
4

Results and Discussion

Based on general tolerance to salt stress, all plants can be roughly divided into two major groups: a) halophytes, that can withstand even 20% of salts in the soil and in most cases, successfully grow in conditions with 2-6% of salts and b) non-halophytes or glycophytes, plants that exhibit various degrees of damage and limited growth in the presence of sodium salts, usually higher than 0.01%. However, there are great differences in the level of salt stress tolerance within both the halophytes and non-halophytes which include sensitive, moderately tolerant and very tolerant species (Dajic, 2006).

To achieve the objective of screening the groundnut varieties for salinity tolerance and to get informations on the morphological, physiological and biochemical mechanisms of salinity tolerance, a series of experiments in the laboratory, pot and in field were carried out.

In phase 1, screening of five different varieties of groundnut (*Arachis hypogaea* L.) seeds for tolerance under different salinity levels (50 mM, 100 mM and 125 mM NaCl) in laboratory condition and a pot culture screening for salinity tolerance of the groundnut (*Arachis hypogaea* L.) varieties selected for the study were done with two different concentrations of sodium chloride solutions namely 50 mM and 100 mM.

In phase 2, the phenotypic and genotypic variance among the five varieties of groundnuts chosen for the study was performed using IRRISAT Mixed Model Analysis software. To analyse the fatty acid composition of groundnut (*Arachis hypogaea* L.) varieties under salt stress, High Pressure Thin Layer Chromatographic and Gas Chromatographic techniques were performed.

In phase 3, to assess the impact of different growth regulating chemicals on mitigating the adverse effects of salt stress, a field study was conducted with two groundnut varieties that have been identified as tolerant and susceptible in phase 1.

To identify the presence of heat shock protein if any, the expression of hsp 70 gene using RT-PCR technique was performed in the tolerant groundnut variety and an insilico approach was done in phase 4 to identify the salt tolerant protein from the available sequence of *Arachis hypogaea* L. by BLAST, in order to predict the conserved regions in it using Clustal W and to predict the domains of the protein in *Arachis hypogaea* L. using DOMPRED.

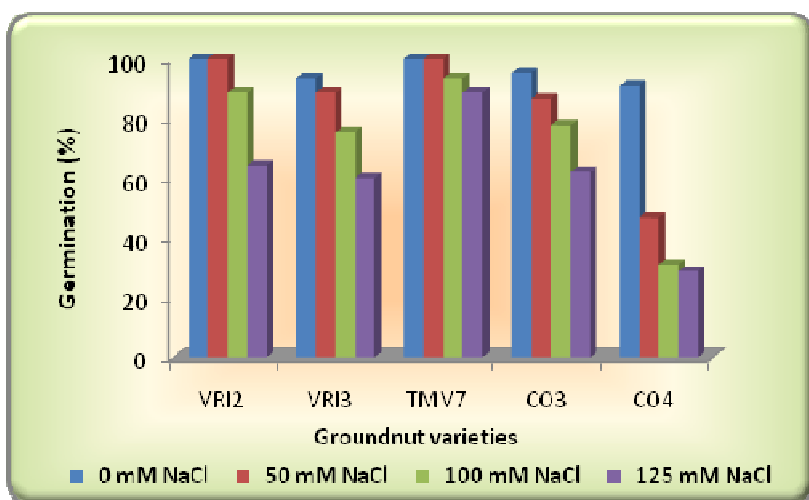
The results are presented and discussed in this chapter.

4.1. Laboratory Screening of Five Different varieties of Groundnut (*Arachis hypogaea* L.) Seeds under Different Salinity Levels

4.1.1. Germination Percentage

Germination percentage is an important index for salt tolerance and the observed values due to stress treatments are depicted in Figure 10. While observing the effect of different salinity stresses imposed using sodium chloride at 50, 100 and 125 mM levels on germination percentage, a significant reduction in germination was noticed at the salinity level of 125 mM in all the varieties. Comparing the performance of varieties at the highest salinity level of 125 mM, it could be observed that the variety CO4 had a significant reduction in its germination, whereas the variety TMV7 had a significant increase than other varieties.

Figure 10
Germination percentage (%) of the selected five groundnut varieties at different salinity levels

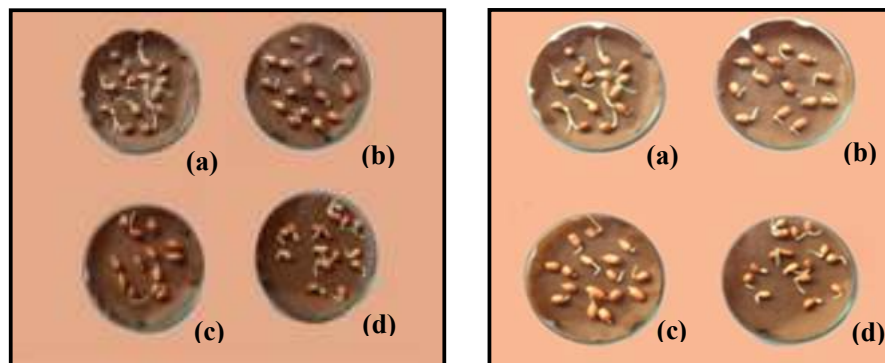


The ability of a seed to germinate and emerge under salt stress indicates that it has genetic potential for salt tolerance and the present study result agrees with the report of Mensah *et al.* (2006), where they observed decreases in percentage germination and seedling emergence with increase in salinity. According to Singh *et al.* (2007), germination percentage along with root length, hypocotyls length and number of secondary roots is considered to determine salinity tolerance index of a plant. Germinability of the groundnut seeds under salt stress conditions is an index of salt tolerance. The present study also revealed significant varietal variations in salt tolerance in terms of germination percentage.

Vadez *et al.* (2005) supported this finding with a view that susceptible varieties expressed their sensitivity to salt stress in germination itself. Kaymakanova (2009) also noticed a drastic reduction in germination of certain bean seed (*Phaseolus vulgaris* L.) cultivars under salinity condition. He explained that limited water uptake by the seeds under saline condition is the direct cause of affecting the germination process. Jamil *et al.* (2006) suggested that seeds must absorb sufficient water and during germination, the tissue water potential plays a critical role in the initiation of activity of the enzyme α -amylase and higher concentration of salt reduces the water potential in the medium which hinders water absorption by germinating seeds and thus reduces germination. It appears that a decrease in germination is related to salinity induced disturbance of metabolic process leading to increase in phenolic compounds. It is assumed that germination rate and the final seed germination decrease with the decrease of the water movement into the seeds during imbibitions. Salinity stress can affect seed germination through osmotic effects. Salt induced inhibition of seed germination could be attributed to osmotic stress or to specific ion toxicity. According to Tobe *et al.* (2001), toxicity due to salinity during seed germination is usually associated with a significant decrease in the seed K^+ content, which could reduce metabolic functions and ultimately reduce germination and seedling growth and osmotic effects because of declining solute potential or toxic effects due to uptake and/or accumulation of some ions in the seed. The germination status of the groundnut varieties chosen for the study is presented in Plate 5.

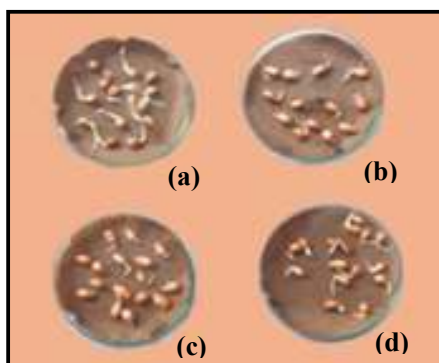
Plate 5

Petriplates showing germination of the seeds of five different varieties of groundnuts at different salinity levels

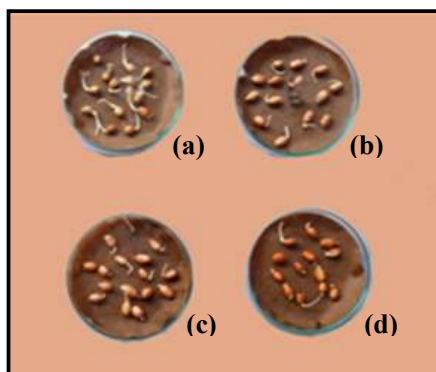


VRI2

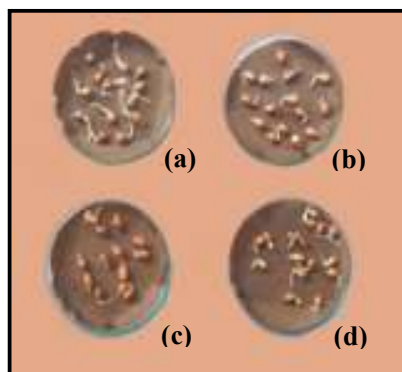
VRI3



TMV7



CO3



CO4

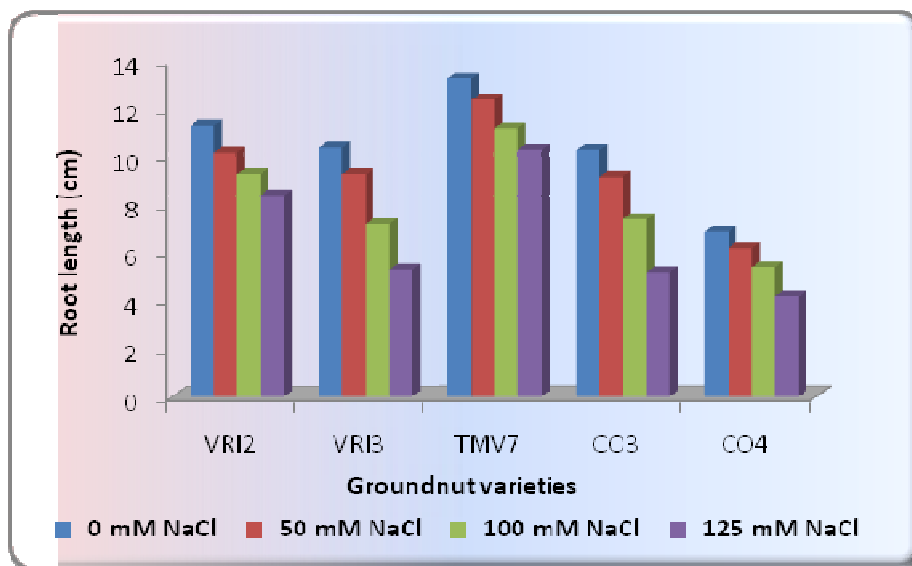
(a) Control (b) 50mM NaCl (c) 100mM NaCl (d) 125mM NaCl

4.1.2. Root Length

The adverse effect of salinity stress at different levels reflected on root growth in terms of length has been presented in Figure 11. There was a significant decrease in the root length at salinity levels 100 and 125 mM NaCl concentration when compared with the salinity level of 50mM NaCl. At the severe stress level of 125 mM, the response of all the varieties was differential. TMV7 showed significantly increased root length of 10.3 cm when compared to other varieties of groundnut. The variety CO4 had a significantly reduced root length of 4.2 cm when compared to control as well as other varieties proving its susceptibility to NaCl stress.

Figure 11

Root length (cm) of the selected five varieties of groundnut seedlings at different salinity levels



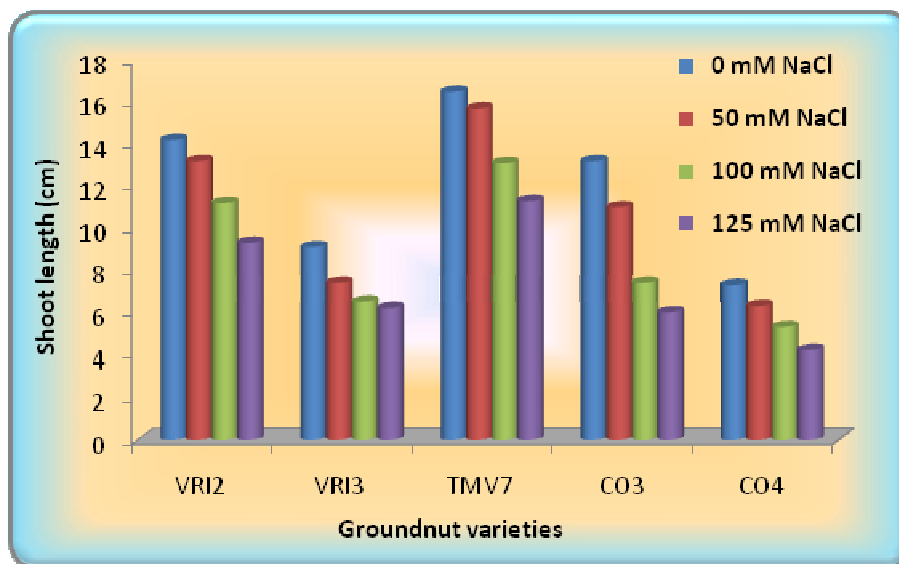
Djanaguiraman *et al.* (2003) suggested that decline in the growth of root and germination percentage of rice genotypes under salt stress condition was attributed to the inhibition of hydrolysis of endosperm reserves and reduced translocation of food reserves from endosperm to embryo.

4.1.3. Shoot Length

The mechanism by which salinity inhibits shoot growth may be due to 1) disturbed photosynthesis, 2) decline in turgor of expanding tissues and insufficient osmoregulation, 3) root sensing and down regulation of shoot growth via a long distance signal and 4) disturbance in mineral supply to the shoot as suggested by Lazof and Bernstein (1999).

Time dependant changes of growth and development of plants exposed to salinity stress have been reviewed (Munns, 2002). In the first few seconds or minutes, cells lose water and shrink, whereas over hours cells regain their volume, but the expansion rates are limited. Over days and weeks, reduced cell elongation and cell division result in slower leaf appearance and inhibition of shoot growth. In the present study, shoot length of 15 days old seedlings was measured and a significant reduction was noticed in all the varieties subjected to different salinity levels (Figure 12). At the highest salinity level of 125 mM, TMV7 recorded significantly higher shoot length of 11.3cm. A significant reduction was observed in CO4 at 125mM salinity level.

Figure 12
Shoot length (cm) of the selected five varieties of groundnut seedlings at different salinity levels



The adverse effects of salt stresses on germination per cent also reflected on shoot growth of certain varieties of groundnut seedlings. CO4 besides poor germination also showed a significant reduction in shoot length under higher level of salinity stress. This was in confirmation with the result of Ghoulam and Foursy,(2002) who found that a high sodium chloride concentration caused reduction in growth parameters such as leaf area, root and shoot length, germination, fresh weight and dry weight and more of solute leakage in case of sugar beet. The present study also revealed that the groundnut genotypes TMV7 and VRI2 maintained better shoot growth under extreme salinity condition. This might be attributed to the inherent mechanism of tolerant varieties in the regulation of entire germination process.

4.1.4. Vigour Index

The effect of salt stress on vigour index of all the varieties was assessed at different salinity levels (Figure 13). The seedling vigour index was significantly altered by all the three levels of salinity. Salinity at 125 mM concentration had a significant reduction in the mean vigour index over control. At severe salinity level of 125mM, all the varieties performed differentially. The maximum vigour index of 1920 was recorded by the genotype TMV7 followed by VRI2 (1141). The vigour index of CO4 was significantly reduced when compared to control. Significant varietal variations were observed in vigour index as a result of salinity stress. Djanaguiraman *et al.* (2003) also conducted similar experiment and reported that some varieties of rice were more sensitive to NaCl.

4.1.5. Stress Tolerance Index

The stress tolerance index (STI) was worked out based on the vigour index of the seedlings as per cent ratio between the values under different stress treatment and control. The results revealed that with the increase in salinity level, there was a significantly decreasing trend in STI in all the varieties, indicating that higher the stress lesser the tolerance (Figure 14). Individual varieties responded differentially with various tolerant indices. At the extreme stress level of 125 mM, TMV7 and VRI2 maintained higher STI of 64 and 45 respectively. CO4 recorded the least STI of 19 showing its susceptibility to salinity stress.

Figure 13

Vigour index of the selected five varieties of groundnut seedling at different salinity levels

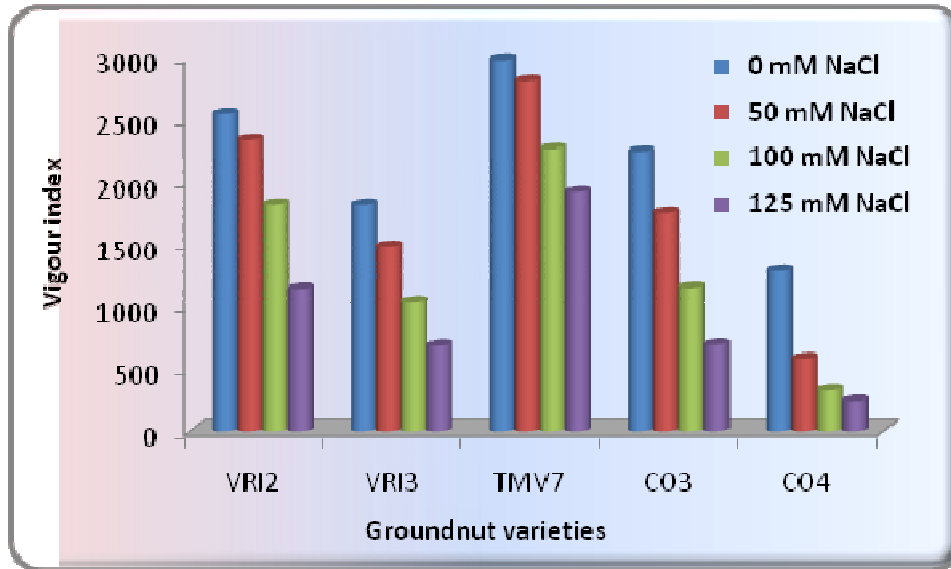
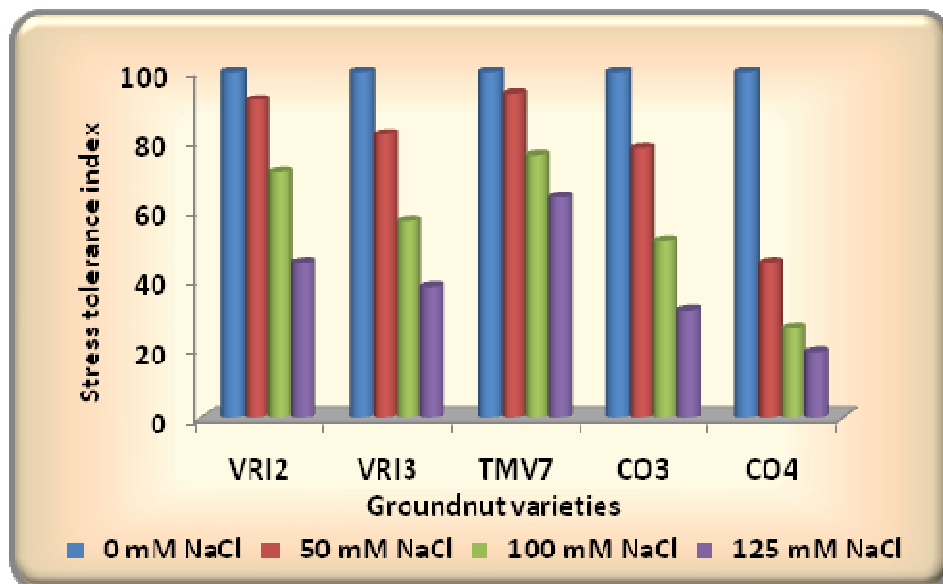


Figure 14

Stress tolerance index of the selected five varieties of groundnut seedling at different salinity levels



4.2. Pot Culture Screening of Groundnut varieties for their Morphological, Physiological and Biochemical Characteristics under Salinity Stress

Based on the *in vitro* screening study with five groundnut varieties, viz., VRI2, VRI3, TMV7, CO3 and CO4, they were further evaluated in pot culture condition for their morphological, physiological and biochemical characteristics along with yield, subjecting to two different levels of salinity (50 and 100mM) stress (Plate 6).

Plate 6

Pot culture experiment with two different salinity levels



Groundnut plants without NaCl salt stress (Control)



Groundnut plants with 50 mM NaCl salt stress (T2)



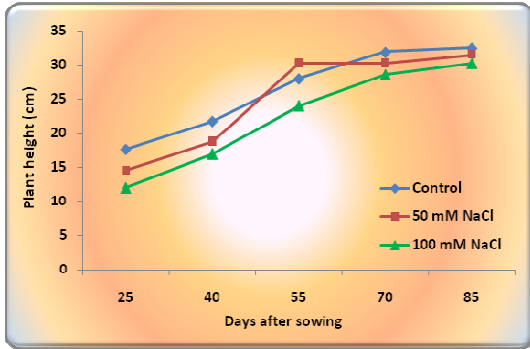
Groundnut plants with 100 mM NaCl salt stress (T2)

4.2.1. Plant Height

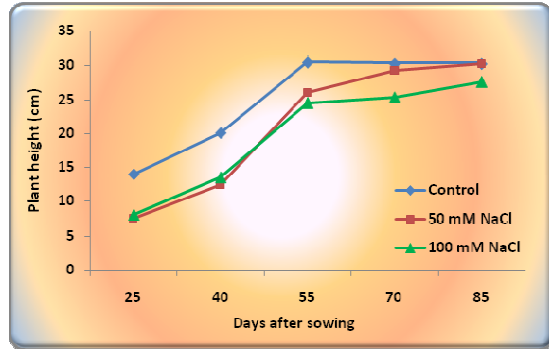
The impact of salt stress on morphological characteristics of five groundnut varieties screened through laboratory experiment was assessed at different growth stages. The plant height at different growth stages indicated a steady increasing trend with the age of the crop. In terms of plant height all the five varieties exhibited significant variations in response to different salinity level (Figure 15).

Figure 15

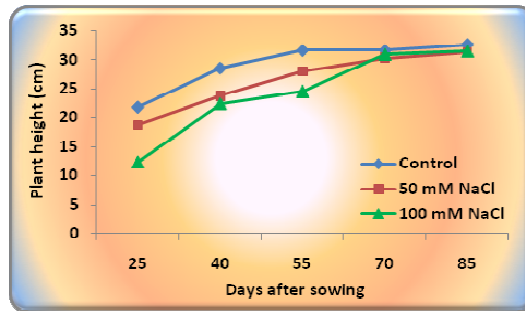
Plant height (cm) of the five selected groundnut varieties at different stages of growth with different levels of salinity stress



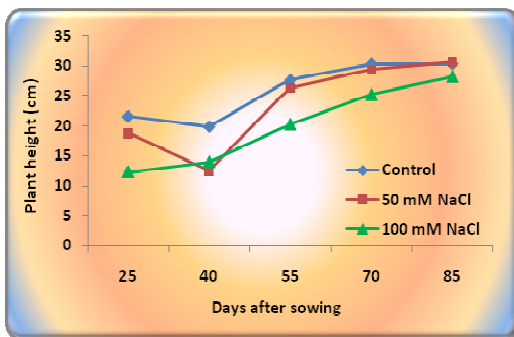
VRI2



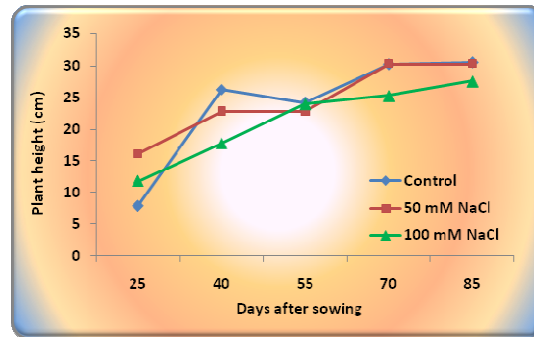
VRI3



TMV7



CO3



CO4

TMV7 variety recorded a maximum plant height of 31.6 cm at 85 DAS with increased salt stress, followed by VRI2 recording plant height of 30.3 cm. The lowest value of 27.6 cm was registered by VRI3 and CO4, which showed a significant reduction when compared to the control. At two salinity levels of 50 mM and 100 mM, there was a significant reduction in plant height when compared to the control. CO4 showed maximum inhibition of growth reduction under higher level of salinity condition (100mM NaCl). These results could be supported by the study conducted by Desire *et al.* (2010) on groundnut variety Pyrieur cultivar at a concentration of 200mM NaCl. Rabie and Almadini (2005) reported that plant growth was basically regulated by change in the osmotic system and its specificity. The disturbance of physiological and biochemical processes in the susceptible cultivars, due to high osmotic stress and low tissue water potential was the direct cause for growth inhibition. These findings strongly support the results of the present study.

4.2.2. Leaf Area

The results of leaf area of the five varieties of *Arachis hypogaea* L. as affected by salinity stress exhibited significant differences throughout the growth stages (Table 8). The time trend variation of leaf area revealed a gradual increase from 25 DAS to 70 DAS with a remarkable reduction at 85 DAS. Among the varieties, CO3 registered a significant increase in leaf area of 939 cm² plant⁻¹ at 70 DAS, which coincided with pod development stage. Higher concentration of salinity at 100mM NaCl in all the five varieties showed a significant reduction of leaf area when compared to the control.

