacy of various growth regulating chemicals on mitigating the stress effects
- To identify the expression of heat shock protein (hsp70) in stress induced *Arachis hypogaea* L.
- To compare the sequence of hsp 70 gene in *Arachis hypogaea* L. with salt tolerant protein sequence of other plants identified, for future studies through *in silico* methods.

The null hypothesis which could be proposed in the different phases of the study are :-

- (i) There is no significant difference in salt tolerance among the five varieties chosen for the study.
- (ii) There is no significant difference among the two salt concentrations (50mM and 100mM NaCl) used in the study with respect to the morphological, physiological and biochemical responses of groundnut.
- (iii) There is no significant difference in the efficacy of various growth regulating chemicals on mitigating the stress effects among the salt tolerant and salt susceptible varieties.
- (iv) There is no significant difference among the aminoacid sequences in heat shock protein 70 of the salt tolerant groundnut variety and the heat shock protein 70 of the other plant varieties whose sequences are available on line.