

Many species of higher plants, including most crops, are subjected to growth inhibition under high salinity conditions. Soil salinity and sodicity cause detrimental effects on plant activities, which are likely to alter the yielding potential of the crops. Hence to identify the physiological parameters, which get altered under salt stress conditions, the degree of damage caused by the stress, to standardize the method of ameliorating the adverse effects of salt, to identify the presence of heat shock protein gene in groundnut and to compare the sequence of the hsp70 gene with other salt tolerant sequence identified so far, the present work was carried out in groundnut under three conditions viz., laboratory, pot culture and field.

In the laboratory experiment, screening of five groundnut varieties namely VRI2, VRI3, TMV7, CO3 and CO4 was done at critical salt concentrations of 50 mM, 100 mM and 125 mM NaCl. The results of the investigation revealed that with increasing concentration of the salt, the germination percentage, seedling growth and vigour index were decreased drastically. Salinity stress at high level caused similar adverse effects on the growth of the seedlings. All the five varieties displayed their differential responses to these stresses. The variety TMV7, showed high degree of tolerance with high germination percentage and low reduction in vigour index. Therefore, this variety along with other four varieties namely VRI3, CO3, CO4 and VRI2 with moderate performance were selected for future evaluation under pot culture condition.

The selected five varieties were subjected to two levels of salinity stress with 50 mM and 100 mM NaCl. The results revealed that high salinity (100 mM) level showed greater inhibitory effect on the overall growth of all the varieties. However, among the varieties evaluated, TMV7 was found to be the most tolerant variety and VRI3, the most sensitive one. Maintenance of optimum leaf area with high chlorophyll, soluble protein and proline contents along with high total dry matter production (TDMP) were observed to be the physiological basis for tolerance to salinity stress.

In the present study the number of stomata were decreased upon stress and their development was affected which was seen from their improper closure which might affect the exchange of gases in the stomata and lead to the inhibition of photosynthesis, transpiration and ultimately affect the plant growth. It is important for plants to be able to balance the amount of CO₂ being brought into the plant with the amount of water escaping as a result of the open stomatal pores.

The five groundnut varieties chosen for the present work were studied for pods and kernel yield as well as other genetic parameters, genotypic coefficients of variation (GCV), phenotypic coefficients of variation (PCV), heritability and genetic advance calculated by the IRRISAT Mixed Model Analysis software. The values revealed a high heritability for the oil content and height of the plants, greater than 0.5, whereas for fatty acid content it was only 0.34. Being an oilseed crop, high heritability estimates for oil are very desirable. Thus the progenies of the groundnut variety TMV7 taken up for the study proved to be tolerant to salt stress suggesting their planting and good performance in salt affected sites. The progenies of the groundnut variety VRI3 were found susceptible to salt stress and this suggested further advancement in generations for enhancing their salt tolerance abilities.

In order to assess the changes in fatty acid profile of groundnut oil of the selected five varieties of groundnuts grown under two different levels of salinity (50 and 100mM NaCl), the oil samples of the selected varieties were analysed for fatty acid profile using High Pressure Thin Layer Chromatography (HPTLC) technique. Based on the morphological and physiological responses of the varieties to salt tolerance, the ranking of the varieties was in the order CO4<VRI3≡VRI2≡CO3>TMV7. The fatty acid profile of the varieties in HPTLC revealed that they play a role in the management of abiotic stresses in groundnut having a protective role in CO4 and a defensive role in VRI2, VRI3 and TMV7. The free fatty acids present in the oil samples of the five selected varieties of groundnut were further analysed by Gas Chromatograph (GC). GC revealed fatty acids specific to different salinity treatments suggesting the need for elaborate studies on these induced acids which could be by products / breakdown products / new

precursors performing protective functions in the seeds of the tolerant variety and defensive in moderately susceptible ones.

In the field trial carried out with the tolerant variety (TMV7) and susceptible variety (VRI3) along with the external application of different concentrations and combination of growth regulators namely brassinolide – 0.5ppm and 1.0ppm, salicylic acid – 50ppm and 100ppm, nutrient mixture - Diammonium phosphate-1% +Potassium Nitrate - 0.5% + Borax-0.2% + Naphthalene Acetic Acid - 20ppm + Salicylic Acid - 50ppm + Brassinolide -1ppm, the results revealed that, among the treatments Brassinolide (BR) 1 ppm was found effective in alleviating the adverse effects of salt stress. The morphological and growth traits such as root length, leaf area index, leaf area duration and total dry matter production were significantly enhanced by brassinolide (1.0 ppm) treatment in both the varieties. Similarly the biochemical constituents namely proline content, chlorophyll and soluble protein contents were improved by the treatment with BR 1 ppm. Most of the metabolic enzymes namely nitrate reductase, catalase, peroxidase, acid phosphatase and polyphenol oxidase were also accelerated by this treatment. Leaf nitrogen, phosphorus, potassium, calcium and magnesium contents were also enhanced by BR 1 ppm.