The groundnut varieties CO3 and CO4 under 50mM NaCl stress showed a significant reduction in leaf area (959 and 900 cm² plant⁻¹ respectively) over the control (1001 and 936 cm² plant⁻¹) at 70 DAS.

It could be inferred that, leaf area, the photosynthetic surface of the plant, was drastically reduced under salinity stresses. The groundnut varieties TMV7 and CO3 performed better by maintaining significantly higher leaf area with lesser reduction in salinity stress. VRI2 and VRI3 however, showed a significant reduction over control under both the salinity stresses induced (50 and 100mM NaCl).

Table 8

Leaf area (cm² plant⁻¹) of the five selected groundnut varieties at different stages of growth with different levels of salinity stress

| Varieties | 25 DAS | | | 40 DAS | | | 55 DAS | | | 70 DAS | | | 85 DAS | | |
|-----------------|-------------|-----------|------------|-------------|-----------|------------|-------------|-----------|------------|-------------|-----------|------------|-------------|-----------|------------|
| | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl |
| VRI2 | 247 | 244 | 239 | 477 | 259 | 246 | 681 | 499 | 319 | 992 | 619 | 450 | 566 | 279 | 200 |
| VRI3 | 223 | 220 | 207 | 397 | 239 | 319 | 529 | 500 | 539 | 905 | 617 | 639 | 452 | 299 | 319 |
| TMV7 | 280 | 240 | 219 | 658 | 629 | 339 | 811 | 759 | 629 | 1023 | 999 | 799 | 679 | 649 | 319 |
| CO3 | 264 | 229 | 220 | 511 | 437 | 399 | 701 | 677 | 639 | 1001 | 959 | 939 | 620 | 599 | 520 |
| CO4 | 223 | 220 | 199 | 459 | 450 | 419 | 651 | 599 | 539 | 936 | 900 | 859 | 535 | 519 | 479 |
| CD(0.05) | 5.26 | | | 2.45 | | | 2.49 | | | 3.85 | | | 1.64 | | |

Values presented are mean of three replicates in each variety in two different salinity levels

DAS- Days after sowing

These results could be supported by Farouk (2011) who reported that plants that were subjected to salt stress showed a greater reduction in leaf area. He also stated that restriction of leaf growth is among the earliest visible effects of many stress conditions, including salinity. Because leaves determine radiation interception and are the main photosynthetic organs, salinity stress effects on leaf expansion and functions are directly related to yield constraints under saline conditions.

4.2.3. Total Dry Matter Production

The total dry matter production of all the varieties showed a progressive increase throughout the growth period, reaching its maximum biomass accumulation at 85 DAS (Table 9). However, salt stress conditions indicated significantly decreased dry matter production compared to the respective control in the four time periods analysed. The variety, TMV7 maintained its superiority and recorded a maximum dry matter production $80.6 \text{ g plant}^{-1}$ followed by CO3 ($79.6 \text{ g plant}^{-1}$), VRI2 ($72.9 \text{ g plant}^{-1}$), CO4 ($71.8 \text{ g plant}^{-1}$) and VRI3 ($64.3 \text{ g plant}^{-1}$) at 85 DAS. All the salinity levels seemed to significantly affect the total dry matter production of all the varieties. Salinity stress of 100mM NaCl showed greater adverse effect on total dry matter production of all the varieties with a significant reduction over the control.

Saline conditions, in general, bring the stunting of plant growth, restrict lateral shoot development, reduce the size of the leaves and decrease the fresh and dry weight of the plants. Muthukumarasamy and Panneerselvam (1997), suggested that NaCl treatment of 100 and 150 mM was sufficient to cause a marked reduction in biomass of green gram. The results of the present study also revealed a significant reduction in total dry matter production due to salinity stress over control.

Desire *et al.* (2010) explained that salinity stress decreased leaf area development and plant height much earlier than any other morphological parameters. Since dry matter accumulation by plant is the product of net photosynthetic rate, the drastic reduction in leaf area might be the main cause for remarkable reduction in dry matter production. In addition to explaining the adverse

Table 9

Total dry matter production (g plant⁻¹) of the five selected groundnut varieties at different stages of growth with different levels of salinity stress

| Varieties | 25 DAS | | | 40 DAS | | | 55 DAS | | | 70 DAS | | | 85 DAS | | |
|-----------------|-------------|-----------|------------|-------------|-----------|------------|-------------|-----------|------------|-------------|-----------|------------|-------------|-----------|------------|
| | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl |
| VRI2 | 7.8 | 7.4 | 6.9 | 18.9 | 17.9 | 17.5 | 34.0 | 33.9 | 31.6 | 52.4 | 52.6 | 51.9 | 73.2 | 73.8 | 72.9 |
| VRI3 | 6.7 | 6.4 | 6.2 | 17.5 | 17.0 | 16.8 | 31.7 | 31.0 | 30.3 | 48.3 | 48.0 | 44.0 | 65.3 | 64.6 | 64.3 |
| TMV7 | 8.1 | 7.9 | 7.9 | 21.9 | 21.6 | 22.3 | 38.3 | 36.8 | 38.3 | 54.1 | 52.6 | 54.6 | 80.0 | 77.6 | 80.6 |
| CO3 | 8.0 | 7.9 | 7.5 | 20.7 | 21.6 | 22.8 | 35.6 | 35.6 | 36.6 | 53.2 | 53.8 | 54.7 | 76.9 | 77.6 | 79.6 |
| CO4 | 7.3 | 6.9 | 7.1 | 17.9 | 15.9 | 14.9 | 32.8 | 31.8 | 32.8 | 51.2 | 51.8 | 54.6 | 69.5 | 69.3 | 71.8 |
| CD(0.05) | 0.26 | | | 0.56 | | | 0.74 | | | 0.60 | | | 0.93 | | |

Values presented are mean of three replicates in each variety in two different salinity levels

DAS- Days after sowing

effects of salt stresses, the present study also revealed remarkable varietal variations on dry matter production in response to stress situations. The groundnut variety, CO4, when subjected to higher level of salinity stress (100mM NaCl) showed a significant reduction when compared to the other four varieties. VRI3, however, recorded the highest reduction under 100mM salinity condition at 85 DAS thereby indicating its susceptibility.

4.2.4. Total Chlorophyll

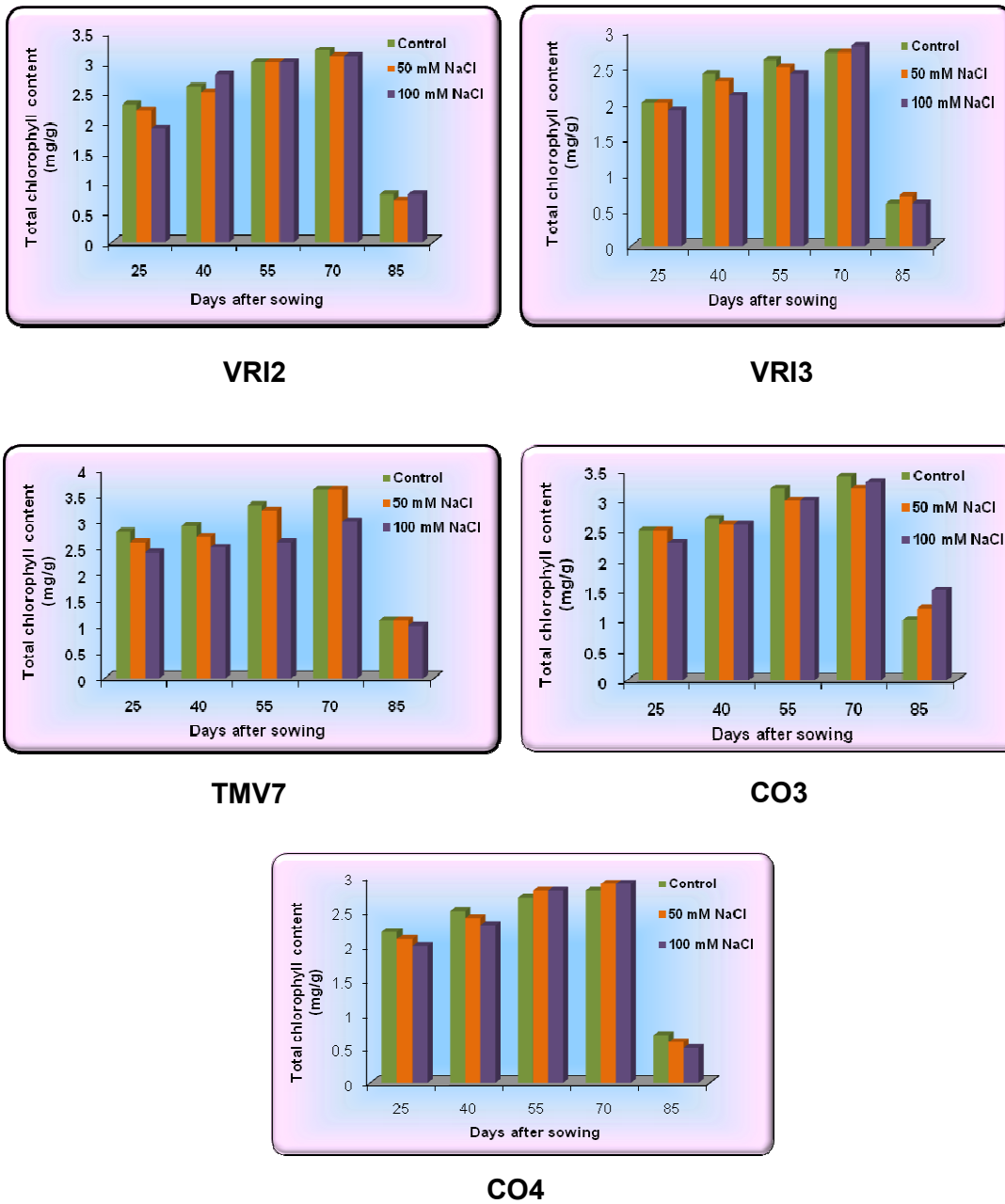
The data on total chlorophyll content of groundnut varieties, revealed an increasing trend from 25 DAS to 70 DAS with a significant reduction at 85 DAS (Figure 16). All the varieties showed significant differences throughout the growth period. Among the varieties of groundnut, CO3 recorded significantly increased chlorophyll content of 3.3 mg g⁻¹ whereas the VRI3 variety had a significantly lower chlorophyll content of 2.8 mg g⁻¹ on 70 DAS. The salinity stress treatments significantly reduced the chlorophyll content particularly at higher concentrations. Therefore, at 70 DAS, the 100mM NaCl treatment recorded significantly reduced chlorophyll content of 2.8 mg g⁻¹, whereas the 50 mM NaCl treatment recorded the chlorophyll content of 2.7 mg g⁻¹ in VRI3. However, at 70 DAS, under unstressed control, a significantly higher chlorophyll content of 3.6 mg g⁻¹ was recorded in TMV7 compared to other varieties at the same period.

Significant variations were also observed among the varietal and treatmental combinations. Groundnut variety TMV7 under 50mM NaCl stress and the variety CO3 under 100mM NaCl level (3.6 mg g⁻¹) and (3.3 mg g⁻¹) had a significant increase in chlorophyll content after 70 days where as the other varieties showed no significant difference when compared to their control.

Chlorophyll pigments play a major role in plant productivity, as it is the only pigment responsible for capturing light energy and using it as a driving force for producing the assimilates. Increasing salinity causes a reduction in chlorophyll content (Sheng *et al.*, 2008) due to suppression of specific enzymes that are responsible for the synthesis of photosynthetic pigments (Murkute *et al.*, 2006). In *Sesbania grandiflora* seedlings, Dhanapackiam and Ilyas (2010) noticed a drastic reduction in the chlorophyll content due to salinity. Salinity through specific ion

Figure 16

Total chlorophyll content (mg g^{-1}) in the five selected groundnut varieties at different stages of growth with different levels of salinity stress



toxicity induces disintegration of thylakoid membranes and causes degradation of chlorophyll pigments. Destruction of this vital pigment under this stress condition is deleterious to plant productivity. As observed in the present study, salinity, particularly at high concentration causes considerable reduction in chlorophyll content. Differential varietal responses were also noticed in terms of chlorophyll loss.

4.2.5. Soluble Protein

The soluble protein content of different varieties of groundnut revealed a significant increase from 25 to 55 DAS, followed by a slow and gradual increase up to 70 DAS with a sharp decline at 85 DAS (Table 10). All the varieties differed significantly in the soluble protein content at all the stages of growth. At 70 DAS, VRI2 recorded a significantly increased soluble protein content of 14.0 mg g⁻¹ followed by VRI3 (13.0 mg g⁻¹) in 100mM salt stress. A significantly reduced soluble protein content of 10.9 mg g⁻¹ was recorded by CO4. Different levels of salinity stress reduced the soluble protein content remarkably. On 70 DAS, all the five varieties recorded significantly decreased soluble protein content with 100mM salt stress compared to their respective controls.

Soluble protein content was estimated in order to find out the photosynthetic capacity of groundnut varieties under various salt stress situations. The groundnut variety CO4, however, recorded significantly lower protein content. Salinity stress resulted in a general decrease of soluble protein content of the leaves. Varieties behaved differentially when subjected to salinity stress at two different levels. These findings were in accordance with the results of Maibangsa *et al.* (2000) who observed a significant reduction in soluble protein content of rice grown under salt stress condition. Yurekli *et al.* (2004) reported that total soluble protein content was significantly decreased in salt sensitive *Phaseolus vulgaris* but increased in salt tolerant *P. acutifolius* plants. Similarly, Porgali and Yurekli (2005) reported that compared with control plants, the protein amount in salt sensitive *L. esculentum* plants decreased with the salt application. In salt tolerant *L. pennellii* plants, total protein amount was more than control plants.

Table 10

Soluble protein content (mg g⁻¹) in the five selected groundnut varieties at different stages of growth with different levels of salinity stress

| Varieties | 25 DAS | | | 40 DAS | | | 55 DAS | | | 70 DAS | | | 85 DAS | | |
|-----------------|--------------|-----------|------------|--------------|-----------|------------|--------------|-----------|------------|--------------|-----------|------------|--------------|-----------|------------|
| | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl |
| VRI2 | 7.2 | 6.9 | 6.7 | 12.4 | 12.0 | 6.9 | 13.8 | 14.0 | 13.2 | 14.4 | 14.5 | 14.0 | 7.5 | 7.4 | 6.9 |
| VRI3 | 6.6 | 6.5 | 6.0 | 11.2 | 10.1 | 10.0 | 12.0 | 12.4 | 12.0 | 13.2 | 13.0 | 13.0 | 5.5 | 6.0 | 6.0 |
| TMV7 | 8.1 | 8.0 | 7.6 | 12.9 | 10.0 | 9.0 | 14.2 | 13.0 | 11.0 | 14.6 | 14.0 | 12.4 | 12.6 | 11.0 | 10.0 |
| CO3 | 7.7 | 7.6 | 7.0 | 12.6 | 10.2 | 8.0 | 14.1 | 13.2 | 9.4 | 14.8 | 13.8 | 11.0 | 8.4 | 9.4 | 9.0 |
| CO4 | 6.8 | 6.8 | 6.0 | 12.2 | 8.0 | 7.8 | 13.2 | 10.1 | 9.9 | 14.1 | 12.0 | 10.9 | 6.1 | 9.0 | 8.8 |
| CD(0.05) | 0.017 | | | 0.026 | | | 0.039 | | | 0.020 | | | 0.018 | | |

Values presented are mean of three replicates in each variety in two different salinity levels

DAS- Days after sowing

Demiral and Turkan (2006) detected that while total soluble protein content of salt tolerant *O. sativa* cv. Pokkali plants increased with salinity, sensitive (*O. sativa* cv. IR-28) rice cultivars showed a decrease under salt stress. Similar results were reported in salt tolerant cultivars of barley, sunflower, finger millet and rice plants (Parvaiz and Satyavati, 2008). Doganlar *et al.* (2010) explained that soluble protein is the most abundant protein containing the enzyme RUBISCO, which is involved in CO₂ assimilation; therefore, the reduction in soluble protein might have a direct adverse effect on photosynthesis.

4.2.6. Proline

There was a gradual accumulation of proline upto 55 DAS with the remarkable reduction in the subsequent stages. All the five varieties differed significantly in proline accumulation at all the growth stages. At 55 DAS, the variety VRI3 recorded the highest accumulation of 781 $\mu\text{g g}^{-1}$ followed by the variety TMV7 (599 $\mu\text{g g}^{-1}$), with the significant difference over other varieties (Table 11). A significantly reduced proline accumulation was recorded by the variety CO4 (491 $\mu\text{g g}^{-1}$). In the later stages of the growth also, all the varieties performed in a similar way as observed on 55th DAS. The two salinity levels induced the proline accumulation of leaf at various concentrations. Mild salinity stress (50mM NaCl) improved the accumulation of proline over the respective control at 55 DAS. All the stress treatments differed significantly with each other and also over control.

Salt stress leads to the suppression of plant growth and development, membrane leakage, ion imbalance or disequilibrium, enhanced lipid peroxidation and increased production of reactive oxygen species like superoxide radicals, hydrogen peroxide and hydroxy radicals, which are scavenged by both enzymatic and non-enzymatic reactions (Roychoudury *et al.*, 2008). In order to maintain homeostasis during stress condition, plants need to have special mechanisms for adjusting internal osmotic conditions and changing of osmotic pressure inside the cell, a process called osmotic adjustment (OA). Stressed plants diminish osmotic potential by accumulating low molecular weight, osmotically active compounds called osmolytes. These compounds, which include simple or complex sugars, sugar alcohols, polyols, inositols, quarternary amino compounds

Table 11

Proline content ($\mu\text{g g}^{-1}$) in the five selected groundnut varieties at different stages of growth with different levels of salinity stress

| Varieties | 25 DAS | | | 40 DAS | | | 55 DAS | | | 70 DAS | | | 85 DAS | | |
|-----------------|-------------|-----------|------------|-------------|-----------|------------|-------------|-----------|------------|-------------|-----------|------------|-------------|-----------|------------|
| | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl | Control | 50mM NaCl | 100mM NaCl |
| VRI2 | 245 | 251 | 259 | 360 | 369 | 381 | 507 | 510 | 519 | 392 | 399 | 419 | 259 | 259 | 319 |
| VRI3 | 255 | 259 | 301 | 325 | 320 | 331 | 470 | 775 | 781 | 349 | 361 | 381 | 193 | 220 | 231 |
| TMV7 | 322 | 325 | 339 | 399 | 401 | 420 | 573 | 579 | 599 | 439 | 451 | 453 | 283 | 280 | 300 |
| CO3 | 307 | 320 | 331 | 376 | 380 | 401 | 540 | 545 | 551 | 403 | 420 | 431 | 271 | 280 | 299 |
| CO4 | 271 | 279 | 301 | 345 | 344 | 349 | 487 | 490 | 491 | 375 | 380 | 386 | 299 | 221 | 229 |
| CD(0.05) | 1.77 | | | 1.53 | | | 1.42 | | | 3.17 | | | 1.66 | | |

Values presented are mean of three replicates in each variety in two different salinity levels

DAS- Days after sowing

like glycine-betaine, proline and higher polyamines (PAs), serve as osmoprotectants under stress conditions, maintain membrane structure and act as free-radical scavengers preventing lipid per oxidation or as regulators of K⁺ channels in stomata (Hasegawa *et al.*, 2000).

Compatible osmotica such as proline and glycine betaine are thought to function as osmoprotectants for proteins (Wang *et al.*, 2011). The level of proline accumulation in plants varies from species to species and can be 100 times greater than in control situation (Nathalie and Christian, 2008). Proline accumulation may be a part of the stress signal influencing adaptive responses (Maggio *et al.*, 2002).

Such substances provide an environment compatible with macromolecular structure and function. Circumstantial evidence indicates that stress induced proline accumulation is adaptive, since they may function as osmoprotectants. Under stress conditions proline serves as a storage sink for carbon and nitrogen and a free radical scavenger. It also stabilizes subcellular structures (membranes and proteins) and buffers cellular redox potential.

As observed in the present study, the salinity stress seemed to induce proline accumulation at various levels. At the time of pegging (55 days after sowing), salinity at lower levels was found to cause an increase in proline accumulation over the control. Therefore it could be ascertained that proline synthesis might have been accelerated at the sub lethal level of stress rather than severe stress. Proline accumulation has been suggested to result from: (a) decreased proline degradation, (b) increased proline biosynthesis, (c) lower proline utilization, and (d) protein degradation (Delauney and Verma, 1993). In higher plants, proline is synthesized via both glutamate (Glu) and ornithine (Orn) pathways. The glutamate pathway is catalyzed by a single bifunctional enzyme, pyrroline-5-carboxylate synthetase (P5CS) and produces gultamic semialdehyde (GSA), which is spontaneously converted to pyrroline-5-carboxylate (P5C) and then reduced to proline (Nathalie and Christian, 2008; Maurizio *et al.*, 2008; Silke *et al.*, 2010).

Summart *et al.* (2010) reported that proline content in the salt stressed cells of Thai rice was approximately 2.0 - 2.5 times higher than that in the control cells.

It is generally assumed that proline is acting as a compatible solute in osmotic adjustment. It may act as an enzyme protectant, stabilizes membranes and cellular structures during hostile conditions, detoxifies free radicals by forming long-lived adducts with them and affects solubility of various proteins by interacting with their hydrophobic residues (Hong *et al.*, 2000). Proline may also serve as an organic nitrogen reservoir ready to be used after stress relief to sustain both amino acid and protein synthesis. An increase in proline content under stress condition may be due to breakdown of proline rich protein or *de novo* synthesis of proline. It could also be due to prevention of feedback inhibition of the biosynthetic enzyme caused by sequestering proline away from its site of synthesis or by relaxed feedback inhibition of the regulatory step enzyme or by decreased activity of enzymes involved in degradation of proline such as proline dehydrogenase and proline oxidase (Girija *et al.*, 2002). The present study reports were similar to those obtained by them in various groundnut genotypes.

In the present study, in order to explore the pattern of proline accumulation with time trend, the estimation was done at all the growth stages. The data revealed that there was no proline accumulation after pegging, indicating that the protein biosynthetic process stopped functioning at this stage. However the maintenance of higher level of proline in some varieties like TMV7 and VRI3 indicated that adaptive mechanism still continued upto pod maturation stage. In the present study, TMV7 and VRI3 performed better throughout the growth stages by maintaining higher proline content, whereas VRI2, CO3 and CO4 expressed their susceptible nature by way of accumulating lesser proline content.

4.2.7. Oil Content

In the present study, the oil content of the five selected groundnut varieties under two different salinity levels was found to be significantly differing among each other (Table 12). The groundnut variety TMV7 was found to have a significantly reduced effect of salinity on its oil content (13%) which proved its tolerance towards salt stress compared to other varieties chosen for the study. All the five varieties were found to have a decreased percentage of oil compared to their respective control, which might be due to the decreased yield of groundnuts because of

salinity stress. Similar result was reported by Ahmed and Mohamed (2009) in *Arachis hypogaea* L. under salinity stress through mutation induction.

Table 12
Oil content (%) in the five selected groundnut varieties with different levels of salinity stress

| Varieties | Oil content (%) | | |
|-----------------|-----------------|-----------|------------|
| | Control | 50mM NaCl | 100mM NaCl |
| VRI2 | 13 | 11 | 8 |
| VRI3 | 20 | 15 | 6 |
| TMV7 | 20 | 18 | 13 |
| CO3 | 19 | 11 | 9 |
| CO4 | 19 | 18 | 6 |
| CD(0.05) | 13.8 | | |

Values presented are mean of three replicates in each variety in two different salinity levels
DAS- Days after sowing

Peanuts are grown worldwide in the tropics and temperate zones primarily as an oilseed crop. Peanut seeds are rich in oil, naturally containing from 47 to 50%. They also make an important contribution to the diet in many countries, being a good source of protein, lipids and fatty acids for human nutrition. Peanut oil is mainly composed of triglycerides and contains small quantities of free fatty acids, diacylglycerols, phosphatides, pigments, flavour compounds and sterols. The saponifiable fraction of peanut oil includes triglycerides, free fatty acids, diacylglycerols and phospholipids. The unsaponifiable fraction is mainly composed of sterols, triterpenic alcohols, tocopherols, tocotrienols, pigments, aliphatic compounds, phenolic compounds and volatile compounds. The major fatty acids present as glycerides in peanut oils are palmitic (C16:0), oleic (C18:1) and linoleic (C18:2) acids that are generally present as triacylglycerols (TGs). In particular the major triacylglycerols are: OOL (17%), PLO (13%), LLO (12%), OOO (10%),

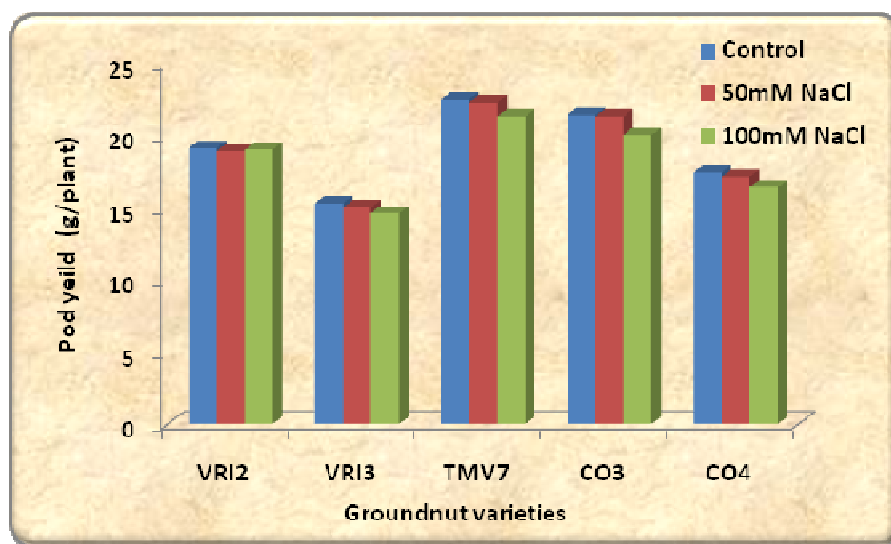
POO (8%), (P=palmitic, O=oleic, S=stearic, and L=linoleic acids). Also free fatty acids and diacylglycerols, called diglycerides, are present in raw peanut oil. Crude oil can have free fatty acids content as low as 0.3%, but most of the commercial oil is in the range 0.5-1.5%. Free fatty acids and diglycerides vary considerably according to peanut maturity (<http://www.mac-oils.eu/ABSpeanut.htm>).

4.2.8. Pod Yield

Per plant pod yield is considered as the major contributing factor for the final crop yield, generally, expressed in unit land area basis. The data on pod yield of groundnut varieties as affected by different salinity status revealed significant differences which are depicted in Figure 17. The groundnut variety TMV7 excelled all the other varieties in registering a significantly higher pod yield of 21.3 g plant⁻¹. This was followed by CO3 (20.0 g plant⁻¹) and VRI2(19.0 g plant⁻¹). The other varieties VRI3 (14.6 g plant⁻¹) and CO4 (16.4 g plant⁻¹) registered significantly lower yield.

Figure 17

Pod yield (g plant⁻¹) of the five selected groundnut varieties with different levels of salinity stress



The adverse effect of stress treatments could be noticed in terms of yield reduction. Higher salinity level of 100 mM was found to reduce the yield in all the varieties except VRI2. The stress treatments were found to affect the pod yield of

all the varieties at different intensities. VRI3 variety proved its sensitivity in terms of its yield reduction at severe stress conditions. No significant reduction was observed between control and 50mM NaCl treatments in all the varieties. There was significant reduction noticed in the yield of all four varieties except VRI3 at 100mM NaCl stress.

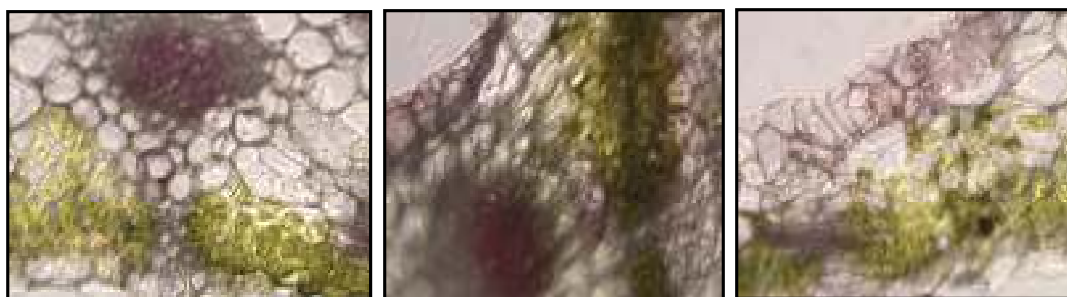
Besides the varietal variations, salinity stress at the two different levels exhibited their significant inhibitory effect on pod yield. Salinity stress at 50 mM level had significantly reduced effect on pod yield. A higher stress level of 100 mM salinity was found to result in a significant reduction over the control. In a study conducted with groundnut and wheat, yield reductions of upto 79 per cent in groundnut and 47 per cent in wheat was observed at the salinity level of 7.5 dSm⁻¹ (Sharma *et al.*, 2003). According to Girdhar *et al.* (2003), the yield reduction in a particular tolerant groundnut genotype, was only 13 per cent, whereas in the susceptible genotypes, the pod yield reduction was more than 50 percent. These findings also strongly supported the results of the present study.

4.2.9. Morphology of Stomata

In the present study the number of stomata was decreased upon stress and their development was affected which was seen from their improper closure which is evident from Plate 7. This might affect the exchange of gases in the stomata and lead to the damage of photosynthesis, transpiration and ultimately affect the plant growth.

Plate 7

Stomatal changes in groundnut leaves under two different salinity levels



(a) Control

(b) 50mM NaCl

(c) 100mM NaCl

It has been reported that salinity limits plant growth and productivity (Ashraf and Fooland, 2006; Ghazi and Al-Karaki, 2006). Salinity stress has proved to decrease photosynthetic capacity, due to the osmotic stress and partial closure of stomata (Drew *et al.*, 1990). It is evident that there are big changes in morphology and anatomy of plants growing in saline soils. The effect of salinity on root (An *et al.*, 2003) and leaf anatomy (Hu and Sehmihalter, 2001; Kilic *et al.*, 2007) of plants had already been reported in previous works. Many researchers reported that with increase in salinity there was a decrease in the development of the xylem (Pimmongkol *et al.*, 2002) and stated that the width of vascular bundles and diameters of rice stems were found to decrease in sodium chloride medium.

The decline in photosynthesis observed in case of salinity could be attributed to stomata factors. During salt stress the concentration of CO₂ in chloroplasts would have decreased because of the reduction in stomata conductance. It also might indicate that reduced photosynthetic carbon assimilation might be attributed to reduced stomata conductance. This tendency, with respect to the water use efficiency, observed in the leaves suggests that the non-stomata factors, in addition to the stomata ones, affected photosynthesis. Limited CO₂ fixation due to stress conditions leads to a decrease in carbon reduction by the Calvin cycle and decrease in oxidizes NADP⁺ to serve as an electron acceptor in photosynthesis (Khan and Panda, 2008). Plants reduce respiration while they withhold water inside their tissues as a response against salt stress. The plant might adapt itself to this event by closing its stomata and reducing stomata number in unit area at increased salt concentrations to protect itself (Turkoglu *et al.*, 2011).

4.3. Analysis of Heritability

Heritability estimates for a trait reflects the ability of the species to pass on the trait to the future generations. The success of any crop improvement program largely depends on the genetic variability present in the population. Heritability estimates are used to determine the amount of variation present in the population. Heritability combined with genetic advance will bring out the genetic gain expected from selection (Shoba *et al.*, 2009).

Table 13

Heritability analysis of the five different groundnut varieties

| | Germination Percentage | Root Length | Height of the plant 90 DAS | No. of leaves 90 DAS | Fat content | Mineral content | Carbohydrate | Protein | Pods | Seeds | Oil |
|---------------------------|------------------------|-------------|----------------------------|----------------------|-------------|-----------------|--------------|---------|------|-------|------|
| s²g | 2.68 | 0.24 | 3.81 | 0.46 | 0.04 | 0.02 | 0.21 | 1.05 | 0.36 | 1.64 | 3.62 |
| SE(s²g) | 0.06 | 0.13 | 0.75 | 0.03 | 0.38 | 0.11 | 0.06 | 0.12 | 0.06 | 0.06 | 0.9 |
| Z | 46.36 | 1.85 | 5.07 | 14.06 | 0.1 | 0.15 | 3.58 | 8.49 | 5.61 | 29.33 | 4.02 |
| s²e | 38.13 | 1.42 | 3.65 | 14.03 | 0.07 | 0.11 | 3.04 | 6.52 | 4.63 | 24.8 | 2.89 |
| h² | 0.07 | 0.15 | 0.51 | 0.03 | 0.34 | 0.12 | 0.06 | 0.14 | 0.07 | 0.06 | 0.56 |

s²g - Genetic variance

SE (s²g) - Standard error of s²g

Z - Test statistic to determine significance of s²g

s²e - Residual/error variance

h² - Heritability of the trait, computed as $s^2g/(s^2g + s^2e)$

The five groundnut varieties chosen for the present work were studied for mean and variability parameters for pods and kernel yield as well as other component characters. The genetic parameters namely variability, Genotypic Coefficients of Variation (GCV), Phenotypic Coefficients of Variation (PCV), heritability and genetic advance as percentage of mean were calculated by the IRRISAT Mixed Model Analysis software which was used to analyse the phenotypic and genotypic variance of the groundnut varieties selected for the present study based on their morphological, physiological and biochemical characteristics. A heritability value of 0.5 is considered very high. The results on the mean performance of all the five varieties selected for the study are presented in Table 13. 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 good performance in salt affected sites, while progenies of the groundnut variety CO4, which were found susceptible to salt stress suggested further advancement in generations for enhancing their salt tolerance abilities.

4.4. Fatty Acid Profile of the Groundnut Oil from the Five varieties of Groundnut Seeds (*Arachis hypogaea* L.)

4.4.1. High Pressure Thin Layer Chromatographic analysis of free fatty acids

Groundnut (*Arachis hypogaea* L.) is among the major oil seeds in the world. China, India and USA are the main producers of groundnuts to the rest of the world (Campos-mondragon *et al.*, 2009). In India groundnuts are used mainly for oil extraction (Ingale and Shrivastava, 2011). Groundnut seed contains 44-56% oil and 22-30% protein on a dry seed basis and is a rich source of minerals (phosphorus, calcium, magnesium and potassium) and vitamins (E, K and B group) (Isleib *et al.*, 2006). Nuts are defined as fruits with less than 50% of water. They have high proportion of fat (45-75%). Fats from nuts have a degree of saturation near to ideal intake in humans. Their high portion of mono unsaturated fatty acid (MUFA) makes nuts an excellent food to weight control and decrease cardiovascular risk. They

have also considerable amounts of many compounds involved in human wellness viz. antioxidant molecules and phytosterols (www.hptlc.com/pdf/2006pdf/pdf10pm/pujol.pdf).
















The genus *Arachis*, a member of the family Leguminosae, is widely distributed in the tropics and moderate regions. Groundnut (*Arachis hypogaea* L.) is an important source of edible oil for millions of people living in the semi tropic region. Groundnuts make an important contribution to the diet in many countries. Groundnut seeds are a good source of protein, lipid and fatty acids for human nutrition. Groundnuts are rich in oil, naturally containing from 47 to 50%. The oil is pale yellow and has the characteristic odour and flavour of peanuts (Ozcan and Seven, 2003). Grosso *et al.* (2000) have reported the oil, protein, ash, carbohydrate contents, iodine value and fatty acid composition of some wild groundnut species (*Arachis*) seeds. Predominant fatty acids as free acids were oleic, linoleic, palmitic and arachidic acids.


In the present study, 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 HPTLC technique. Pink and red coloured zones were present in all groundnut oil sample tracks in visible light and it was observed from the chromatogram that confirmed the presence of fatty acids in the given oil samples of the five selected varieties of groundnuts under two salinity levels namely 50mM and 100 mM NaCl (Table 14).

Three additional fatty acids were found in the oil sample of variety VRI2 under 100mM salinity level at Rf value of 0.62, 0.79 and 0.90 respectively. Two fatty acids that were found in control sample of VRI3 groundnut oil variety (0.32 and 0.36) were not present in the oil samples of the same varieties treated with 50 and 100mM NaCl. Similarly, one fatty acid that was seen in the control oil sample (Rf 0.87) of VRI3, was not present in the same variety oil at 50mM salinity level. Two additional fatty acids were found in the 50mM and 100mM NaCl treated groundnut oil samples of variety VRI3 at Rf values of 0.60 and 0.79 respectively.

Table 14

Fatty acid profile of the five varieties of groundnut oil under salt stress as revealed by HPTLC analysis

| Treatments | VRI2 | VRI3 | TMV7 | CO3 | CO4 |
|-------------|---|---|---|--|---|
| 0 mM NaCl |  |  |  |  |  |
| 50 mM NaCl |  |  |  |  |  |
| 100 mM NaCl |  |  |  |  |  |

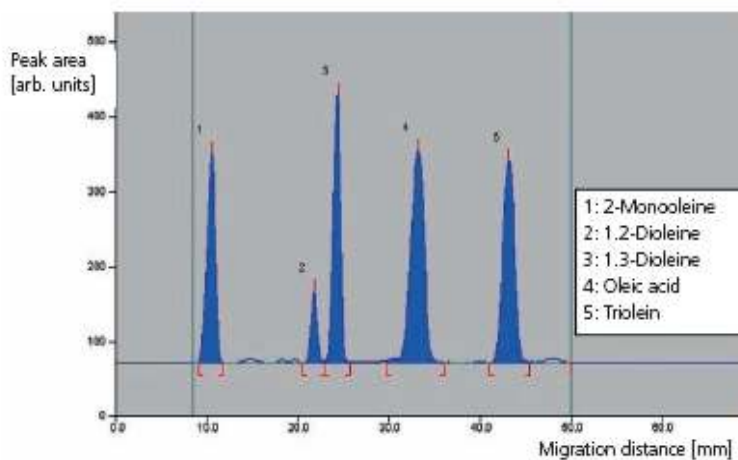


Peak of one fatty acid seen at Rf 0.92 in the control sample of TMV7 variety of groundnut was not present in both of its 50 and 100mM salt treated varieties. One additional fatty acid peak was observed in TMV7 variety at 100 mM NaCl salinity level at Rf 0.79. No changes in the fatty acid profile in the control and treated plants of CO3 were observed. One fatty acid (Rf 0.61) found in control was expressed at 50mM NaCl stress of CO4 but in the same variety at 100mM NaCl level it was not present (Plate 8 and Figure 18).

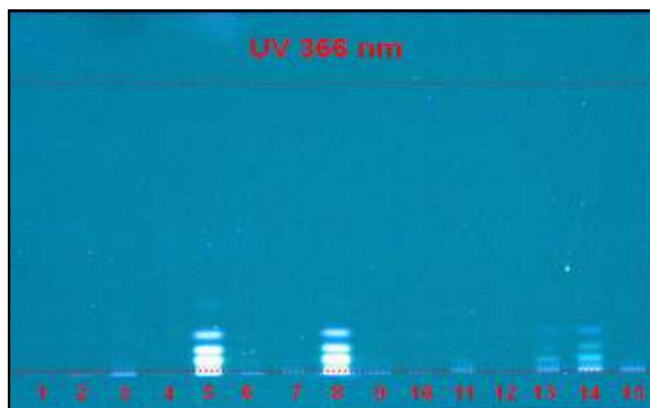
Considering all these facts, it is obvious that one additional fatty acid was common in three groundnut oil varieties of VRI2, VRI3 and TMV7 compared to the other two varieties used namely CO3 and CO4. The same was found in the control sample of CO4 variety but totally absent in CO3 variety. Based on the morphological and physiological responses of the varieties to salt tolerance, the ranking of the varieties for salt tolerance is of the order CO4<VRI2≡VRI3≡CO3>TMV7. The fatty acid profile of the varieties in HPTLC reveals a similar pattern in the case of one particular unknown fatty acid with Rf value at 0.79. This is indicative of the fact that fatty acids play a role in the management of abiotic stresses in groundnut. This specific unknown fatty acid would have played a protective role in CO4 and a defensive role in VRI2, VRI3 and TMV7.

Plate 8

Densitogram of the fatty acids profile of the oil from the five selected varieties of groundnut under salt stress



Densitogram of a lipid standard mixture

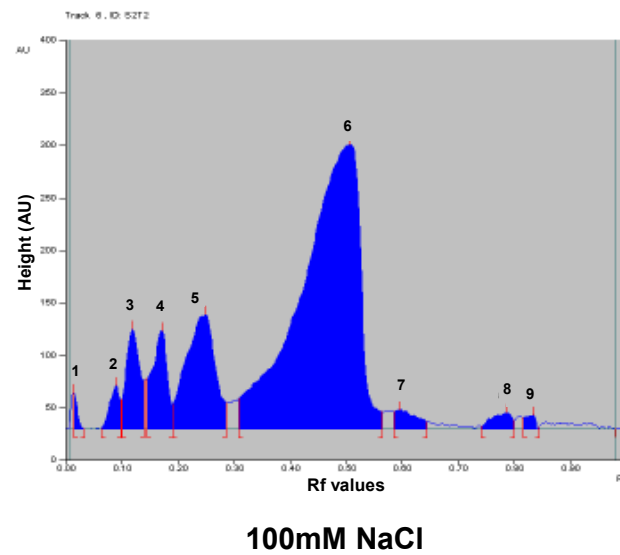
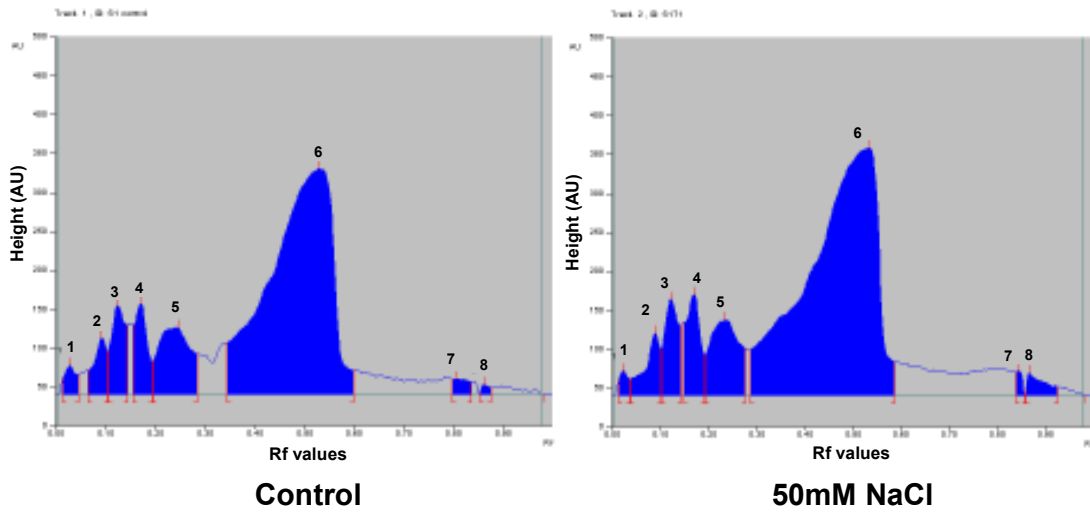


Numbers 1-15 : Oil samples from five selected groundnut varieties at two different salinity levels (50 and 100mM NaCl) including control

Bands : Movement of fatty acids from groundnut oil samples in HPTLC plate visualised at UV 366 nm

Figure 18a

Densitogram display of fatty acid profile present in VRI2 groundnut oil under two different salinity levels

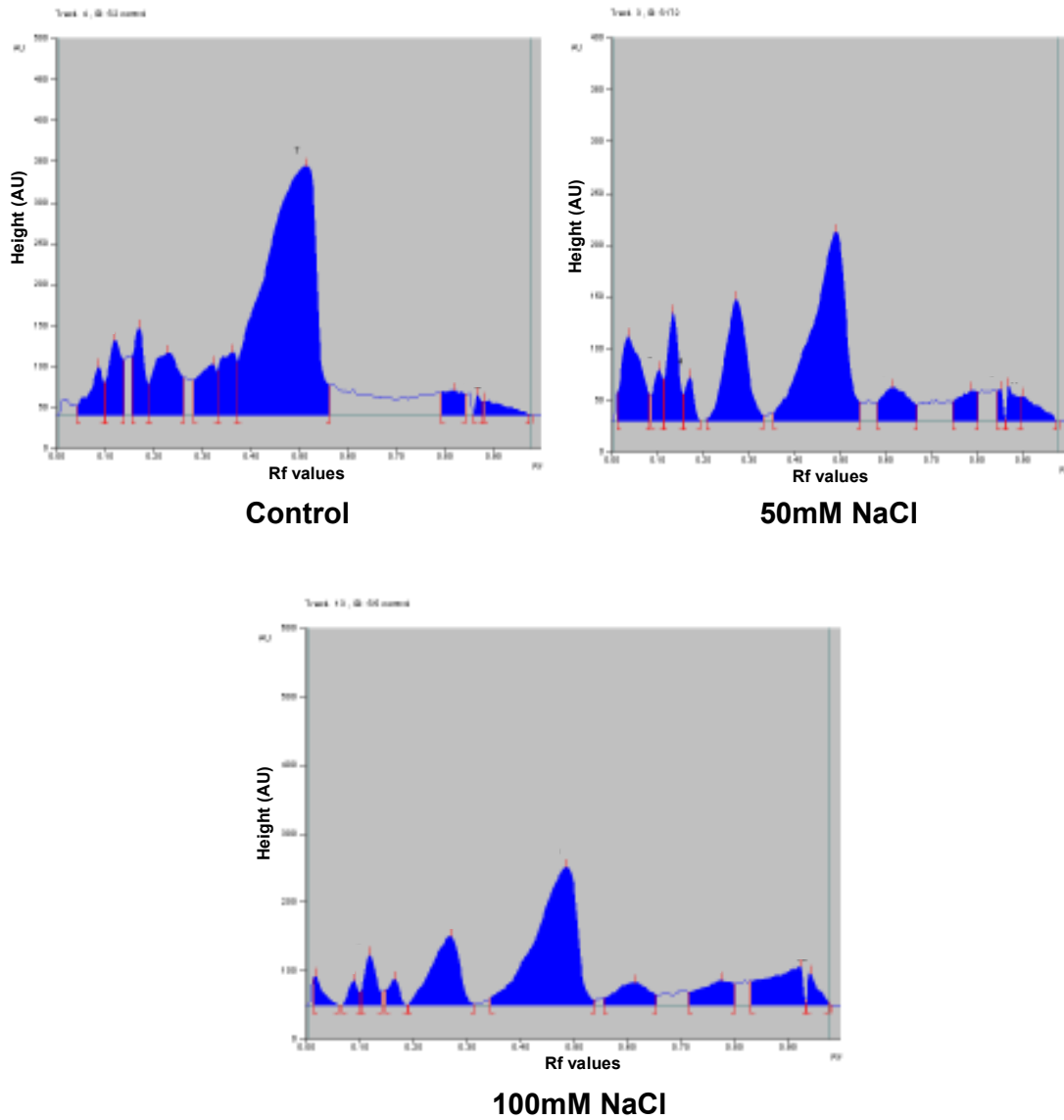


Numbers 1-9 in graph - peaks of fatty acids at different Rf values

1 – 4 : Free fatty acids 5 – Phospholipids 6 – Glycerides 7 – 9: Sterols

Figure 18b

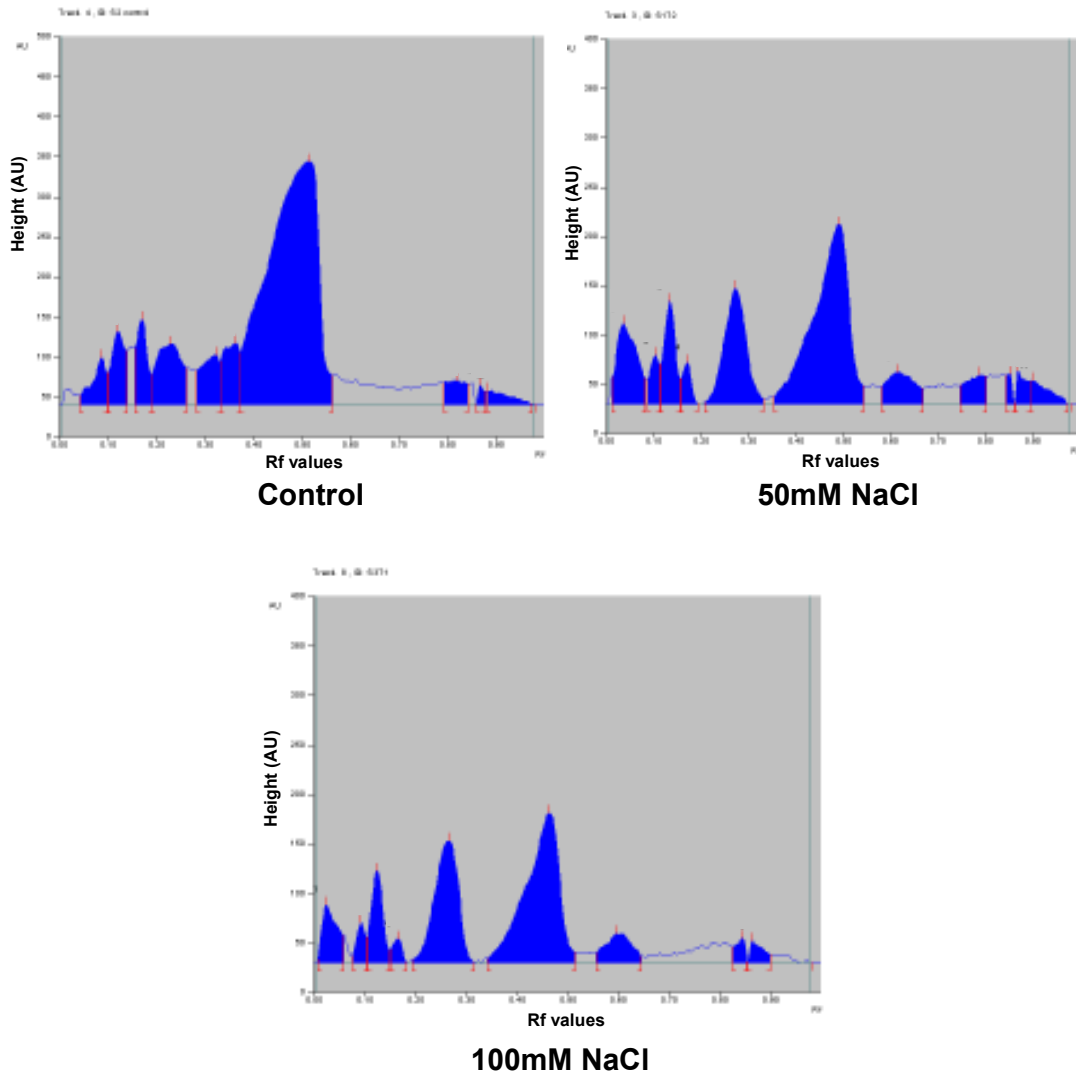
Densitogram display of fatty acid profile present in VRI3 groundnut oil under two different salinity levels