Flower production and peg number were improved by BR treatment in both the varieties. Because of high fertility coefficient, the number of pods produced per plant was also increased considerably. The role of BR in facilitating the partitioning of assimilates could also be revealed through improved seed weight. The overall performance of BR, therefore, reflected on pod yield of groundnut in both the varieties. Therefore, application of BR 1 ppm through foliar spray at preflowering, pegging and pod formation stages effectively enhanced the overall physiological efficiency of the crop and improved the yield in both the tolerant and sensitive varieties of groundnut grown under salt stress conditions.

Salinity, one of the abiotic stresses, is the primary cause of crop loss worldwide, reducing average yields. Identifying the mechanism of plant response to stress and their roles in acquired stress tolerance is thus of great practical and

basic importance. Hsps / chaperones are known to be expressed in plants when they experience stress in response to a wide range of environmental aspects, such as water stress, salinity, osmotic, cold and oxidative stresses. Based on these facts, in order to study the expression pattern of heat shock protein (hsp) 70, if any in the groundnut variety TMV7 (which was identified as the tolerant one in the pot and field study), the RNA of that particular variety was isolated and RT-PCR was run with tubulin as endogenous control. The isolated RNA was treated with DNase for cDNA synthesis which was performed using Revert Aid H Minus First Strand cDNA synthesis kit. Due to noise during amplification, the differential expression of hsp 70 could not be quantified. However, the expression of hsp 70 gene was noticed during salt stress of 100 mM concentration which proved the tolerance of the groundnut genotype TMV7 to salt stress and this had paved the way for further analysis of hsp family in groundnut under stress and their quantification.

The results of the present study showed similarity relationships between the heat shock protein sequence used as primer and 114 other sequences of similar properties. Computer database comparisons were performed using BLAST (Basic Local Alignment Search Tool) and the conserved domains were also identified using DMPRED software. Though 114 sequences showed similarity in properties, the maximum coverage of similarity existed closely with *Arabidopsis thaliana* which proved the existence of heat shock protein in many species.

Germination study, pot culture, heritability analysis, field trial and heat shock protein expression analysis proved that among the five varieties of groundnuts used in the study, TMV7 was found to be salt tolerant grown under 100mM NaCl stress whereas the susceptible variety was found to be VRI3. The yield under the influence of plant growth regulators also proved that TMV7 gave better yield of groundnut compared to the other susceptible variety VRI3.

As far as the four null hypotheses proposed in the beginning of the study are concerned,

- (i) The first null hypothesis proposed in the study that, there is no significant difference in salt tolerance among the five varieties chosen for the study is

rejected as there were differences observed among the five varieties in screening for tolerance by *in vitro* method in laboratory condition.

- (ii) The second null hypothesis proposed that 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 is also rejected as there were significant differences among the two salt concentrations in the selected parameters that were carried out in pot culture.
- (iii) The third null hypothesis that 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 is also rejected as the treatment using Brassinolide 1ppm showed significant increase in the selected parameters studied in the field.
- (iv) The fourth null hypothesis that there is no significant difference among the aminoacid sequences in heat shock protein 70 between the protein under study and the sequence available on line is accepted as there was sequence similarity among them.

From the work carried out it can be recommended that the groundnut variety TMV7 can be used frequently by the farmers of Viraliyur, Thondamuthur as it was found to be the most tolerant one compared to the other varieties used. It is also recommended that the TMV7 groundnut variety can be used in salt affected soils and places where saline water is being used to irrigate land.

The susceptible variety VRI3 can be studied in depth at its salt tolerance level by focusing the expression pattern of the heat shock protein and the gene responsible for it. Another way is crossing the susceptible and tolerant variety of groundnuts in order to have a new combination of genes and a study can be conducted in analysing its performance under stress. The transgenic mode of introducing salt tolerant gene and its pattern of expression can be carried out.

Contribution to Knowledge

- The study revealed that salinity can have an adverse effect on the yield of the crop
- Heat shock proteins can be used as biochemical markers for screening tolerant varieties for deployment in salt affected lands which comprise about 70 % land area in Tamil Nadu
- Heat shock proteins were identified as one of the major contributing factors for enhancing salinity tolerance in groundnut
- The findings of the study may enable to generate new crop varieties using biotechnological approaches