Numbers 1-9 in graph- peaks of fatty acids at different Rf values
1 – 4 : Free fatty acids 5 – Phospholipids 6 – Glycerides 7 – 11: Sterols

Figure 18c

Densitogram display of fatty acid profile present in TMV7 groundnut oil under two different salinity levels

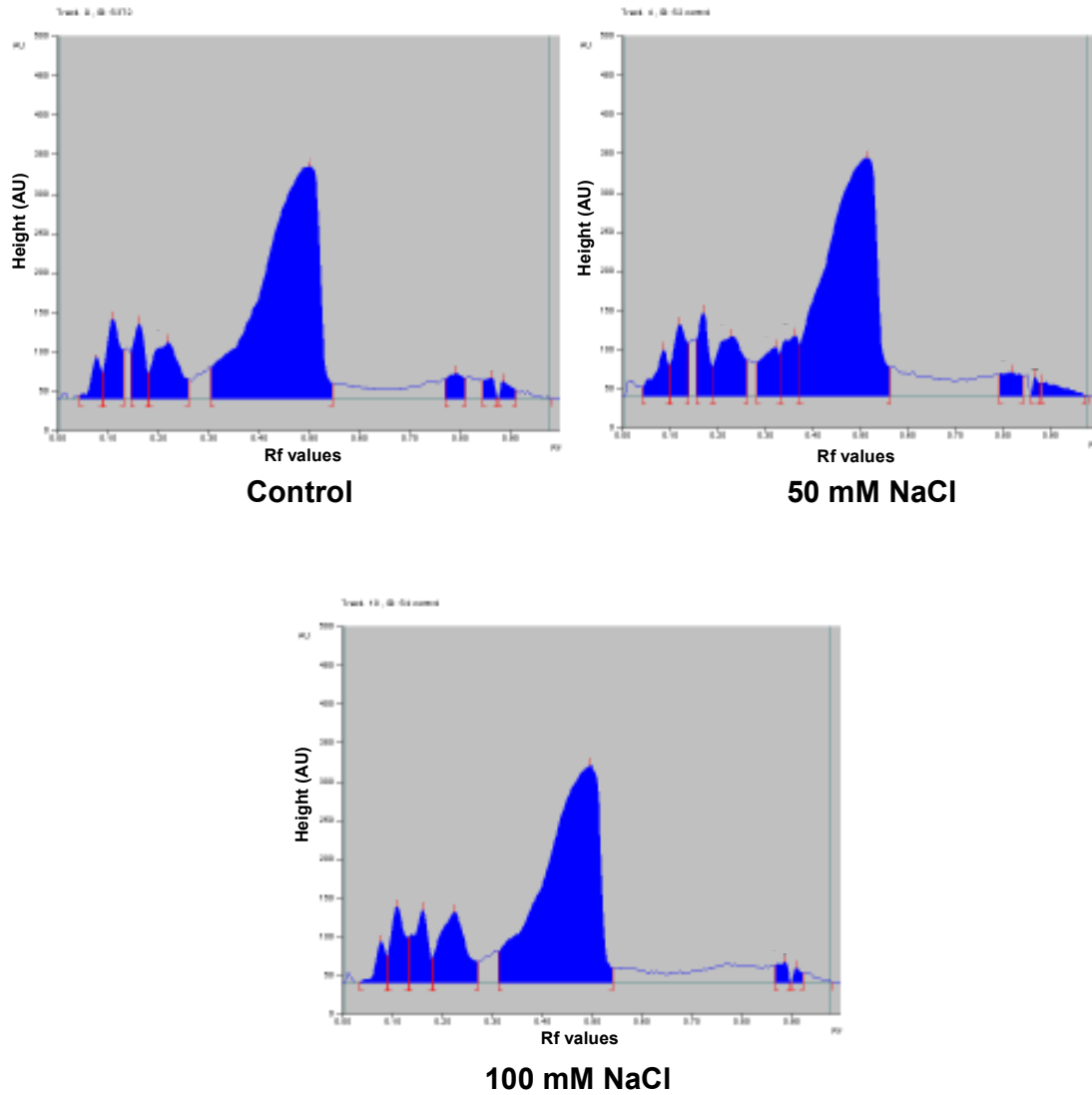


Numbers 1-9 in graph- peaks of fatty acids at different Rf values

1 – 4 : Free fatty acids 5 – Phospholipids 6 – Glycerides 7 – 11: Sterols

Figure 18d

Densitogram display of fatty acid profile present in CO3 groundnut oil under two different salinity levels



Numbers 1-9 in graph- peaks of fatty acids at different Rf values
1 – 4 : Free fatty acids 5 – Phospholipids 6 – Glycerides 7 – 10: Sterols

Figure 18e

Densitogram display of fatty acid profile present in CO4 groundnut oil under two different salinity levels

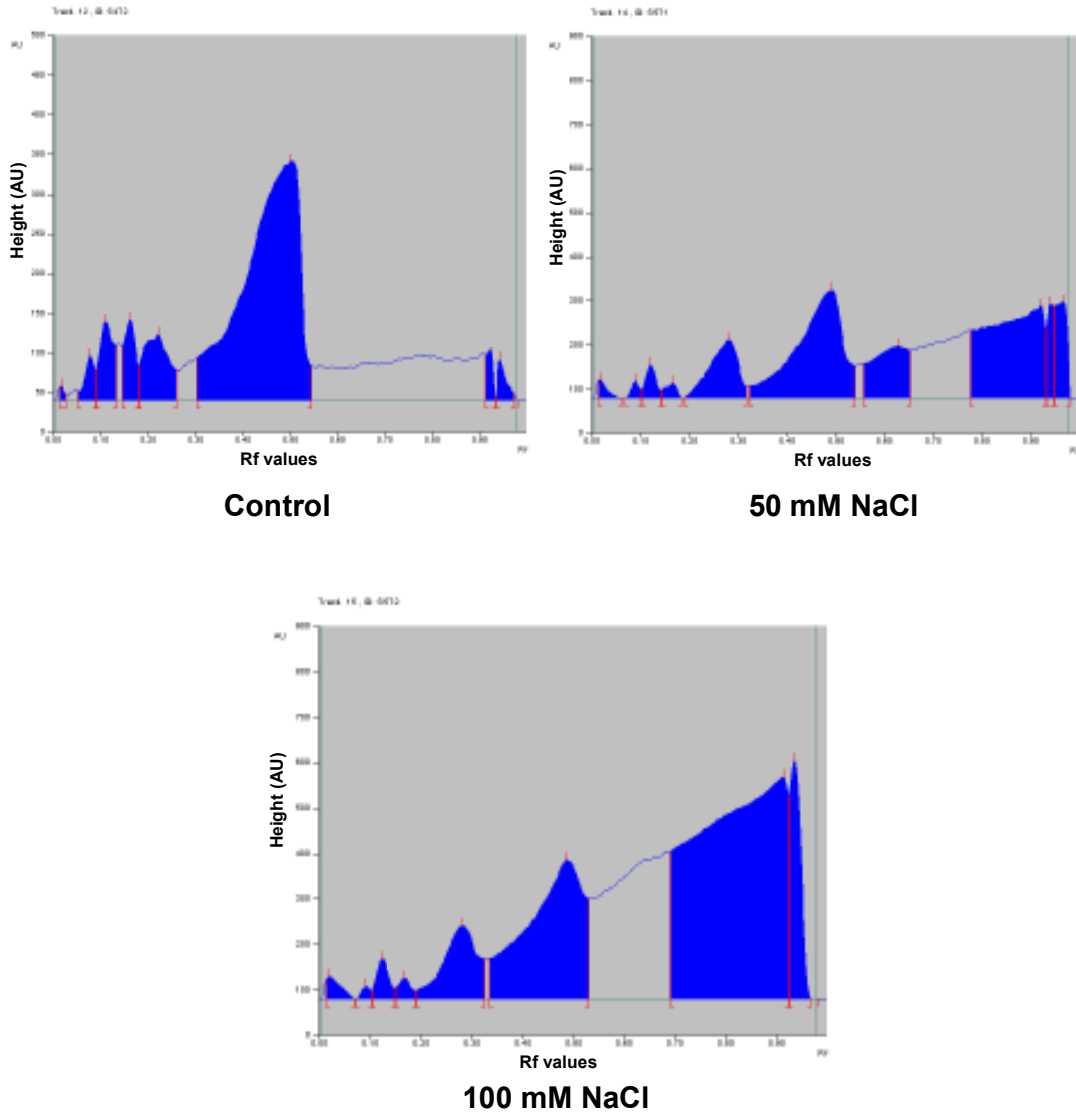
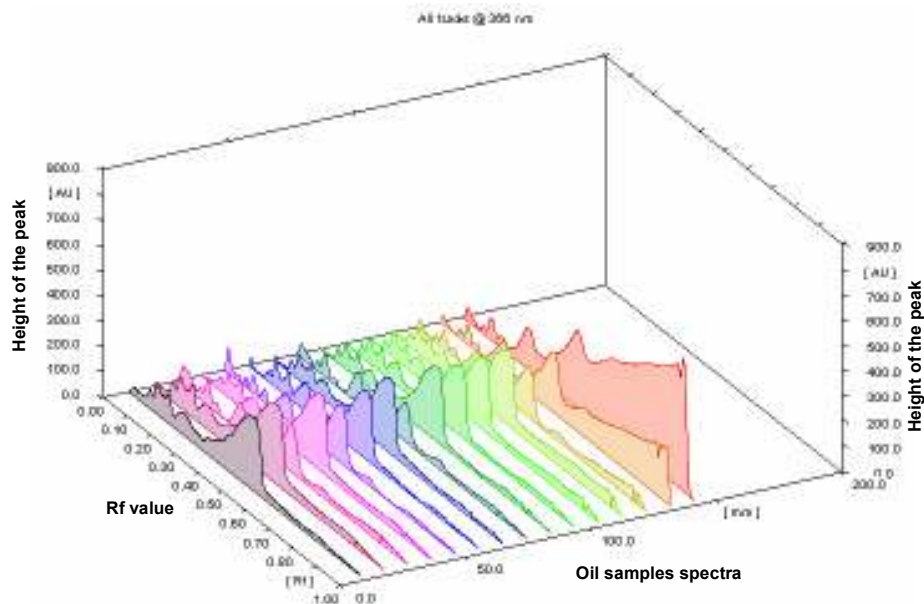


Figure 18f

Overall map of fatty acid profile present in the five selected groundnut oil samples under two different salinity levels



4.4.2. Gas Chromatographic Analysis of Free Fatty acids

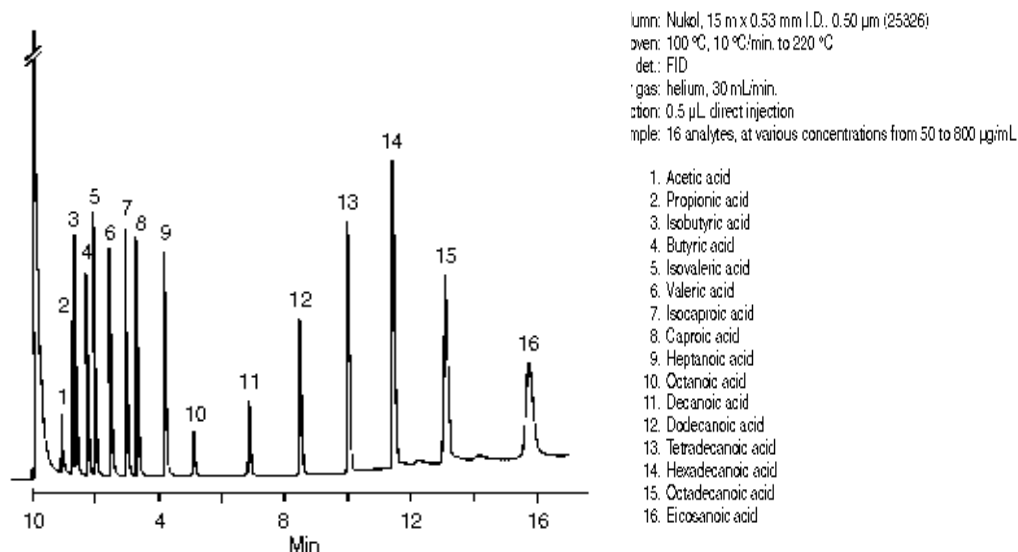
Groundnut oil is an excellent food oil, with good flavour and high quality with its low free fatty acid value. Vegetable oils generally primarily consist of triglycerides but several other compounds are also present. Some of these additional compounds such as diglyceride, tocopherols, sterols and sterols ester need not necessarily be removed during processing. Carefully separated, however, some of these additional compounds, particularly the phosphatides are valuable raw materials (Belcher, 2008).

Although there are many methods that are described in the literature that may be used to analyze fatty acids of vegetable oils, high precision in gas chromatographic analysis of fatty acids is possible with careful attention to details during sample preparation, injection, and chromatograph and data collection. Small theoretical correction factors only should be necessary in most circumstance (Christie, 1993). Also the qualitative and quantitative

determination of the constitution is done by gas chromatography of the compound or their derivatives (Cert *et al.*, 2000). Works have been done in the past and many are still on by lipid analysts to explore the potentials of vegetable oils. The importance of analyzing vegetable oils cannot be overemphasized because in analyzing them, the major feature influencing the physical and chemical properties, their application and uses are obtained. The analysis of phospholipids is important because they contribute to the stability and quality of edible oils, fats and fatty foods through their anti-oxidative activities (Aluyor *et al.*, 2009).

The fat content in groundnut has been largely studied. In general, groundnuts contain 50-55% fat of which approximately 30% is linoleic acid and 45% is oleic acid. High-oleic groundnuts rather than normal groundnuts have increased shelf life and thus proved to improve the oxidative stability of peanut products (Isleib *et al.*, 2006). Based on this fact, in the present study, the free fatty acids present in the oil samples of the five selected varieties of groundnut were further analysed by Gas Chromatographic technique using standard linoleic, oleic, arachidic and stearic acids (Figure 19).

Figure 19
Gas chromatogram of standard peaks of the selected fatty acids



The result revealed that the varieties VRI2 and VRI3 did not show any specific trend in the changes in the levels of saturated and unsaturated fatty acids. The unsaturated fatty acid levels in TMV7 and CO3 were found to increase with increasing salt concentration. Reduction in all the fatty acid profiles studied was observed in treated samples compared to the control in the case of CO4 (Figure 20).

Figure 20a

Gas chromatogram of oil from groundnut variety VRI2

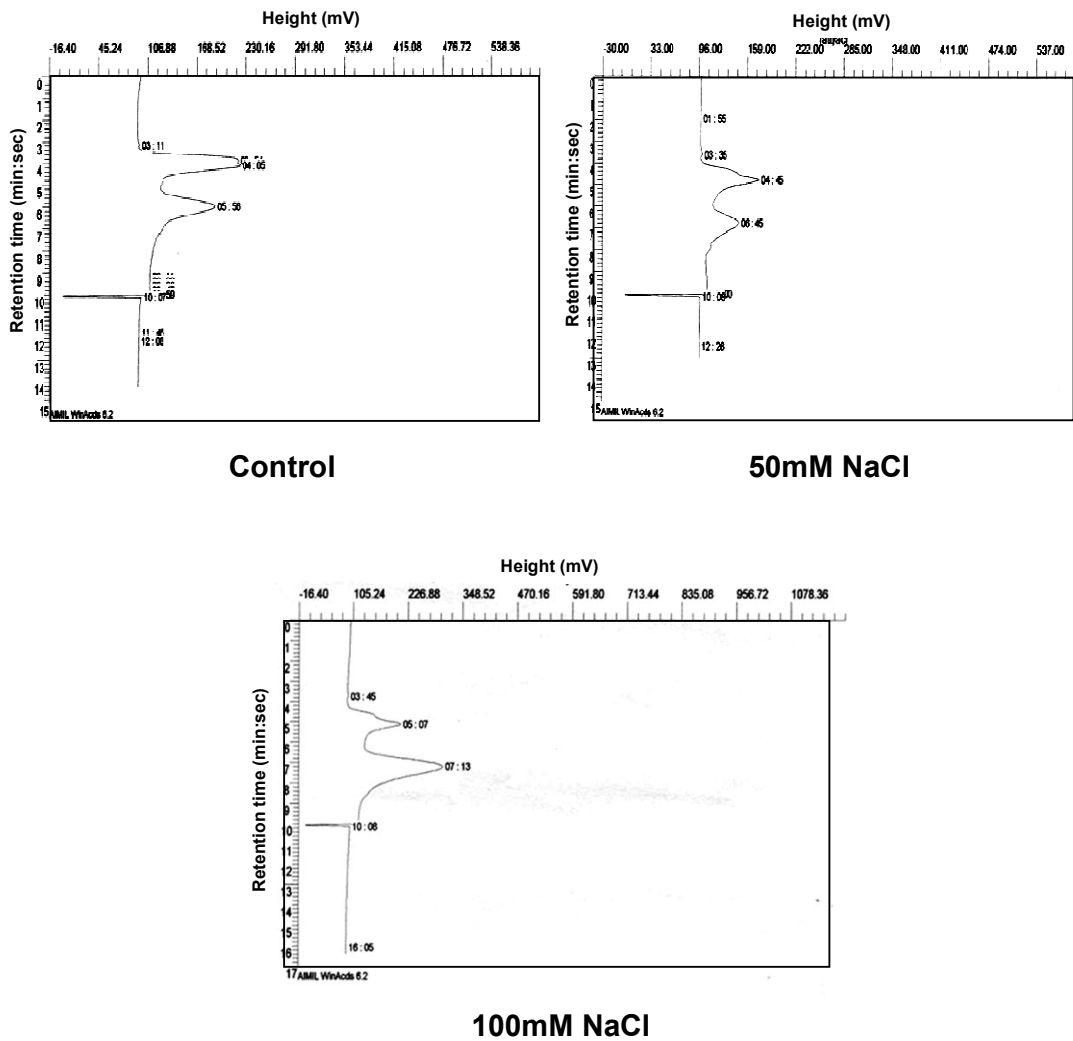


Figure 20b

Gas chromatogram of oil from groundnut variety VRI3

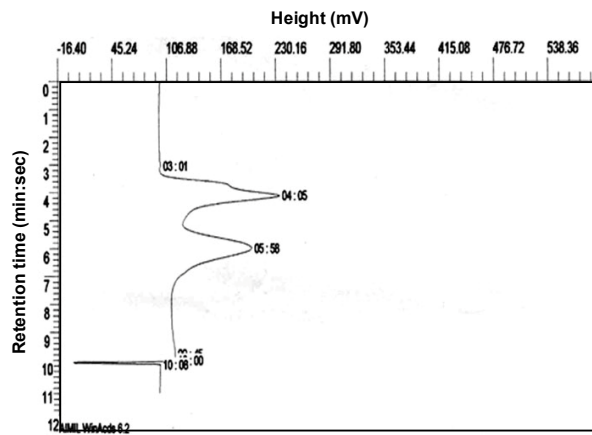
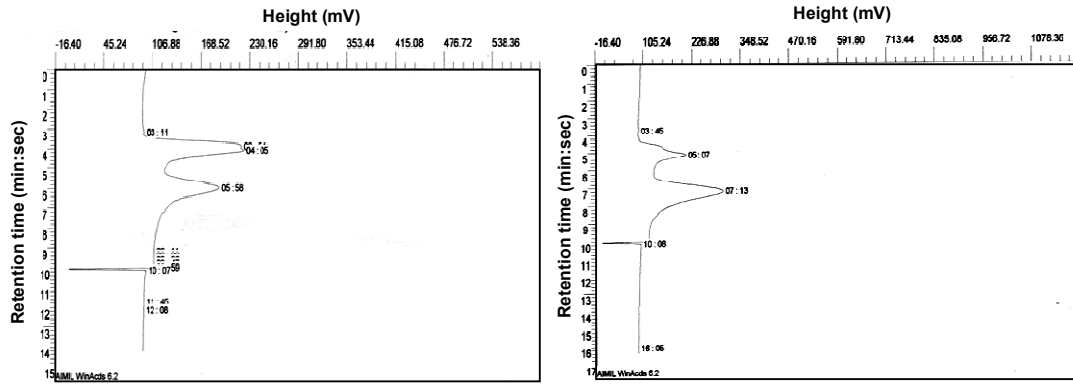


Figure 20c

Gas chromatogram of oil from groundnut variety TMV7

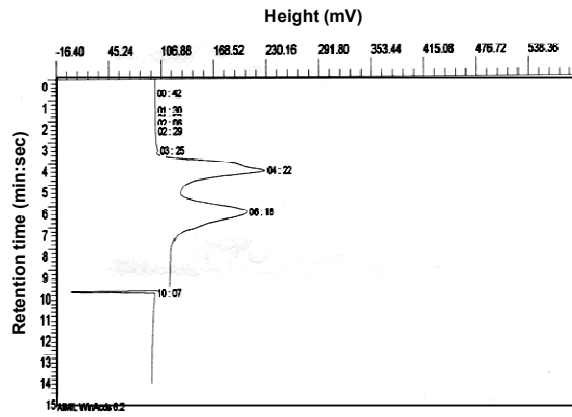
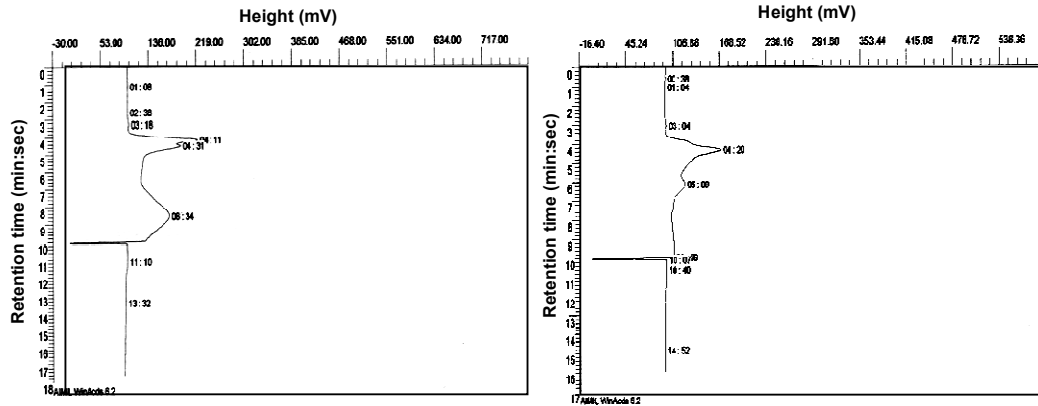


Figure 20d

Gas chromatogram of oil from groundnut variety CO3

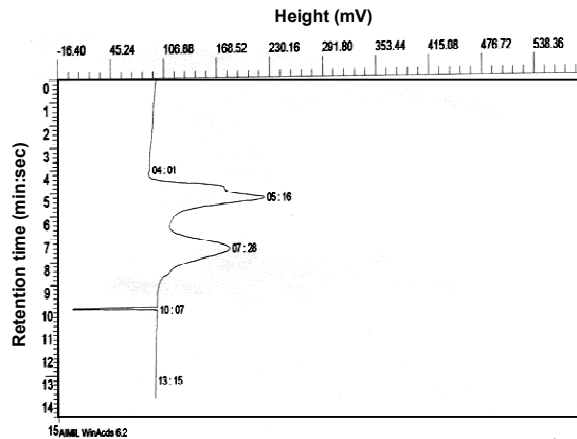
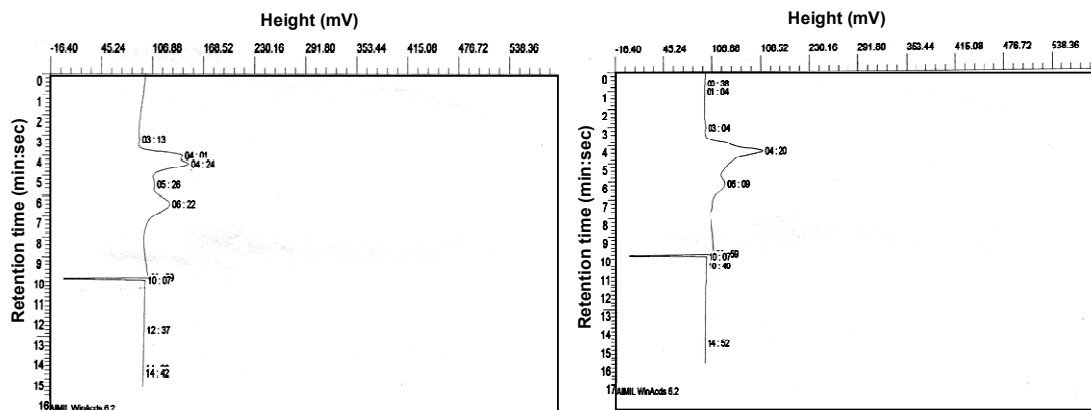
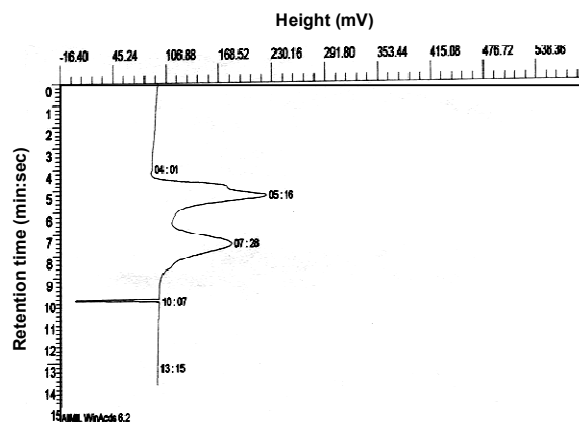
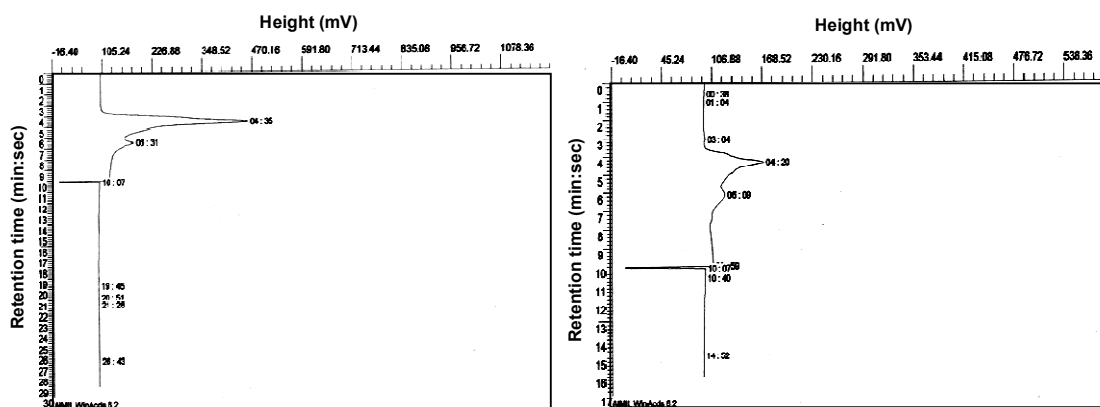


Figure 20e

Gas chromatogram of oil from groundnut variety CO4



The trend followed was similar to the observations made using HPTLC technique wherein CO4 showed reduced fatty acids in sodium chloride treated samples compared to the control. HPTLC revealed presence of additional fatty acids specific to different salinity treatments suggesting 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.

4.5. Field Experiment with the Groundnut varieties (identified as tolerant and susceptible in pot study) with selected Plant Growth Regulating Chemicals

A change in endogenous levels of phytohormones under stress condition has been considered as a step towards adaptation to the adverse conditions. The obstructions and dysfunctions in plants life form under stress may be related to disturbed endogenous levels / balances of phytohormones which can be minimised through exogenous supply of plant growth regulators (PGRs), particularly when the developmental window is open. The exogenous application of PGRs may be quite effective in alleviation of stress responses and use of exogenous PGRs to mitigate the stress effects is of practical significance (Afzal *et al.*, 2005). The overarching goal of crop establishment is to achieve rapid and uniform germination, followed by rapid and uniform seedling emergence plus autotrophy. Seeds are particularly vulnerable to stresses encountered between sowing and seedling establishment. Poor germination and seedling establishment are the results of soil salinity. It is an enormous problem adversely affecting growth and development of crop plants and results into low agricultural production. It is thought that the depressive effect of salinity on germination could be related to a decline in endogenous levels of hormones (Gurmani *et al.*, 2011).

However, incorporation of plant growth regulators in many vegetables crops has improved seed performance. Typical responses to priming are faster, closer spread of times to emergence, overall seedbed environments and wider temperature range of emergence, leading to better crop stands, and hence improved yield and harvest quality, especially under suboptimal and stress conditions in the field (Halmer, 2004).

Recently, investigations have focused more on the mechanisms of salt tolerance in plants (Dajic, 2006; Munns and Tester, 2008). Some researchers have used PGRs for reducing or eradicating the negative effects of salinity (Gurmani *et al.*, 2011). Phytohormones were suggested to play important roles in stress responses and adaptation (Sharma *et al.*, 2005; Shaterian *et al.*, 2005).

Concerted attempts have been made to mitigate the harmful effects of salinity by application of plant growth regulators. Afzal *et al.* (2005) suggested that endogenous hormone level is affected by many environmental stresses. However, external application of appropriate growth regulators optimizes physical metabolic conditions for germination. It is also possible that under high salt concentrations naturally present hormones may be suppressed and that treated with plant growth regulators might supply sufficient hormones for normal growth.

The exogenous application of PGRs, auxins (Khan *et al.*, 2004), gibberellins (Afzal *et al.*, 2005), cytokinins (Gul *et al.*, 2000) produces some benefit in alleviating the adverse effects of salt stress and also improves germination, growth, development and seed yields and yield quality

(Egamberdieva, 2009). To assess the influence of selected plant growth regulating chemicals on alleviating the adverse effects of salinity, field study was conducted using plant growth regulators (Brassinolide – 0.5 ppm and 1.0 ppm and Salicylic acid – 50 ppm and 100 ppm) and nutrient mixture (Diammonium phosphate-1% + Potassium Nitrate - 0.5% + Borax-0.2% + Naphthalene Acetic Acid - 20ppm + Salicylic Acid - 50ppm + Brassinolide -1ppm) as spray. The groundnut variety TMV7, identified as tolerant variety and VRI3, as susceptible variety, through pot study, were employed in this study (Plate 9).

Plate 9

Field study at Viraliyur, Thondamuthur



The influence of plant growth regulators and nutrients sprayed at preflowering, pegging and pod formation stages on the two groundnut varieties, TMV7 and VRI3 exhibited significant variations at all phonological stages. The results of the study are presented below:

4.5.1. Soil Characteristics of the Field chosen for the Study

The soil in Viraliyur, near Thondamuthur, Coimbatore is naturally sodic in condition. The mechanical compositions, physical and chemical properties of this sodic soil were analyzed before the start of the experiment and data are presented in Table 15.

Table 15
Soil characteristics of the experimental field

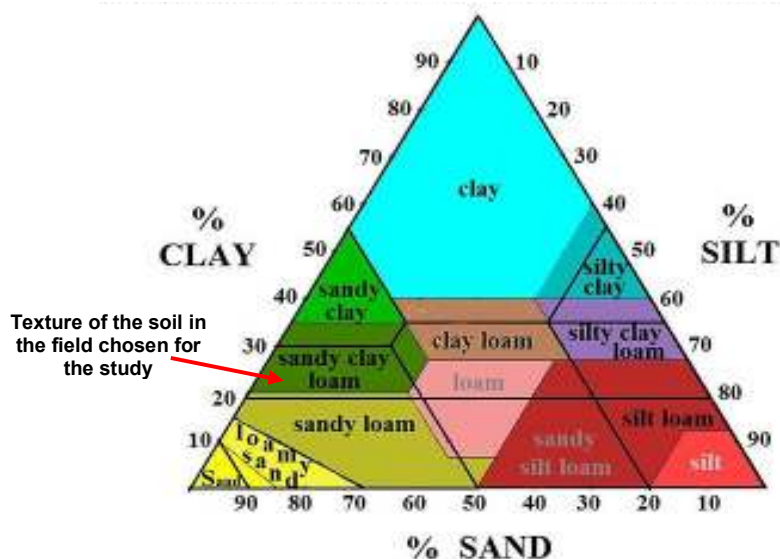
| Characters | Values |
|---|-----------------|
| Mechanical Composition | |
| Coarse sand (%) | 27.0 |
| Fine sand (%) | 38.0 |
| Silt (%) | 15.0 |
| Clay (%) | 20.0 |
| Textural class | Sandy clay loam |
| Chemical properties | |
| pH | 8.82 |
| Electrical Conductivity (dSm ⁻¹) | 0.46 |
| Exchangeable Sodium Percentage (%) | 16 |
| Cation Exchange Capacity (cmol (p ⁺) kg ⁻¹) | 18 |
| Available nitrogen (kg ha ⁻¹) | 168 |
| Available phosphorus (kg ha ⁻¹) | 13.0 |
| Available potassium (kg ha ⁻¹) | 131 |

4.5.2. Assessment of Textural Class of the Soil used in the Field Study using United States Department of Agriculture (USDA) Triangle

After computing the relative percentage of different groups namely clay, silt and sand, the textural class of the soil can be determined using the triangular textural diagram given by the United States Department of Agriculture (1960). The left angle in the triangle represents 100% sand while the right and top angles represent 100% silt and 100% clay respectively. While using this diagram the points corresponding to the percentage of clay, silt and sand present in the soil under consideration are located on the clay, silt and sand lines, respectively. Lines are then projected inward, parallel in the first case to the sand side of the triangle and in the second case parallel to the clay side and in the third case parallel to the silt side. The name of the compartment in which the three lines intersect is the textural name of the soil (Mani *et al.*, 2007).

In the present study, the soil chosen for the field trial was found to have 38 % of sand, 20% of clay and 15 % of silt. These data were then analysed in the USDA triangle to locate for its textural class.

Figure 21
USDA triangle showing different textural classes of soil



It was found to lie in the sandy clay loam soil area in the triangle (Figure 21).

4.5.3. Morphological Characters of the two varieties of Groundnut Plants grown in the Field

4.5.3.1. Plant height

Amirjani (2010) reported a reduction in soybean plant height with increasing salinity levels. The time trend of plant height of groundnut varieties revealed a progressive increase from preflowering to maturity stages (Figure 22a and 22b). Comparison of two varieties at main plot level showed that TMV7 recorded significantly increased plant height than VRI 3. Application of stress ameliorative chemicals namely Brassinolide(BR)-0.5 ppm and 1ppm, Salicylic acid (SA)- 50 and 100ppm and nutrient mixture (Diammonium phosphate-1% +Potassium Nitrate - 0.5% + Borax-0.2% + Naphthalene Acetic Acid - 20ppm + Salicylic Acid - 50ppm + Brassinolide -1ppm) at sub plot level distinctly influenced the plant height in these two varieties. Among the sub plot treatments, BR 1ppm recorded a significantly increased plant height followed by BR 0.5ppm. Other treatment, namely nutrient mixture showed significantly lesser effect on plant height compared to BR 1ppm and BR 0.5ppm treatments. The interaction effects were significant from pegging stage onwards.

Figure 22a

Plant height (cm) of the groundnut variety TMV7 at different stages of growth on treatment with selected plant growth regulators

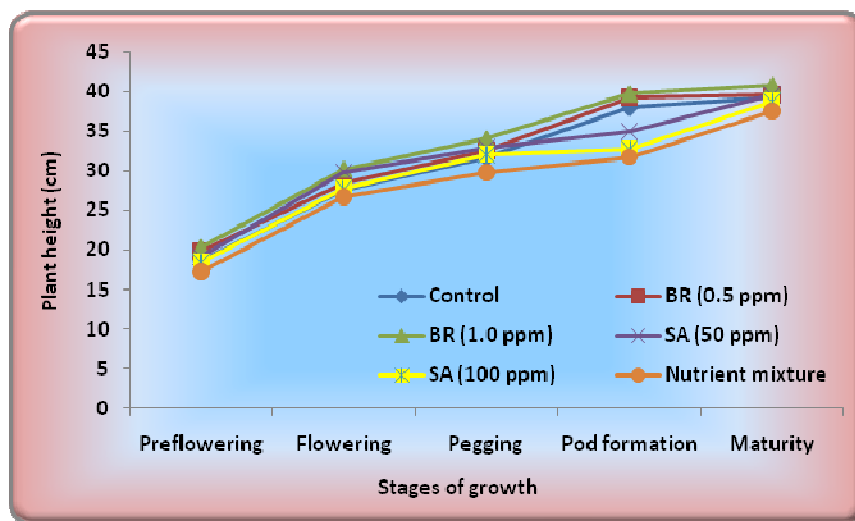
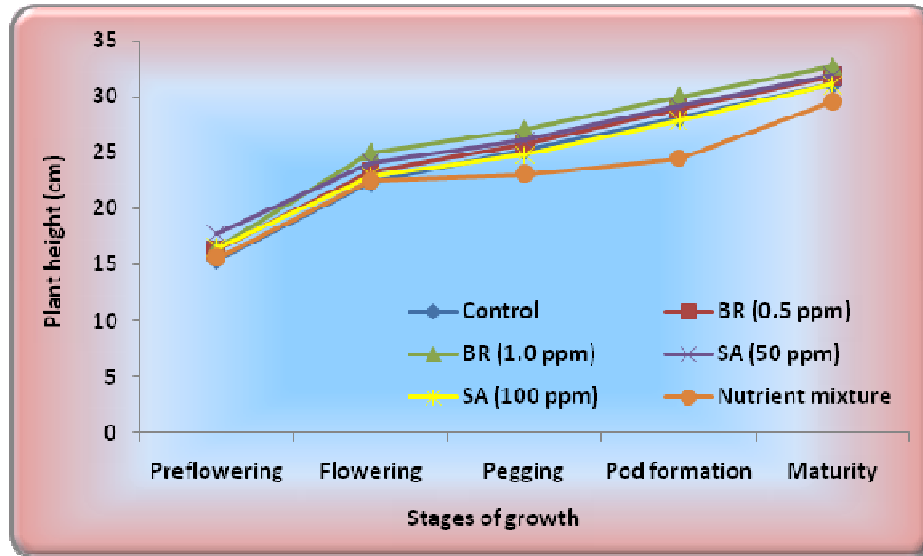


Figure 22b

Plant height (cm) of the groundnut variety VRI3 at different stages of growth on treatment with selected plant growth regulators



The interaction effects were significant at all growth stages. Among the interaction treatments, the groundnut variety TMV7 with BR 1ppm treatment recorded the maximum plant height of 40.8 cm. This was very closely followed by TMV7 with BR 0.5 ppm treatment (39.7cm) and SA 50 ppm treatment (39.7 cm). Considering the other variety VRI3, with SA 50ppm treatment it recorded significantly higher plant height of 31.9 cm than the control. The effect of other treatments showed non significant difference over control.

Comparing the two varieties, TMV7 was much more influenced by the plant growth regulating chemicals. Among the treatments, BR 1 ppm was more effective in improving the height than the other treatments. The treatments BR 0.5 ppm, SA 50 ppm and SA 100 ppm were also found better in improving the plant height significantly over control. However, foliar spray of nutrient mixture was found to be ineffective and showed a little effect on plant growth. These results were in agreement with the findings of Behnamnia *et al.* (2009) in *Lycopersicon esculentum* L. who explained that brassinolide could counteract the salinity induced growth inhibition by accelerating the cell division process. Junko *et al.* (2005) further supported that brassinolide application on leaves not only induced stem

elongation but also enhanced root nodule formations in soybean, thereby, improved the nitrogen usage efficiency.

Jumali *et al.* (2011) showed that most genes responding to acute salicylic acid treatment are related to stress and signaling pathways which eventually led to cell death. This include genes encoding chaperone, heatshock proteins (hsp's), antioxidants and genes involved in secondary metabolite biosynthesis, such as sinapyl alcohol dehydrogenase (SAD), cinnamyl alcohol dehydrogenase (CAD) and Cytochrome P450 (CYP 450). The role of salicylic acid in defense mechanism to alleviate salt stress in plants was studied (Afzal *et al.*, 2006; Hussein *et al.*, 2007). The ameliorative effects of salicylic acid have been well documented including salt tolerance in many crops such as bean (Azooz, 2009), tomato (Tari *et al.*, 2002) and maize (Gunes *et al.*, 2007). Shakirova *et al.* (2003) reported that salicylic acid induced increase in the resistance of wheat seedlings against salinity.

4.5.3.2. Root length

Figures 23a and 23b depict the significant differences between the two main plot treatments and among the subplot treatments. Comparing the two main plots, TMV7 recorded significantly higher root length of 26.4 cm than VRI3 (23.5 cm) at pod formation stage. Among the sub plot treatments, treatment of BR 1ppm registered a significantly increased root length over the control. This was followed by BR 0.5ppm (25.8 cm) and SA 50 ppm (25.4 cm) respectively over the control in the mature plants.

The groundnut variety TMV7 with BR 1ppm treatment performed better than the other treatments with the production of significantly increased roots. Similarly the interaction between the variety VRI3 and sub plots revealed that VRI3 with BR 1ppm treatment exhibited a significant improvement over the other treatments.

Sodium chloride salinity had a major effect on decreasing the root growth, which in turn had an inhibitory effect on shoot growth. The relative root length of oat seedlings allowed identification of more tolerant and non-tolerant accessions at different concentration of NaCl (Asif *et al.*, 2003). There are reports that the growth inhibition in pepper under saline environment was due to disturbed balance of hormones (Houimli *et al.*, 2008).

Figure 23a

Root length (cm) of the groundnut variety TMV7 at different stages of growth on treatment with selected plant growth regulators

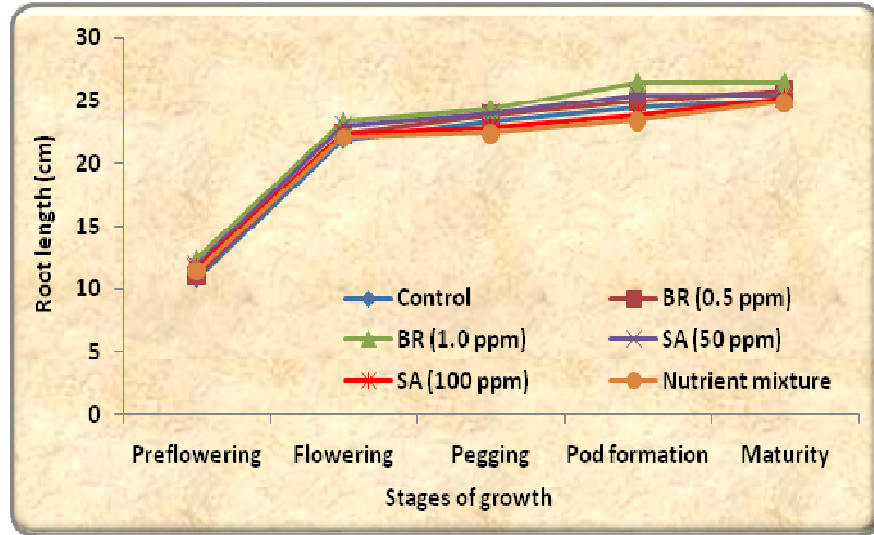
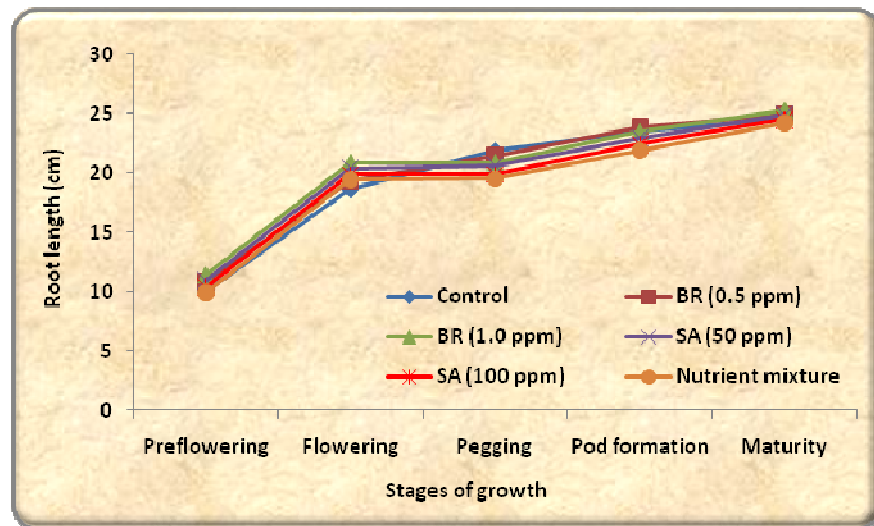


Figure 23b

Root length (cm) of the groundnut variety VRI3 at different stages of growth on treatment with selected plant growth regulators



Growth regulators are known to mitigate the adverse effect of salinity in *Cucumis sativus* (Yu *et al.*, 2004). In the present study, BR 1ppm showed its maximum effect in TMV7 and VRI3 in improving the root growth compared to the

control. Reduction in plant growth by means of salt stress has also been reported for a number of plant species in scientific studies (Essa, 2002; Rameeh *et al.*, 2004; Cicek and Cakirlar, 2002; Li *et al.*, 2006, Kusvuran *et al.*, 2007 and Tunçtürk *et al.*, 2008). The results confirmed these earlier observations of growth reduction due to NaCl treatment. Probably the negative effect of salinity on plants would have provoked osmotic potential by salt in the culture medium, such that the root cells could not obtain the required water from the medium (Mer *et al.*, 2000).

4.5.4. Growth Parameters

4.5.4.1. Specific leaf weight

The data on specific leaf weight (SLW), indicating the thickness of leaf, revealed an increasing trend upto pegging stage and declined gradually in the subsequent stages (Table 16). Two main plot treatments differed significantly at all growth stages. During pegging, the groundnut variety TMV7 recorded a significantly increased specific leaf weight (12.83 mg cm^{-2}) than VRI3. At the subsequent pod formation and maturity stages also TMV7 maintained a significant increase in SLW of 7.1 and 4.96 mg cm^{-2} respectively over VRI3. All the subplot treatments showed significantly improved effects on SLW in TMV7. The interaction effects of varieties and treatments were also significant at all the growth stages. The performance of the groundnut variety TMV7 was here again found to be better than VRI3. The other varietal interaction treatment, VRI3 with BR 0.5ppm, also recorded a significantly increased value of 8.83 mg cm^{-2} than the control. The treatment with nutrient mixture for the groundnut variety VRI3 showed non significant effect on SLW at flowering and pod formation stages.

The ameliorative chemicals used in the study showed positive effect on SLW and BR 1 ppm exhibited maximum effect in both the varieties. Krishnan and Kumari (2008) supported this finding with the result that the positive effect of plant growth regulators on SLW was attributed to the enhancement of phloem transport and their possible role in membrane permeability. In view of a number of studies it is evident that exogenous application of Brassinolide induces abiotic stress tolerance in plants, e.g., exogenous application of Brassinolide increased salinity tolerance in rice (Anuradha and Rao, 2001) and chickpea (Ali *et al.*, 2007).

Table 16

Specific leaf weight (mg cm⁻²) of the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|-------------|------|-------------|------|---------------|------|-------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 3.43 | 3.13 | 9.36 | 6.66 | 12.03 | 8.53 | 6.70 | 6.03 | 4.36 | 4.10 |
| BR (0.5 ppm) | 3.50 | 3.46 | 9.43 | 6.96 | 12.33 | 8.83 | 6.96 | 6.26 | 4.56 | 4.23 |
| BR (1.0 ppm) | 3.83 | 3.70 | 9.83 | 7.26 | 12.83 | 8.50 | 7.10 | 6.46 | 4.96 | 4.40 |
| SA (50 ppm) | 3.46 | 3.46 | 9.56 | 7.03 | 12.33 | 8.23 | 6.76 | 6.50 | 4.56 | 4.23 |
| SA (100 ppm) | 3.06 | 3.13 | 9.40 | 6.96 | 12.10 | 8.10 | 6.63 | 6.23 | 4.26 | 4.16 |
| Nutrient mixture | 2.93 | 2.90 | 9.16 | 6.66 | 11.93 | 7.90 | 6.46 | 6.03 | 4.10 | 3.90 |
| CD(0.05) | 0.12 | | 0.19 | | 0.15 | | 0.12 | | 0.17 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

4.5.4.2. Leaf area

Rajpar *et al.* (2006) reported that increase in soil salinity decreased the height of wheat. Ghoulam and Foursy (2002) reported that high NaCl concentrations caused a great reduction in growth parameters of sugar beet cultivars such as leaf area, fresh and dry weight of leaves and roots but the leaf number was less affected. In the present study the effect of ameliorative chemicals on leaf area at different growth stages of groundnut varieties has been presented in Table 17. Leaf area was found to increase gradually from preflowering to pod formation stage and decline thereafter towards maturity. The results also revealed that variety TMV7 recorded significantly increased leaf area of 942 mg cm⁻² during pod formation stage than VRI3, which recorded the leaf area of 910 mg cm⁻². Among the sub plot treatments, BR 1ppm was more effective in improving the leaf area over the control at pod formation stage. This treatment was followed by BR 0.5ppm (940 mg cm⁻²) and SA 50ppm (939 mg cm⁻²).

The groundnut variety TMV7 with BR 1ppm treatment maintained its superiority over other interaction treatments, which recorded the significantly increased leaf area of 942 mg cm⁻². Among the other varietal combinations, VRI3 with BR 1ppm treatment recorded a significant increase in leaf area over the control.

Leaf area is considered to be the primary site of the photosynthetic activity. Leaf area development, in general, was very sensitive to even low level of salt stress as reported by Eleiwa *et al.* (2011) and Hussein *et al.* (2007) on fenugreek plants who stated that foliar application of Brassinolide showed a significant increase in growth parameters as compared with untreated control plants. Spraying Brassinolide has been shown to affect an increase in the quantum yield of electron transport in photosystem II reaction and the maximum carboxylation of ribulose 1,5-bisphosphate, processes. Application of homobrassinolide resulted in increasing the relative water content, nitrate reductase activity, chlorophyll content and photosynthesis under stress conditions. It also improved membrane stability. These beneficial effects resulted in higher leaf area, biomass production, and yield related parameters in treated plants (Eleiwa *et al.*, 2011). De Pascale *et al.* (2003) reported a decline in leaf area of pepper (*Capsicum annum*) subjected to salt stress.

Table 17

Leaf area (cm² plant⁻¹) of the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|-------|-------------|-------|-------------|-------|---------------|-------|-------------|-------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 316.9 | 245.5 | 666.6 | 527.6 | 777.1 | 734.0 | 935.3 | 905.1 | 702.3 | 377.0 |
| BR (0.5 ppm) | 317.3 | 247.1 | 668.3 | 529.3 | 779.2 | 737.5 | 940.0 | 908.0 | 704.2 | 379.0 |
| BR (1.0 ppm) | 318.4 | 250.1 | 672.1 | 531.0 | 780.0 | 740.0 | 942.0 | 910.0 | 706.0 | 380.0 |
| SA (50 ppm) | 315.8 | 245.5 | 671.0 | 530.0 | 778.3 | 735.2 | 939.1 | 907.9 | 705.1 | 378.0 |
| SA (100 ppm) | 314.4 | 243.1 | 670.1 | 528.2 | 775.3 | 732.1 | 935.2 | 906.3 | 703.1 | 376.0 |
| Nutrient mixture | 311.6 | 241.0 | 665.6 | 527.5 | 775.0 | 730.7 | 932.0 | 904.2 | 700.6 | 375.1 |
| CD(0.05) | 0.37 | | 0.35 | | 0.66 | | 0.40 | | 0.56 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

The reduction in leaf growth under salt stress was induced by lack of adequate tissue potassium ion concentration. According to the results obtained in the present study, BR 1 ppm expressed its maximum effect by increasing the leaf area of both TMV7 and VRI3 varieties over the control.

4.5.4.3. Leaf area index (LAI)

The time trend of LAI showed similar pattern as that of leaf area, with a steady increase up to pod formation stage and then a decline at maturity (Table 18). Between the main plots, the groundnut variety TMV7 recorded significantly increased LAI (3.17) than VRI3 (3.03) at pod formation stage with BR 1.0 ppm. At maturity, there was a significant reduction in LAI in VRI3 when compared to TMV7. Among sub plot treatments, BR 1ppm performed better with the significant increase in LAI at the pod formation stage than the other treatments, which was followed by SA 50ppm. Effects of BR 0.5 ppm and SA 100 ppm were on par.

The interaction treatment of the groundnut variety TMV7 with BR 1ppm recorded a significantly increased LAI (3.17) over the unsprayed control. This was followed by the treatment of SA 50ppm (3.15) over the control.

In the present study the beneficial effect of BR on LAI, the principal factor influencing the canopy net photosynthesis, could be revealed from the results. As per the report of Zheng *et al.* (2008) salt-tolerant cultivars of winter wheat maintained higher leaf area index (LAI) and leaf area duration (LAD) than salt-sensitive cultivars during the reproductive period at all levels of salt concentration. The salinity effect on leaf area and dry matter appeared 20 to 40 days later and finally caused a decrease of about 15%. The decrease in yield of grains was about 28%, although the average soil salinity, expressed as electrical conductivity, only equaled 2.4 dS/m for the most saline treatment (Khatkar and Khusud, 2000).

Table 18

Leaf area index of the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|--------------|------|--------------|------|---------------|------|--------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 1.05 | 0.83 | 2.20 | 1.75 | 2.59 | 2.44 | 3.11 | 3.01 | 2.33 | 1.25 |
| BR (0.5 ppm) | 1.08 | 0.84 | 2.23 | 1.77 | 2.63 | 2.45 | 3.13 | 3.02 | 2.35 | 1.28 |
| BR (1.0 ppm) | 1.11 | 0.88 | 2.25 | 1.80 | 2.64 | 2.49 | 3.17 | 3.03 | 2.37 | 1.29 |
| SA (50 ppm) | 1.11 | 0.85 | 2.24 | 1.79 | 2.61 | 2.44 | 3.15 | 3.03 | 2.34 | 1.28 |
| SA (100 ppm) | 1.07 | 0.85 | 2.23 | 1.74 | 2.58 | 2.42 | 3.13 | 3.01 | 2.31 | 1.24 |
| Nutrient mixture | 1.05 | 0.82 | 2.21 | 1.73 | 2.57 | 2.40 | 3.11 | 3.00 | 2.31 | 1.22 |
| CD(0.05) | 0.016 | | 0.018 | | 0.016 | | 0.025 | | 0.016 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

4.5.4.4. Leaf area duration (LAD)

The results of LAD revealed that there was a gradual and steady increase from preflowering stage to pod formation stage with a decline at maturity (Table 19). Main plot and sub plot treatments differed significantly at all growth stages

Table 19

Leaf area duration (days) of the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | |
|-------------------------|--------------|------|--------------|------|--------------|------|---------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 32.9 | 25.8 | 48.0 | 42.0 | 57.0 | 54.5 | 54.5 | 42.9 |
| BR (0.5 ppm) | 33.1 | 26.1 | 48.3 | 42.5 | 57.9 | 55.1 | 55.1 | 43.1 |
| BR (1.0 ppm) | 34.3 | 26.8 | 48.9 | 42.8 | 58.1 | 54.4 | 55.6 | 43.4 |
| SA (50 ppm) | 33.8 | 26.2 | 48.3 | 42.5 | 57.9 | 53.6 | 54.9 | 42.9 |
| SA (100 ppm) | 33.5 | 25.9 | 48.0 | 42.1 | 57.5 | 53.0 | 54.4 | 42.3 |
| Nutrient mixture | 33.1 | 25.7 | 47.4 | 41.9 | 57.1 | 53.4 | 54.0 | 42.0 |
| CD(0.05) | 0.016 | | 0.018 | | 0.016 | | 0.025 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium

nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

The longest LAD of 58.1 was recorded by TMV7 between pegging and pod formation stages, whereas VRI3 recorded LAD of 54.5. Nutrient mixture showed a lesser LAD of 57.1, which was on par with the control. The groundnut variety TMV7, recorded a significantly increased LAD of 58.1 compared with that of the control with BR 1ppm.

Leaf area duration is another important factor determining the growth and productivity of crops. Higher LAI and LAD could enable the new cultivars to capture more light energy, prolong reproductive period and therefore increase photosynthetic production accumulation. Those provided basis of higher yield

formation for crops, which was evidently verified in soybean plants (Liu *et al.*, 2005).

The present study revealed a longest LAD of 58 days at the time of pegging and pod formation stages in TMV7, whereas in VRI3, LAD was 55 days. It was also observed that at the final stage of growth, reduction in LAD was more in VRI3 than in TMV7. Zheng *et al.*(2008) supported the findings of the present study with the result that foliar spray of BR increased LAD through increasing leaf number and leaf area in groundnut crop.

4.5.4.5. Total dry matter production

The data on total dry matter production revealed a progressive increase with the age of the crop. The main plot and sub plot treatments differed significantly throughout the growth period (Table 20). Here again the variety TMV7 outperformed with the better dry matter production of 82.83 g plant⁻¹ at maturity stage, whereas VRI3 recorded significantly lesser TDMP (71.5 g plant⁻¹) with 1 ppm of BR when compared to TMV7. The sub plot treatment, BR1 ppm was found to be effective in terms of total dry matter production among all treatments. The nutrient mixture treatment has no significant influence on VRI3 compared to its control.

The quantity of biomass produced as a result of conversion of solar energy into photosynthates, is utilized for growth and maintenance of respiration. Under salt stress conditions, plants require additional energy to cope with the adverse situation. This additional energy would have to be diverted from photosynthates, which would, in turn, be available for maintaining the growth of plants. Therefore, diversion of energy under salt stress condition might lead to reduction in the normal plant processes with lesser biomass accumulation and poor yield. In the present study, sodicity stress resulted in a major decline of dry matter production in both the varieties. Eker *et al.* (2006) also observed a significant reduction in dry weight of hybrid maize under NaCl salinity. As observed in the present study, most of the stress alleviating chemicals was found effective in improving the dry matter accumulation. The maximum effect was observed with BR 1ppm followed by BR 0.5 ppm, SA 50ppm and SA 100ppm.

Table 20

Total dry matter production (g plant⁻¹) of the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|-------------|-------|-------------|-------|---------------|-------|-------------|-------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 6.96 | 6.26 | 21.83 | 18.03 | 33.16 | 29.16 | 52.83 | 49.06 | 82.10 | 71.00 |
| BR (0.5 ppm) | 7.40 | 6.46 | 21.96 | 18.33 | 34.20 | 29.70 | 53.23 | 49.66 | 82.26 | 71.26 |
| BR (1.0 ppm) | 7.83 | 6.73 | 22.10 | 18.60 | 34.56 | 30.16 | 53.70 | 49.16 | 82.83 | 71.50 |
| SA (50 ppm) | 7.30 | 6.53 | 21.66 | 18.40 | 34.06 | 29.66 | 53.13 | 49.16 | 82.13 | 71.16 |
| SA (100 ppm) | 7.06 | 6.23 | 21.76 | 18.26 | 33.90 | 29.73 | 53.13 | 48.73 | 81.96 | 71.06 |
| Nutrient mixture | 6.83 | 5.90 | 21.40 | 18.10 | 33.26 | 29.26 | 52.63 | 48.33 | 82.06 | 69.16 |
| CD(0.05) | 0.15 | | 0.32 | | 0.41 | | 0.40 | | 0.36 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

It was found that the reduction of the biomass in beans under saline condition was indicative of several growth limitations and salinity had adverse effects not only on the biomass, but also on other morphological parameters such as plant height, number of leaves, root length and shoot / root ratio (Kaymakanova and Stoeva, 2008). Houimli *et al.* (2010) reported that 24-epibrassinolide treatment might help alleviate the negative effect of salinity on the growth and fruit yield of pepper plant.

4.5.5. Physiological and Biochemical Components

4.5.5.1. Chlorophyll a content

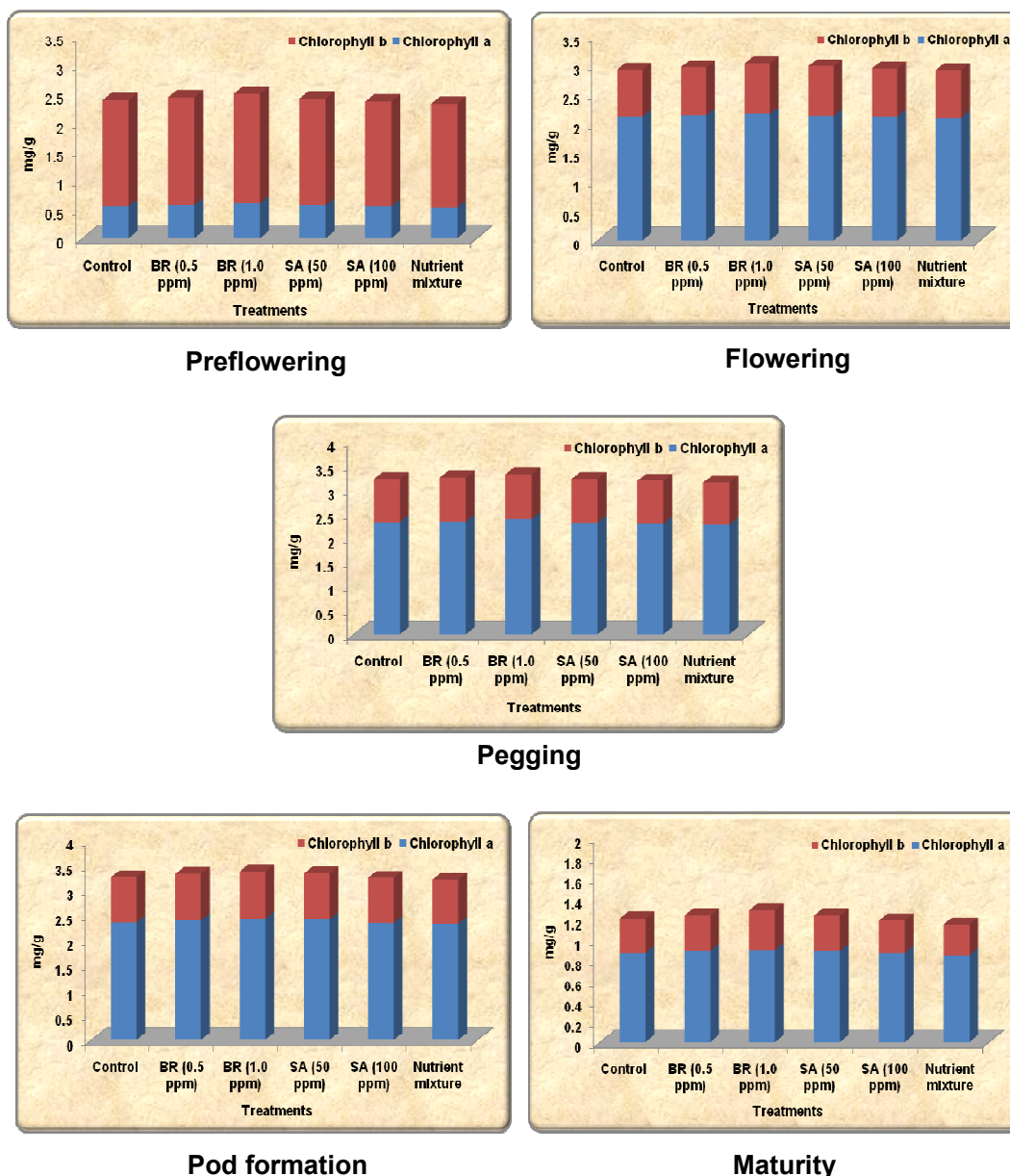
The results of chlorophyll a content exhibited an increasing trend upto pod formation stage with a drastic reduction at maturity (Figure 24a). Both the main plot treatments differed significantly at all the growth stages. During pod formation stage, the groundnut variety TMV7 recorded significantly higher chlorophyll a content (2.42 mg g^{-1}) than the variety VRI3 (2.32 mg g^{-1}) with BR 1ppm. At maturity, there was a less significant reduction in chlorophyll content TMV7 (0.90 mg g^{-1}) than VRI3 (0.72 mg g^{-1}). The sub plot treatments also differed significantly at all stages. During pod formation stage, treatment with BR 1ppm registered significantly higher chlorophyll a content (2.42 mg g^{-1}), followed by SA 50ppm (2.41 mg g^{-1}) than the control (2.35 mg g^{-1}). Treatment with nutrient mixture recorded a significantly reduced chlorophyll a content of 2.32 mg g^{-1} which was not significantly different from SA 100 ppm in TMV7 during pod formation.

4.5.5.2. Chlorophyll b content

Chlorophyll b is one of the eight different chlorophyll molecule groups existing in plant kingdom and it accounts for the largest chlorophyll molecule group existing in mesophyl tissue after chlorophyll a. The determined chlorophyll b content by analyzing its percentage is a good indicator for resistance against salinity (Demiroglu *et al.*, 2001). In the present study, similar to chlorophyll a, chlorophyll b also exhibited an increasing trend from preflowering to pod formation stage with a decline at maturity (Figure 24b).

Figure 24a

Chlorophyll a and b in groundnut variety TMV7 at different stages of growth on treatment with selected plant growth regulators



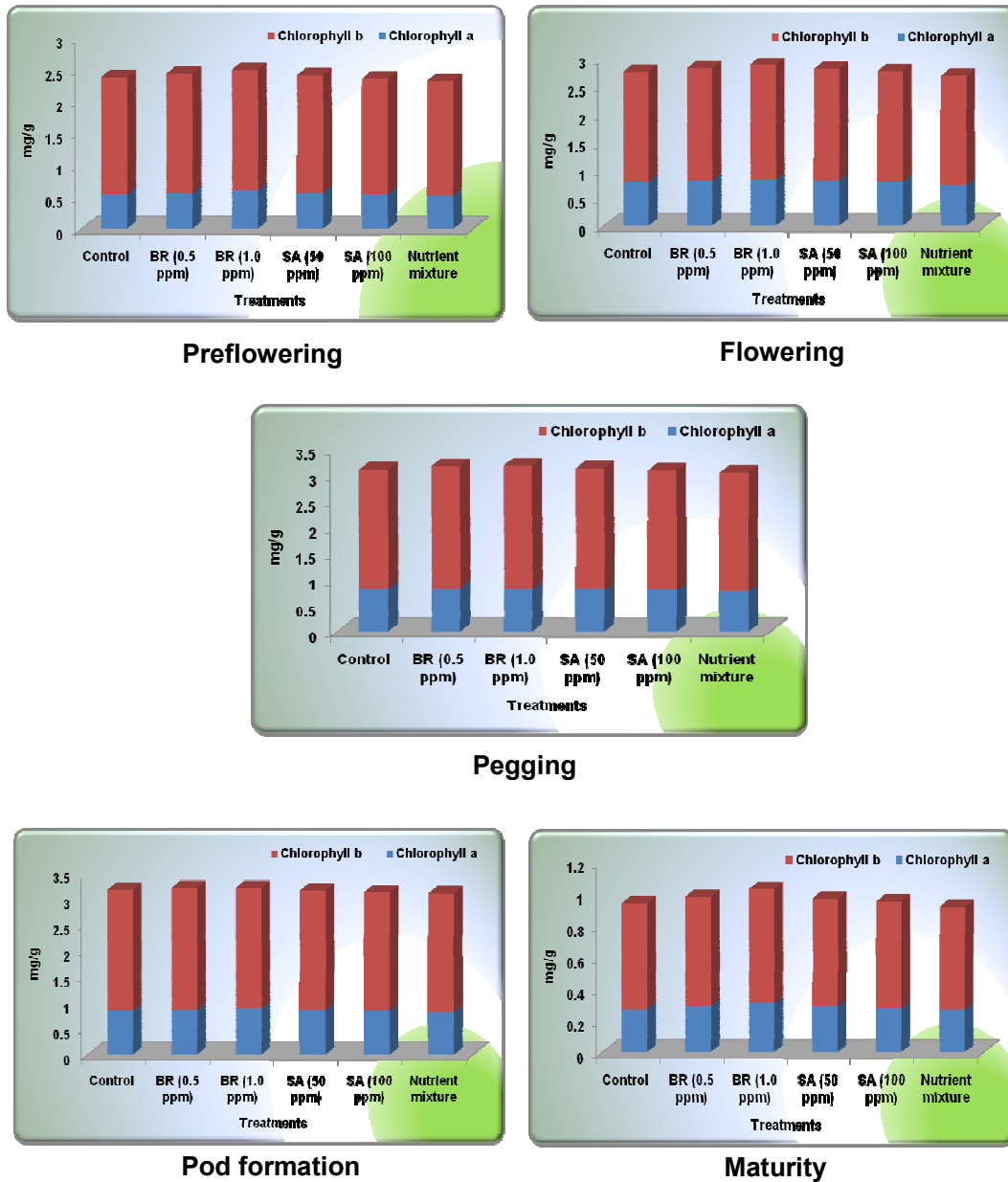
BR - Brassinolides SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%) + Borax(0.2%) + Naphthalene Acetic Acid (20ppm) + SA(50ppm)+ BR(1ppm)

The height of each vertical subdivided bar denotes the total chlorophyll content of that group

Figure 24b

Chlorophyll a and b in groundnut variety VRI3 at different stages of growth on treatment with selected plant growth regulators



BR - Brassinolides SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%) + Borax(0.2%) + Naphthalene Acetic Acid (20ppm) + SA(50ppm)+ BR(1ppm)

The height of each vertical subdivided bar denotes the total chlorophyll content of that group

Main plot and sub plot treatments differed significantly in chlorophyll b content. The difference between the groundnut varieties TMV7 and VRI3 during pod formation stage was wide, higher value being recorded by TMV7 (0.95 mg g^{-1}) than VRI3 (0.89 mg g^{-1}) with BR 1 ppm. Among sub plots, BR 1ppm was effective in increasing chlorophyll b content (0.95 mg g^{-1}) than the other treatments and control. It was reported by Sayed and Sayed (2011), that NaCl stress reduced plant growth of bean plants (*Vicia faba* L.) through reduction of both the chlorophyll content.

4.5.5.3. Total chlorophyll content

The data on total chlorophyll content reflected the variations in chlorophyll a and b contents in different stages of growth in (Table 21). Main plots differed significantly at all the growth stages. During pod formation stage, TMV7 outperformed with higher total chlorophyll content of 3.35 mg g^{-1} than VRI3 (3.22 mg g^{-1}). Significant differences in total chlorophyll content among sub plot treatments revealed the various effects of foliar spray treatments on the two main plots. Both BR 1ppm and SA 50 ppm recorded significantly higher total chlorophyll content in TMV7 at pod formation stage. The treatment with nutrient mixture was on par with the control of TMV7 at pod formation stage (3.29 mg g^{-1}).

Increased accumulation of sodium and chloride ions in the tissues inhibits biochemical processes, particularly, related to photosynthesis through direct ion toxicity. Therefore, a considerable reduction in the level of chlorophyll, soluble carbohydrates and protein content was noted due to salinity (Taffouo *et al.*, 2010). Similar results were reported for total leaf chlorophyll concentration of cucurbit species (Taffouo *et al.*, 2008), bambara groundnut landraces (Taffouo *et al.*, 2010) and lentil plants (Turan *et al.*, 2007). The decrease in chlorophyll content under salt stress is a commonly reported phenomenon and in various studies, because of its adverse effects on membrane stability (Ashraf and Bhatti, 2000). They also explained that inhibition of photosynthesis due to salinity stress involves the disruption of chloroplast membrane structure with the reduction of chloroplast lipid levels and alteration of the ratio of individual polar lipids.

Table 21

Total chlorophyll content (mg g⁻¹) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|--------------|------|--------------|------|---------------|------|--------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 2.59 | 2.40 | 2.97 | 2.76 | 3.22 | 3.13 | 3.29 | 3.16 | 1.21 | 0.94 |
| BR (0.5 ppm) | 2.61 | 2.42 | 2.99 | 2.74 | 3.23 | 3.16 | 3.32 | 3.21 | 1.20 | 0.96 |
| BR (1.0 ppm) | 2.64 | 2.45 | 3.02 | 2.70 | 3.25 | 3.19 | 3.35 | 3.22 | 1.22 | 0.99 |
| SA (50 ppm) | 2.62 | 2.42 | 3.01 | 2.69 | 3.26 | 3.14 | 3.33 | 3.19 | 1.19 | 0.95 |
| SA (100 ppm) | 2.58 | 2.39 | 2.96 | 2.69 | 3.24 | 3.11 | 3.30 | 3.17 | 1.19 | 0.92 |
| Nutrient mixture | 2.56 | 2.39 | 2.95 | 2.64 | 3.22 | 3.09 | 3.29 | 3.15 | 1.17 | 0.91 |
| CD(0.05) | 0.015 | | 0.019 | | 0.012 | | 0.018 | | 0.018 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

Most of the detrimental effects of salinity stress could be alleviated by the usage of plant growth regulators in various crops like soybean (Garg and Chawla, 2002), peanut (Prakash *et al.*, 2003) and mustard (Hayat *et al.*, 2001). A decline in total chlorophylls under saline conditions has been noticed in several salt sensitive species such as greengram, asparagus, chickpea, sorghum and salvadora (Azooz *et al.*, 2004; Dagar *et al.*, 2004). Parvaiz and Riffat (2005) also observed a decrease in chlorophyll content in *Pisum sativum* with increase in salt concentration from 50 to 200mM. A significant decrease in the content of pigment fractions and consequently of the total chlorophyll content as compared with control plants with increasing salinity in *Calotropis procera* have been reported by Al-Sobhi *et al.* (2006).

Plant growth regulating chemical brassinolide is known to impart tolerance to salinity stress through modification of metabolic processes and BR 1mg⁻¹ sprayed twice on 25 and 35 DAS enhanced the total chlorophyll content in groundnut. In the present study also a remarkable increase in chlorophyll a, b and total chlorophyll content was observed due to BR 1 ppm treatment in both the varieties. This increase in chlorophyll content might be due to increased chlorophyll synthesis, since BR promotes cell division and cell expansion in expanding leaves, the differences in cell size and number, could account for the differences in chlorophyll content between BR and untreated control plants. The stimulation of the accumulation of chlorophyll by the brassinosteroids under salt stress was observed by other researchers (Anuradha and Rao 2003 and Ali *et al.* 2007). These results might suggest that 24-epibrassinolide protects photosynthetic apparatus from salt induced oxidative stress.

4.5.5.4. Soil plant analysis development (SPAD)

The chlorophyll meter or SPAD meter is a simple, portable diagnostic tool that measures the greenness or relative chlorophyll content of leaves. Meter readings are given in terms of Minolta Company-defined SPAD (Soil Plant Analysis Development) values that indicate relative chlorophyll contents. There is a strong linear relationship between SPAD values and leaf nitrogen concentration, but this relationship varies with crop growth stage and/or variety mostly because of leaf thickness or specific leaf weight.

Table 22

SPAD values of the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|-------------|------|-------------|------|---------------|------|-------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 30.2 | 26.3 | 33.3 | 30.0 | 35.6 | 31.9 | 29.5 | 27.8 | 26.1 | 24.6 |
| BR (0.5 ppm) | 32.6 | 28.2 | 34.4 | 32.2 | 36.4 | 32.5 | 29.0 | 29.5 | 25.4 | 27.5 |
| BR (1.0 ppm) | 33.3 | 30.3 | 35.8 | 31.3 | 37.5 | 33.4 | 35.1 | 27.9 | 33.2 | 26.9 |
| SA (50 ppm) | 30.1 | 29.4 | 32.4 | 29.8 | 35.3 | 32.3 | 33.2 | 27.5 | 32.1 | 26.4 |
| SA (100 ppm) | 29.5 | 27.5 | 31.5 | 29.2 | 33.1 | 31.2 | 32.0 | 25.4 | 30.9 | 24.5 |
| Nutrient mixture | 27.6 | 25.4 | 30.3 | 27.4 | 32.0 | 28.9 | 30.3 | 23.5 | 29.5 | 22.0 |
| CD(0.05) | 0.23 | | 0.26 | | 0.25 | | 0.20 | | 0.18 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

The linear relationship between nitrogen and SPAD values has led to the adaptation of the SPAD meter to assess crop nitrogen status and to determine the need of the plant for additional nitrogen fertilizer. SPAD readings indicate that plant nitrogen status and the amount of nitrogen to be applied are determined by the physiological nitrogen requirement of crops at different growth stages. SPAD value is the indirect estimation of chlorophyll content (Gholizadeh *et al.*, 2009). In the present study SPAD value was found to be increased up to pegging stage and was found to decline towards maturity (Table 22). TMV7 variety recorded significantly increased value of 35.8 at pegging stage. Among the sub plot treatments, BR 1ppm was found to be effective followed by BR 0.5ppm (34.4).

4.5.5.5. Chlorophyll fluorescence (Fv/Fm)

Photosynthesis is one of the main metabolic processes determining crop production and it is directly affected by drought (Guang-cheng *et al.*, 2011). As water stress progresses, the plants must dissipate excess light energy, since photosynthesis is unable to efficiently utilize photochemical energy (Ennahli and Earl, 2005). In this way, the photosynthetic apparatus may be damaged eventually, imposing an additional non-stomatal limitation to the process. Chlorophyll fluorescence parameters can evaluate the influence of environmental stress on growth and yield, since these traits were found to be closely correlated with the rate of carbon exchange (Fracheboud *et al.*, 2004; Guo and Li, 2000) and they could serve as reliable indicators to evaluate the metabolic imbalance of photosynthesis and yield performance under water stress (Guang-cheng *et al.*, 2011).

Fv/Fm ratio estimated during various growth stages revealed that the ratio was low at initial stage and as the growth advanced towards pegging stage, there was an increase (Table 23). The ratio started declining at the time of pod formation. The groundnut variety TMV7 recorded an appreciable increase in photochemical effects with 0.83 over VRI3 at pegging stage. The sub plot treatment BR 0.5 ppm showed elevated chlorophyll fluorescence to the highest level of 0.83 at pegging stage. The other treatments also recorded significantly higher values at the same stage. These two treatments (BR 0.5 and BR 1ppm) performed well throughout the crop growth period.

Table 23

Chlorophyll fluorescence values of the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|--------------|------|--------------|------|---------------|------|--------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 0.67 | 0.64 | 0.76 | 0.74 | 0.83 | 0.78 | 0.73 | 0.71 | 0.66 | 0.63 |
| BR (0.5 ppm) | 0.72 | 0.65 | 0.77 | 0.76 | 0.83 | 0.79 | 0.73 | 0.73 | 0.66 | 0.62 |
| BR (1.0 ppm) | 0.77 | 0.68 | 0.78 | 0.75 | 0.83 | 0.79 | 0.75 | 0.74 | 0.68 | 0.61 |
| SA (50 ppm) | 0.69 | 0.66 | 0.76 | 0.73 | 0.82 | 0.78 | 0.75 | 0.72 | 0.67 | 0.59 |
| SA (100 ppm) | 0.65 | 0.65 | 0.74 | 0.72 | 0.80 | 0.76 | 0.74 | 0.71 | 0.66 | 0.58 |
| Nutrient mixture | 0.62 | 0.62 | 0.73 | 0.70 | 0.78 | 0.75 | 0.73 | 0.70 | 0.65 | 0.57 |
| CD(0.05) | 0.001 | | 0.001 | | 0.003 | | 0.001 | | 0.001 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

Table 24

Stomatal diffusive resistance ($s\ cm^{-1}$) of the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|--------------|------|--------------|------|---------------|------|--------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 0.28 | 0.27 | 0.39 | 0.37 | 0.57 | 0.54 | 1.00 | 0.98 | 0.44 | 0.42 |
| BR (0.5 ppm) | 0.30 | 0.29 | 0.42 | 0.39 | 0.57 | 0.55 | 1.02 | 1.01 | 0.44 | 0.43 |
| BR (1.0 ppm) | 0.31 | 0.32 | 0.45 | 0.42 | 0.60 | 0.59 | 1.05 | 1.02 | 0.49 | 0.44 |
| SA (50 ppm) | 0.27 | 0.29 | 0.42 | 0.40 | 0.60 | 0.54 | 1.01 | 0.99 | 0.45 | 0.42 |
| SA (100 ppm) | 0.25 | 0.26 | 0.40 | 0.37 | 0.56 | 0.51 | 1.00 | 0.96 | 0.42 | 0.39 |
| Nutrient mixture | 0.23 | 0.24 | 0.38 | 0.35 | 0.54 | 0.48 | 0.98 | 0.94 | 0.41 | 0.37 |
| CD(0.05) | 0.013 | | 0.010 | | 0.018 | | 0.021 | | 0.027 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

Table 25

Transpiration rate of the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|--------------|------|--------------|------|---------------|------|--------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 52.7 | 55.8 | 49.1 | 51.1 | 34.2 | 38.9 | 32.7 | 34.5 | 25.3 | 26.7 |
| BR (0.5 ppm) | 51.6 | 55.7 | 49.3 | 51.0 | 34.1 | 38.7 | 32.6 | 34.2 | 25.2 | 26.6 |
| BR (1.0 ppm) | 51.0 | 55.6 | 49.2 | 50.8 | 33.9 | 38.3 | 32.5 | 34.1 | 25.1 | 26.5 |
| SA (50 ppm) | 53.0 | 55.9 | 50.1 | 51.2 | 34.5 | 38.9 | 32.8 | 34.6 | 25.3 | 26.7 |
| SA (100 ppm) | 53.4 | 56.2 | 50.4 | 52.4 | 34.7 | 39.1 | 33.1 | 34.9 | 25.6 | 26.9 |
| Nutrient mixture | 53.7 | 56.4 | 50.7 | 52.5 | 35.0 | 39.2 | 33.4 | 34.9 | 25.7 | 27.0 |
| CD(0.05) | 0.017 | | 0.021 | | 0.045 | | 0.013 | | 0.033 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

4.5.5.6. Stomatal diffusive resistance

Stomatal diffusive resistance showed an increasing trend upto pod formation stage and declined during maturity. There existed significant differences in main plot and sub plot treatments at all the stages (Table 24). In main plot treatment, the groundnut variety TMV7 had a significantly increased stomatal diffusive resistance of 1.05 s cm^{-1} than VRI3 (1.02 s cm^{-1}) with BR 1 ppm. The susceptible variety VRI3 with BR 1ppm treatment also showed its efficiency in offering higher resistance than the other treatments.

4.5.5.7. Transpiration rate

The time trend of transpiration rate revealed a gradual decrease throughout the growth period of the crop. Between the two main plot treatments (two varieties of groundnut), TMV7 recorded significantly low rate of transpiration over VRI3 at all the growth stage. All the sub plot treatments with plant growth regulators at different concentrations, significantly differed from the control by maintaining low rate of transpiration (Table 25).

4.5.5.8. Soluble protein

Progressive increasing trend of soluble protein content was observed upto pod formation stage with a remarkable decline towards maturity (Table 26). The two main plot treatments differed significantly, with the higher value of 14.01 mg g^{-1} being recorded by the groundnut variety TMV7 at pod formation stage. Among the sub plot treatments, Brassinolide (BR) 0.5ppm treatment was significantly increased than the unsprayed control.

The performance of the groundnut variety TMV7 with BR 1ppm (14.01 mg g^{-1}) was significantly better than the other treatments to increase the soluble protein content over the control at the time of pod formation. Similarly, the effect of BR 1ppm on the variety VRI3 was found to have significantly increased soluble protein content over the control.

Salinity, by and large, seemed to depress the protein synthesis and accelerate their degradation and disturbed the soluble amino acid protein ratio in plants. Breakdown of polysomes accompanied by an increased level of nitrate

Table 26

Soluble protein content (mg g⁻¹) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|--------------|------|--------------|-------|---------------|-------|--------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 7.45 | 7.13 | 9.11 | 8.61 | 11.39 | 10.29 | 13.98 | 12.59 | 7.24 | 6.74 |
| BR (0.5 ppm) | 7.51 | 7.11 | 9.13 | 8.64 | 11.40 | 10.26 | 13.99 | 12.62 | 7.27 | 6.70 |
| BR (1.0 ppm) | 7.53 | 7.15 | 9.15 | 8.65 | 11.42 | 10.24 | 14.01 | 12.64 | 7.29 | 6.69 |
| SA (50 ppm) | 7.48 | 7.12 | 9.11 | 8.63 | 11.40 | 10.23 | 13.96 | 12.60 | 7.26 | 6.69 |
| SA (100 ppm) | 7.47 | 7.09 | 9.09 | 8.61 | 11.39 | 10.20 | 13.93 | 12.62 | 7.25 | 6.65 |
| Nutrient mixture | 7.43 | 7.05 | 9.07 | 8.59 | 11.38 | 10.19 | 13.91 | 12.58 | 7.22 | 6.64 |
| CD(0.05) | 0.017 | | 0.016 | | 0.016 | | 0.019 | | 0.016 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

reductase was demonstrated during salt stress. Decline in total soluble protein content was showed in *Lycopersicon esculentum*, *Oryza sativa*, *Vicia faba*, *Amaranthus tricolor* and *Brugiera parviflora* plants under NaCl stress (Al-aghabary *et al.*, 2004; Parida and Das, 2005; Parvaiz and Satyavati, 2008; Wang and Nil, 2000).

Moderate salinity in some cases might increase total protein but innumerable reports revealed a decrease in protein and nucleic acid content in root, leaves and embryo axis of mungbean, chickpea, pea and cowpea under NaCl and Na₂SO₄ salinity (Doganlar *et al.*, 2010). The present study results also revealed a considerable decline in soluble protein content of the two groundnut varieties TMV7 and VRI3 grown without receiving any ameliorative treatments. However, the varieties which were imposed with ameliorative chemicals, responded differentially by showing enhanced level of soluble protein at various concentrations. BR 1 ppm, BR 0.5 ppm and SA 50 ppm were effective in increasing the soluble protein content over control in TMV7. In pearl millet, a low concentration of BR (0.1 ppm) caused a remarkable enhancement in soluble protein content of the leaf at 60 DAS (Sivakumar *et al.*, 2002). Increase in total soluble protein content was reported at high NaCl concentration and decrease at low concentration in *Pancratium maritimum* plants (Khedr *et al.*, 2003). In contrast to this, increases in protein contents were reported in *Arabidopsis thaliana* and *Fragaria ananassa* cv. *Camarosa* (El-Baz *et al.*, 2003).

4.5.5.9. Proline

Increased levels of proline, under salt stress, have been reported in two wheat cultivars (Khatkar and Khuhad, 2000). It was suggested that proline accumulation may be caused by increasing proteolysis or by decreasing protein synthesis. The highest concentration of proline under salt stress is found to be favourable to plants as proline participates in the osmotic potential of leaf and thus in the osmotic adjustment. Besides the role of osmolyte, proline can also confer enzyme protection and increase membrane stability under various conditions. Proline accumulation may also help in non-enzyme free radical detoxifications (Khan *et al.*, 2002). As lipid peroxidation is the symptom

Table 27

Proline content ($\mu\text{g g}^{-1}$) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|------|-------------|------|-------------|------|---------------|------|-------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 351 | 263 | 403 | 325 | 589 | 422 | 410 | 269 | 276 | 229 |
| BR (0.5 ppm) | 354 | 263 | 407 | 329 | 592 | 425 | 413 | 273 | 279 | 231 |
| BR (1.0 ppm) | 357 | 265 | 409 | 331 | 594 | 428 | 419 | 274 | 283 | 235 |
| SA (50 ppm) | 349 | 264 | 407 | 329 | 593 | 425 | 415 | 271 | 280 | 234 |
| SA (100 ppm) | 349 | 261 | 403 | 327 | 589 | 423 | 413 | 270 | 278 | 232 |
| Nutrient mixture | 347 | 261 | 402 | 324 | 589 | 420 | 410 | 269 | 275 | 230 |
| CD(0.05) | 2.70 | | 1.64 | | 1.07 | | 1.86 | | 1.52 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

mostly ascribed to oxidative damage, it is often used as an indicator of increased damage (Khan and Panda, 2008; Hernandez *et al.*, 2000; Meloni *et al.*, 2003; Bor *et al.*, 2003; Sayed and Sayed, 2011).

In the present study, proline content showed an increasing trend as the growth stage advanced up to pegging stage and declined towards maturity. The groundnut variety TMV7 had significantly increased proline content of $594 \mu\text{g g}^{-1}$ followed by VRI3 ($428 \mu\text{g g}^{-1}$) at pegging stage (Table 27). Among the treatments, the variety TMV7 with BR 1ppm treatment recorded significantly increased proline content ($419 \mu\text{g g}^{-1}$) at pod formation stage. As evidenced from the data, the variety VRI3 with BR 1ppm treatment showed significant improvement in proline content over the control. Summart *et al.* (2010) advocated that proline might function as a source of solute for intercellular osmotic adjustment under saline conditions. He also suggested that proline might serve as a storage compound for reduced carbon and nitrogen during stress. Free proline accumulation was generally more in the most tolerant cultivars, suggesting its correlation with salinity tolerance of the plants. In the present study also, TMV7 the tolerant variety accumulated higher amount of proline, particularly at pegging stage than VRI3. However, when the cultivar was subjected to ameliorative treatments, proline accumulation was further accelerated. Comparing the two varieties, TMV7 was highly responsive to ameliorative treatments than VRI3 in terms of proline accumulation. This finding could reveal the beneficial effect of plant growth regulators in improving the tolerance of the crops through osmotic adjustment by means of proline accumulation as noticed in VRI3, the most susceptible variety to salinity. Houimli *et al.* (2010) reported that proline accumulation is a common metabolic response of higher plants to salinity stress and the foliar spraying of 24-epibrassinolide reduced the proline accumulation induced by salt stress in pepper plants.

4.5.6. Antioxidant Enzymes

4.5.6.1. Indole acetic acid (IAA) oxidase activity

IAA oxidase activity was found to increase remarkably from preflowering to pegging stage and decrease towards maturity. This trend could be revealed from the data with increased unoxidised auxin content up to pegging stage followed by decreased content towards maturity (Table 28).

Table 28

Indole acetic acid oxidase activity (μg of unoxidised auxin $\text{g}^{-1} \text{h}^{-1}$) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|-------|-------------|-------|-------------|--------|---------------|-------|-------------|-------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 53.97 | 48.58 | 81.64 | 75.79 | 117.30 | 112.98 | 45.79 | 38.69 | 25.34 | 21.09 |
| BR (0.5 ppm) | 54.16 | 48.78 | 81.72 | 75.16 | 117.81 | 112.61 | 45.96 | 38.67 | 25.44 | 21.08 |
| BR (1.0 ppm) | 55.16 | 50.16 | 82.21 | 74.97 | 118.19 | 112.43 | 47.24 | 39.10 | 25.51 | 21.08 |
| SA (50 ppm) | 54.69 | 48.67 | 81.74 | 73.08 | 118.00 | 110.99 | 47.07 | 38.99 | 25.24 | 20.97 |
| SA (100 ppm) | 53.76 | 48.23 | 81.07 | 72.83 | 117.41 | 110.95 | 46.70 | 38.73 | 24.66 | 20.94 |
| Nutrient mixture | 53.23 | 47.83 | 80.98 | 72.41 | 117.21 | 110.74 | 46.23 | 38.21 | 24.74 | 20.73 |
| CD(0.05) | 0.24 | | 0.20 | | 0.05 | | 0.11 | | 0.31 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)
 Values presented are mean of three replicates in each variety

Comparing the main plot treatments, VRI3 recorded significantly lesser activity than TMV7 at all growth stages. Sub plot treatments also revealed significant variations. At pegging stage, the enzyme activity was very much controlled by BR 1ppm treatment with significantly increased auxin content of 118.19 μg of unoxidised auxin $\text{g}^{-1}\text{h}^{-1}$. Similarly SA 50ppm and BR 0.5 ppm treatments registered significantly reduced rate of enzyme activity with high auxin contents of 118.0 and 117.8 μg of unoxidised auxin $\text{g}^{-1}\text{h}^{-1}$ respectively. All the interaction treatments were also found to differ significantly.

The inhibitory effect of IAA oxidase on auxin and in turn, growth was demonstrated by various workers (Sivakumar *et al.*, 2002; Vijayalakshmi and Srinivasan, 2001; Garg and Chawla, 2002). The enhancement of the activity of this enzyme under stress condition might be one of the reasons for stress induced stunted plant growth. In the present investigation, a considerable reduction in auxin content was observed under sodicity condition as the result of elevation of the enzyme activity. Similarly with increasing level of salinity up to 12dSm^{-1} caused an enhancement in IAA oxidase activity in groundnut as observed by Senthil *et al.* (2005). Plant growth regulating chemicals play distinct role in suppressing the activity of IAA oxidase. In the present investigation, BR 1 ppm and salicylic acid 50 ppm seemed to reduce the IAA oxidase activity over the control in VRI3. As brassinolide acts synergistically with auxin, BR application caused a remarkable reduction in the activity of IAA oxidase enzyme as observed in the present study.

4.5.6.2. Polyphenol oxidase activity

Polyphenol oxidase activity was increased up to pegging stage and decreased towards maturity (Table 29). Main plot treatments differed significantly at all the stages of growth. Significantly increased enzymatic activity was maintained by TMV7 than VRI3 throughout the growth period. All the sub plot treatments also exhibited their significant differences. At pegging stage, higher polyphenol oxidase activity was registered by BR 1ppm treatment ($0.638\Delta \text{OD } 403\text{nm min}^{-1} \text{g}^{-1}$) followed by BR 0.5ppm ($0.627\Delta \text{OD } 403\text{nm min}^{-1} \text{g}^{-1}$) and SA 50ppm ($0.628\Delta \text{OD } 403\text{nm min}^{-1} \text{g}^{-1}$) respectively.

Table 29

Polyphenol oxidase activity (Δ OD 403nm min⁻¹ g⁻¹) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|---------------|-------|---------------|-------|---------------|-------|---------------|-------|---------------|-------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 0.481 | 0.458 | 0.577 | 0.554 | 0.620 | 0.558 | 0.482 | 0.459 | 0.407 | 0.391 |
| BR (0.5 ppm) | 0.489 | 0.464 | 0.585 | 0.560 | 0.627 | 0.562 | 0.483 | 0.458 | 0.411 | 0.394 |
| BR (1.0 ppm) | 0.494 | 0.470 | 0.589 | 0.563 | 0.638 | 0.562 | 0.492 | 0.460 | 0.415 | 0.401 |
| SA (50 ppm) | 0.489 | 0.466 | 0.581 | 0.561 | 0.628 | 0.559 | 0.489 | 0.457 | 0.412 | 0.398 |
| SA (100 ppm) | 0.487 | 0.459 | 0.577 | 0.554 | 0.628 | 0.554 | 0.487 | 0.456 | 0.408 | 0.396 |
| Nutrient mixture | 0.485 | 0.456 | 0.574 | 0.550 | 0.621 | 0.550 | 0.481 | 0.450 | 0.404 | 0.390 |
| CD(0.05) | 0.0027 | | 0.0019 | | 0.0044 | | 0.0023 | | 0.0018 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

Morris *et al.* (2005) reported that PPO activity in red clover leaves converts certain phenol compounds to highly reactive quinones in the presence of oxygen. Quinones readily bind to proteins to form complexes, which are more resistant to breakdown by plant and microbial enzymes. Sivasankaramoorthy *et al.* (2011) reported that increased PPO activity under salinity stress may indicate the oxidation and degradation of the toxic substances such as phenolic compounds, which are generally accumulated during salt stress. In the present study, a significantly low rate of PPO activity was observed under sodicity condition. The enzyme activity was however accelerated when the two cultivars were treated with BR 1ppm. VRI3, the susceptible variety was greatly influenced by BR treatment with increase in enzyme activity over control.

4.5.6.3. Nitrate reductase activity

The data on nitrate reductase activity revealed an increasing trend only upto pegging stage. Later there was reduction in enzymatic activity towards maturity (Table 30). Here again TMV7 performed better with significantly increased values than VRI3 at all the stages of growth. All the sub plot treatments also showed significant variations at all the growth stages. At pegging BR 1ppm treatment recorded significantly increased value of $114.27 \mu\text{g NO}_2 \text{ g}^{-1} \text{ h}^{-1}$. The nitrate reductase activity in both the treatments of BR 0.5ppm and SA 50ppm were on par. Significantly lowest value of $113.46 \mu\text{g NO}_2 \text{ g}^{-1} \text{ h}^{-1}$ was recorded by nutrient mixture.

In plants, nitrogen assimilation is regulated by the activation of the enzyme nitrate reductase. This enzyme plays a constructive role in nitrogen utilization by the plants through nitrogen metabolism. Nitrate reductase is the most affected enzyme by salinity. Singla and Garg (2005) also recorded a suppression of nitrate reductase activity and the effect was significantly higher by sodium chloride. The loading of nitrate into the root is also thought to be a highly sensitive step. Salinity may strongly affect the overall nitrate assimilation process because nitrate is required to induce nitrate reductase, the key enzyme of nitrate assimilation process (Tischner, 2000). Nitrate reductase activity in leaves is largely dependent on nitrate flow from roots and is severely affected by NaCl salt stress (Abd - El Baki *et al.*, 2000; Silveira *et al.*, 2001).

Table 30

Nitrate reductase activity ($\mu\text{g NO}_2 \text{ g}^{-1} \text{ h}^{-1}$) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|-------|--------------|-------|--------------|--------|---------------|-------|--------------|-------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 81.07 | 67.43 | 99.61 | 95.46 | 114.19 | 110.54 | 33.29 | 27.19 | 18.60 | 16.06 |
| BR (0.5 ppm) | 81.10 | 67.45 | 99.62 | 95.54 | 114.23 | 110.53 | 33.35 | 27.20 | 18.63 | 16.11 |
| BR (1.0 ppm) | 81.13 | 67.48 | 99.66 | 95.59 | 114.27 | 110.57 | 33.39 | 27.23 | 18.65 | 16.15 |
| SA (50 ppm) | 81.10 | 67.43 | 99.62 | 95.40 | 114.23 | 110.45 | 33.30 | 27.23 | 18.59 | 16.11 |
| SA (100 ppm) | 81.09 | 67.42 | 99.60 | 95.38 | 114.24 | 110.40 | 33.29 | 27.20 | 18.55 | 16.09 |
| Nutrient mixture | 80.99 | 67.38 | 99.58 | 95.36 | 113.46 | 110.39 | 33.27 | 27.20 | 18.50 | 16.03 |
| CD(0.05) | 0.027 | | 0.019 | | 0.244 | | 0.048 | | 0.036 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)
 Values presented are mean of three replicates in each variety

As observed in the present study, sodicity seemed to reduce nitrate reductase activity in leaves of the two groundnut cultivars, with more adverse effect on VRI3. Petronia *et al.* (2005) correlated the lower enzyme activity with a decrease in nitrate concentration in salt stressed condition. The ameliorative chemicals, particularly plant growth regulators play their beneficial role in improving the overall biochemical processes, which are generally inhibited by stress conditions. The significant effect of Brassinolide (BR) in stimulating the enzyme activity could be explained through the results obtained from the present study. In both the varieties, BR 1 ppm could improve the enzyme activity over stressed plants. A stimulated nitrate reductase activity was also observed in salicylic acid treated pearl millet by Sivakumar *et al.* (2002). Similar finding was also made in the present study, in which soluble protein 50 ppm was more effective than another level of 100 ppm. The positive effect of salicylic acid on NRase activity was due to its role in activation of nitrate reductase protein and prevention of enzyme degradation by proteolysis. This might also be involved in the enhancement of enzyme synthesis or its maintenance in active form and thus might have a protective role on nitrate reductase activity.

4.5.6.4. Peroxidase activity

Reactive oxygen species (ROS) are regarded as the main source of damage to cells under biotic and abiotic stresses (Candan and Tarhan, 2003; Bor *et al.*, 2003; Gara *et al.*, 2003; Mittler, 2002; Vaidyanathan *et al.*, 2003). ROS's are partially reduced forms of atmospheric oxygen, which are produced in vital processes such as photorespiration, photosynthesis and respiration (Mittler, 2002; Uchida *et al.*, 2002). To produce water in these processes, four electrons are required for perfect reduction of oxygen. But ROS typically results from the transfer of one, two and three electrons, respectively, to O₂ to form superoxide (O₂^{•-}), peroxide hydrogen (H₂O₂) and hydroxyl radical (HO[•]) (Mittler, 2002). Fortunately, plants have developed various protective mechanisms to eliminate or reduce ROS, which are effective at different levels of stress-induced deterioration (Beak and Skinner, 2003). H₂O₂ is eliminated by various antioxidant enzymes such as catalases and peroxidases which convert H₂O₂ to water (Gara *et al.*, 2003).

Table 31

Peroxidase activity (Δ OD 430 nm min⁻¹ g⁻¹) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|---------------|-------|---------------|-------|---------------|-------|---------------|-------|---------------|-------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 0.551 | 0.493 | 0.680 | 0.599 | 0.777 | 0.715 | 0.487 | 0.459 | 0.392 | 0.371 |
| BR (0.5 ppm) | 0.559 | 0.501 | 0.687 | 0.609 | 0.781 | 0.721 | 0.490 | 0.464 | 0.399 | 0.375 |
| BR (1.0 ppm) | 0.562 | 0.509 | 0.701 | 0.612 | 0.784 | 0.730 | 0.499 | 0.467 | 0.403 | 0.381 |
| SA (50 ppm) | 0.560 | 0.501 | 0.698 | 0.609 | 0.781 | 0.729 | 0.490 | 0.461 | 0.399 | 0.374 |
| SA (100 ppm) | 0.553 | 0.495 | 0.693 | 0.600 | 0.779 | 0.724 | 0.487 | 0.458 | 0.399 | 0.367 |
| Nutrient mixture | 0.551 | 0.488 | 0.690 | 0.597 | 0.776 | 0.722 | 0.483 | 0.459 | 0.394 | 0.360 |
| CD(0.05) | 0.0024 | | 0.0023 | | 0.0023 | | 0.0016 | | 0.0025 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

In the present study the time trend of peroxidase activity also revealed a gradual increase up to pegging with a decline towards maturity. The main plot treatments and sub plot treatments varied significantly at all growth stages (Table 31). TMV7 recorded significantly increased activity (0.784Δ OD 430 nm $\text{min}^{-1} \text{g}^{-1}$) at pegging stage than VRI3 (0.723Δ OD 430 nm $\text{min}^{-1} \text{g}^{-1}$). The influence of BR 1ppm was much better than the other treatments. BR 0.5ppm and SA 50ppm were on par at the same stage. In the interaction effect, the varietal and ameliorative treatment combination showed significant variations with respect to the enzyme activity.

4.5.6.5. Acid phosphatase activity

Acid phosphatase activity of the root increased steadily up to pegging stage with a decline towards maturity (Table 32). TMV7 and VRI3 differed significantly at all the growth stages, with significantly higher rates maintained by TMV7 throughout the growth periods. All the sub plot treatments influenced the enzyme activity significantly. At pegging stage BR 1ppm showed its greater influence ($19.43 \mu\text{g}$ of pi released $\text{min}^{-1} \text{g}^{-1}$) followed by SA 50ppm ($19.30 \mu\text{g}$ of pi released $\text{min}^{-1} \text{g}^{-1}$). BR 0.5ppm and SA100ppm were on par at this stage.

4.5.6.6. Catalase activity

A high level of H_2O_2 directly inhibits CO_2 fixation (Yamazaki *et al.*, 2003). Catalase activity exhibited similar pattern of peroxidase activity during the entire growth period (Table 33). Both the main plots varied significantly. At the time of pegging, at which the enzymatic activity was at maximum, TMV7 showed its superiority ($84.27 \mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{g}^{-1}$) compared to VRI3 ($72.26 \mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{g}^{-1}$). All the sub plot treatments also varied significantly. BR 1ppm, as an effective treatment, registered significantly higher activity than the other treatments and control at pegging stage.

The interaction effect between varieties and treatments also varied significantly. The highest enzyme activity of $84.27 \mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{g}^{-1}$ was registered by TMV7 with BR 1ppm at pegging with significantly higher rate over the other treatments. VRI3 with BR 1ppm treatment ($72.26 \mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{g}^{-1}$) also registered comparatively higher activity over the other treatments.

Table 32

Acid phosphatase activity ($\mu\text{g of Pi released min}^{-1} \text{g}^{-1}$) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|-------|--------------|-------|--------------|-------|---------------|-------|--------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 13.17 | 12.79 | 14.83 | 14.40 | 19.13 | 17.30 | 13.70 | 12.99 | 8.32 | 7.30 |
| BR (0.5 ppm) | 13.25 | 13.19 | 15.22 | 14.43 | 19.24 | 17.39 | 13.79 | 13.02 | 8.44 | 7.56 |
| BR (1.0 ppm) | 13.73 | 13.43 | 15.54 | 14.53 | 19.43 | 17.44 | 13.78 | 13.10 | 8.55 | 7.65 |
| SA (50 ppm) | 13.23 | 13.20 | 15.20 | 14.21 | 19.30 | 17.34 | 13.65 | 12.96 | 8.42 | 7.41 |
| SA (100 ppm) | 13.01 | 13.01 | 15.11 | 14.11 | 19.24 | 17.21 | 13.54 | 12.98 | 8.32 | 7.32 |
| Nutrient mixture | 12.73 | 12.65 | 15.09 | 14.01 | 19.11 | 17.20 | 13.42 | 12.86 | 8.22 | 7.20 |
| CD(0.05) | 0.022 | | 0.026 | | 0.017 | | 0.043 | | 0.018 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)
 Values presented are mean of three replicates in each variety

Table 33

Catalase activity ($\mu\text{g H}_2\text{O}_2 \text{ min}^{-1} \text{ g}^{-1}$) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Preflowering | | Flowering | | Pegging | | Pod formation | | Maturity | |
|-------------------------|--------------|-------|--------------|-------|--------------|-------|---------------|-------|--------------|-------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 61.15 | 47.74 | 71.90 | 62.16 | 83.81 | 72.02 | 62.41 | 54.01 | 49.37 | 37.97 |
| BR (0.5 ppm) | 61.24 | 47.78 | 71.99 | 62.28 | 84.11 | 72.11 | 62.55 | 53.78 | 49.78 | 38.00 |
| BR (1.0 ppm) | 61.58 | 47.98 | 72.44 | 62.33 | 84.27 | 72.26 | 62.67 | 54.21 | 49.64 | 38.11 |
| SA (50 ppm) | 61.44 | 47.83 | 71.74 | 62.24 | 84.14 | 72.13 | 62.42 | 54.20 | 49.42 | 37.88 |
| SA (100 ppm) | 61.34 | 47.78 | 71.63 | 62.10 | 84.10 | 72.12 | 62.30 | 54.17 | 49.35 | 37.68 |
| Nutrient mixture | 61.24 | 47.68 | 71.52 | 61.99 | 83.98 | 71.99 | 62.20 | 54.11 | 49.22 | 37.57 |
| CD(0.05) | 0.043 | | 0.036 | | 0.030 | | 0.345 | | 0.192 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

Active oxygen species (AOS), such as the superoxide radical (O_2^-), H_2O_2 and the hydroxyl radical (OH.) are generated as byproducts of normal metabolism in different subcellular compartments including the chloroplasts, mitochondria, peroxisomes and plasma membrane - linked electron transport system (Esfandiari *et al.*, 2007). These AOS can damage DNA, proteins, chlorophyll and membrane functions. Furthermore, the imposition of biotic or abiotic stress may give rise to an excessive concentration of AOS, resulting in oxidative damage at a cellular level. To mitigate and repair damage initiated by AOS, plants have developed a complex antioxidant system (Del Rio *et al.*, 2002; Sampayo *et al.*, 2003). Salt stress induces accumulation of reactive oxygen species (ROS) that are detrimental to cells as they cause oxidative damage to membrane lipids, proteins and nucleic acids (Hernandez *et al.*, 2001). Plants employ some detoxifying enzymes such as catalase, peroxidase, superoxide dismutase, etc to scavenge the free radicals from the metabolic sites and thereby show tolerance to osmotic stresses (Roxas *et al.*, 2000).

The two groundnut varieties selected in the present study showed differential responses to the stress ameliorative chemicals. TMV7 though maintained higher activity than VRI3, the response to growth regulating chemicals was more in VRI3. In both the varieties BR and salicylic acid were found to be effective in enhancing the enzymatic activity remarkably.

4.5.7. Nutrient Content of the two varieties of Groundnut Plants grown in the Field chosen for the Study

The uptake and distribution of different minerals and salts are known to have bearing on the overall growth and development processes of plants. Under stress conditions, inhibition of uptake, transport and assimilation of different minerals and disturbances in their balances have been commonly observed. The phenomenon is more pronounced under soil stresses like salinity and alkalinity. Under such conditions, soils contain extreme ratios of Na^+/Ca^{2+} , Na^+/K^+ , Ca^{2+}/Mg^{2+} , Cl^-/NO_3^- or HCO_3^-/NO_3^- . Therefore plants growing in salt affected soils are subjected primarily to four types of stresses acting on biophysical and metabolic components of plant growth: osmotic stress, specific ion toxicities, ionic imbalance and developmental disturbance (Grattan and Grieve, 1999). These conditions restrict the nutrient

availability and uptake, leading to the development of nutritional disorders and reduction of crop growth and quality. The beneficial effects of phytohormones on overcoming the adverse effects of salt stress and improving the nutrient uptake by the plants were thoroughly discussed by various workers in different crops. Ions at high concentrations in the external solution (e.g. Na⁺ or Cl⁻) are taken up at high rates, which may lead to excessive accumulation in tissues. These ions may inhibit the uptake of other ions into the root and their transportation to the shoot. There is a potential for many nutrient interactions in salt stressed plants which may have important consequences for growth (Cramer *et al.*, 1985). Some researcher (Li *et al.*, 2006; Kusvuran *et al.*, 2007; Hu *et al.*, 2006; Amador *et al.*, 2007) reported that salinity had a major effect on the uptake and internal concentrations of mineral elements and plant growth in many plants.

4.5.7.1. Leaf nitrogen content

The nitrogen content of leaf was estimated at the time of flowering, pod formation and maturity stages to study the pattern of uptake and utilization by the two varieties as influenced by the ameliorative chemicals namely Brassinolide – 0.5 ppm and 1.0 ppm, Salicylic acid – 50 ppm and 100 ppm and nutrient mixture (Diammonium phosphate - 1% +Potassium Nitrate - 0.5% + Borax-0.2% + Naphthalene Acetic Acid - 20ppm + Salicylic Acid - 50ppm + Brassinolide -1ppm (Table 34). The data revealed an increasing trend from flowering to pod formation stages with a sharp decline at maturity.

The two main plot treatments differed significantly at all growth stages, while sub plot treatments showed their significant effect at flowering and pod formation stages and the effect was non significant at maturity. Comparing the two main plot treatments, TMV7 recorded significantly increased nitrogen content of 28.4 mg g⁻¹ at pod formation stage while VRI3 registered 27.6 mg g⁻¹. Among the sub plot treatments, BR 1ppm recorded the maximum nitrogen content of 28.4 mg g⁻¹ at pod formation stage, which was followed by VRI3 (27.6 mg g⁻¹). Nitrogen content in both differed significantly at 5 percent level and were significantly higher than the control. Among the chemicals, brassinolide 1 ppm was found to be highly effective in increasing the nitrogen content of the leaf in TMV7 and VRI3, over the control.

Table 34

Leaf nitrogen content (mg g^{-1}) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Flowering | | Pod formation | | Maturity | |
|-------------------------|-------------|------|---------------|------|-------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 16.5 | 15.0 | 27.8 | 26.9 | 14.2 | 13.9 |
| BR (0.5 ppm) | 16.9 | 15.4 | 27.9 | 27.1 | 14.6 | 14.4 |
| BR (1.0 ppm) | 17.3 | 16.0 | 28.4 | 27.6 | 14.8 | 14.9 |
| SA (50 ppm) | 16.7 | 15.1 | 27.9 | 27.0 | 14.3 | 14.5 |
| SA (100 ppm) | 16.3 | 15.0 | 27.4 | 26.9 | 14.0 | 14.1 |
| Nutrient mixture | 16.1 | 14.5 | 27.1 | 26.1 | 13.6 | 13.7 |
| CD(0.05) | 0.23 | | 0.14 | | 0.17 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%) + Borax(0.2%) + Naphthalene Acetic Acid(20ppm) + SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

4.5.7.2. Leaf phosphorus content

Sayed and Sayed (2011) reported that salinity stress increased the accumulation of sodium, phosphorus, iron, manganese and chloride in leaf, stem, root and legumes. In the present study, the data on phosphorus content of leaf exhibited an increasing trend from flowering to pod formation stage with a decline at maturity (Table 35). The two main plot treatments differed significantly at all the three stages of growth. TMV7 performed better with the leaf phosphorus content of 4.4 mg g^{-1} than VRI3, which showed 3.9 mg g^{-1} at the time of pod formation. At maturity stage TMV7 showed higher decline in phosphorus content than VRI3. All the sub plot treatments differed significantly at all the stages. Among the treatments, BR 1ppm was found effective with phosphorus content of 4.4 mg g^{-1} followed by BR 0.5ppm (3.9 mg g^{-1}) at pod formation stage.

Table 35

Leaf phosphorus content (mg g⁻¹) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Flowering | | Pod formation | | Maturity | |
|-------------------------|-------------|------|---------------|------|-------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 3.03 | 2.53 | 3.66 | 3.26 | 1.26 | 1.43 |
| BR (0.5 ppm) | 3.43 | 3.36 | 3.93 | 3.66 | 1.56 | 1.83 |
| BR (1.0 ppm) | 3.93 | 3.76 | 4.40 | 3.96 | 2.00 | 2.16 |
| SA (50 ppm) | 3.70 | 3.26 | 3.90 | 3.66 | 1.53 | 1.96 |
| SA (100 ppm) | 3.53 | 2.96 | 3.73 | 3.36 | 1.33 | 1.83 |
| Nutrient mixture | 3.23 | 2.76 | 3.26 | 2.93 | 1.16 | 1.46 |
| CD(0.05) | 0.12 | | 0.13 | | 0.15 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%) + Borax(0.2%) + Naphthalene Acetic Acid(20ppm) + SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

4.5.7.3. Leaf sodium content

Generally, salt-tolerant plants differed from salt sensitive ones mainly in having a low rate of Na⁺. It was suggested that the capacity of ion accumulation of plants is related to their tolerance to salt stress. It was found that tolerant species accumulated lower Na⁺, and the decrease of K⁺ was lower than in the sensitive species (Essa, 2002; Yasar *et al.*, 2006; Kusvuran *et al.* 2007).

The time trend of sodium content revealed a differential pattern with a very slight and gradual increase from flowering to maturity stages (Table 36). Main plot treatments and sub plot treatments varied significantly at all the stages of growth. The groundnut variety TMV7 recorded significantly increased sodium content throughout the crop growth. Among the sub plot treatments, BR 1ppm treatment

recorded significantly increased sodium content at flowering, pod formation and maturity stages respectively. Treatment with nutrient mixture showed significantly reduced content of 1.0, 1.8 and 2.6 mg g⁻¹ at these three stages respectively.

Table 36

Leaf sodium content (mg g⁻¹) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Flowering | | Pod formation | | Maturity | |
|------------------|-----------|------|---------------|------|----------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 0.8 | 1.0 | 1.5 | 2.0 | 2.8 | 3.1 |
| BR (0.5 ppm) | 1.1 | 1.5 | 2.0 | 2.4 | 3.2 | 3.5 |
| BR (1.0 ppm) | 1.6 | 1.9 | 2.5 | 3.0 | 3.8 | 3.9 |
| SA (50 ppm) | 1.3 | 1.4 | 2.2 | 2.5 | 3.4 | 3.3 |
| SA (100 ppm) | 1.1 | 0.9 | 2.0 | 2.3 | 3.0 | 3.0 |
| Nutrient mixture | 1.0 | 0.7 | 1.8 | 2.0 | 2.6 | 2.7 |
| CD(0.05) | 0.18 | | 0.14 | | 0.17 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%) + Borax(0.2%) + Naphthalene Acetic Acid(20ppm) + SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

Both the varieties of groundnut TMV7 and VRI3 with BR 1ppm treatment recorded a significant increase in the sodium content of 3.8 and 3.9 mg g⁻¹ at maturity stage respectively. The other treatments showed significant reduction in sodium content in the leaf at maturity stage compared to BR 1ppm.

An excess of sodium ions in the soil solution can be inhibitory to various physiological processes of plants. Comparison of two groundnut varieties for their response to sodicity in the present study revealed that VRI3, being a sensitive variety accumulated significantly higher sodium in the leaves than TMV7. However this undesirable nature of sensitivity to salinity could be overcome by the treatments with growth regulating chemicals. Brassinolide 1 ppm showed its higher effect in VRI3 in

suppressing the accumulation of sodium. Salicylic acid also showed its beneficial effect on decreasing sodium content of the leaves. The effect was however more pronounced in sensitive variety than in tolerant one.

The work by Bandeh-Hagh *et al.* (2008) with canola, Yasar *et al.* (2006) with green bean, Wang and Han (2007) with alfalfa and Li *et al.* (2006) with soybean, reported similar results and indicated that the distribution of Na⁺ ions vary among the organs of plants and genotypes that tolerate salt well. It can be postulated that K⁺/Na⁺ ratios might be valid selection criteria for assessing salinity tolerance of different crop species. Previous studies have shown that high K⁺/Na⁺ ratio shows a positive relationship with salt tolerance (Essa, 2002; Kusvuran *et al.*, 2007). These findings of the present study are in agreement with the other reports suggesting that salt stress reduces the K⁺/Na⁺ ratio of green bean (Yasar *et al.*, 2006), melon (Kusvuran *et al.*, 2007), wheat (Hu *et al.*, 2006) and legume (Amador *et al.*, 2007). These results indicated that salt tolerance mechanisms may display differences according to cultivars.

4.5.7.4. Leaf potassium content

It was noticed that accumulation of ion in root, shoot and leaves changed under salt stressed plants (Yasar *et al.*, 2006). The K⁺ content in plant tissues represents the main cation in plant cells and it is an important component of the cell osmotic potential (Tuncturk *et al.*, 2011). Potassium content of the leaf increased from flowering to pod formation stage with a decline at maturity (Table 37). The two main plots exhibited significant variations at all the three growth stages. At all the stages, TMV recorded significantly higher values than VRI3. The sub plot treatments also differed significantly. At pod formation stage, when potassium content was maximum, BR 1ppm registered significantly increased potassium content of 8.9 mg g⁻¹.

At pod formation stage, TMV7 with BR 1ppm treatment registered significantly increased value of 8.9 mg g⁻¹ of leaf potassium followed by TMV7 with BR 0.5ppm treatment (8.6 mg g⁻¹). Among the other varietal interactions, VRI3 with BR 1ppm treatment registered significantly higher content of 7.9 mg g⁻¹ of potassium than the other treatments.

Table 37

Leaf potassium content (mg g^{-1}) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Flowering | | Pod formation | | Maturity | |
|------------------|-----------|------|---------------|------|----------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 6.10 | 5.83 | 8.03 | 7.36 | 5.56 | 4.96 |
| BR (0.5 ppm) | 6.43 | 6.06 | 8.63 | 7.73 | 6.33 | 5.40 |
| BR (1.0 ppm) | 6.90 | 6.33 | 8.93 | 7.96 | 6.66 | 6.26 |
| SA (50 ppm) | 6.66 | 5.96 | 8.53 | 7.40 | 6.06 | 5.90 |
| SA (100 ppm) | 6.36 | 5.76 | 8.26 | 6.90 | 5.90 | 5.46 |
| Nutrient mixture | 6.03 | 5.46 | 8.06 | 6.76 | 5.53 | 4.93 |
| CD(0.05) | 0.16 | | 0.11 | | 0.12 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%) + Borax(0.2%) + Naphthalene Acetic Acid(20ppm) + SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

Many highly calcareous soils have high total phosphorus content and most of this phosphate has precipitated as calcium phosphate in the soil in forms relatively unavailable for plant uptake (Mc Laughlin, 2002). Achilea, (2002) reported that salination (50 mmol/l NaCl) of the nutrient solution of greenhouse tomatoes markedly decreased K content of the leaves, fruit-set rate, number of flowers, dry weights of the plants, fruit sizes and stem heights . Tzortzakis (2010) also reported that salinity reduced significantly the elemental (K, Ca, and Na) uptake after the 5th week and became minimal following the 6th week in endive plants. Similar observations were recorded in the present study. The two groundnut varieties subjected to sodicity condition showed a considerably lesser accumulation of phosphorus in leaves than the varieties administrated with ameliorative chemicals. When the two groundnut varieties were treated with plant growth regulating chemical and nutrients, the uptake process as well as accumulation was enhanced

through improved root growth. As the result of 30 to 40 per cent improvement in root growth, the phosphate content of leaf was increased by about 20 to 30 per cent in the two groundnut varieties treated with foliar spray of brassinolide 1 ppm.

In the present study, TMV7, regarded as tolerant variety, had considerably higher potassium content than VRI3. The increased potassium uptake might have eventually reduced the toxic ions entering into the plants from the soil solution. Besides this inherent nature, growth-promoting chemicals play their effective role in improving the nutrient uptake efficiency of the crops. The effect, in general, is more pronounced in susceptible varieties than in tolerant ones. This was clearly evident from the results of the present investigation. VRI3, considered as susceptible variety, responded more to the chemical manipulations. Application of BR 1ppm to this susceptible variety improved the potassium uptake by about 22 per cent over control at the time of pod formation stages. This chemical also showed similar effect on the other variety TMV7.

The salt tolerant genotype had a greater K^+ accumulation capacity. Similar results were reported with different green bean cultivars (Yasar *et al.*, 2006), soybean cultivars (Essa, 2002) and canola cultivars (Bandeh-Hagh *et al.*, 2008).

4.5.7.5. Leaf calcium content

Calcium has been shown to ameliorate the adverse effects of salinity on plants (Amador *et al.*, 2007). It has been suggested that high calcium levels can protect the cell membrane from the adverse effects of salinity. The effect of salinity on the nutrient composition of plant tissues, especially the concentration of calcium (Ca^{2+}) and potassium (K^+), has been extensively investigated, and several researchers have confirmed that the detrimental effects of salinity on plant growth may occur through an ionic imbalance, particularly of Ca^{2+} and K^+ (Essa, 2002; Kusvuran *et al.*, 2007; Yasar *et al.*, 2006). Some species and cultivars can maintain higher growth under saline conditions by accumulating fewer toxic ions and maintaining a high tissue Ca^{2+} concentration (Essa, 2002). The calcium content of leaf increased from flowering to pod formation stage and declined at maturity (Table 38). The main plots of the two varieties of groundnuts varied significantly at all the three stages of growth. The variety TMV7 registered significantly higher

calcium content of 16.0 mg g⁻¹ at pod formation stage. The sub plot treatments also exhibited significant differences at all the three stages of growth. At pod formation stage, the treatment BR 1ppm (15.5) and SA 50 ppm (14.0) registered the highest values. All the other treatments also recorded significantly higher values than control.

Table 38

Leaf calcium content (mg g⁻¹) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Flowering | | Pod formation | | Maturity | |
|-------------------------|-------------|-------|---------------|-------|-------------|-------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 13.66 | 13.06 | 15.03 | 14.20 | 13.30 | 13.10 |
| BR (0.5 ppm) | 13.96 | 13.60 | 15.46 | 14.66 | 13.66 | 13.70 |
| BR (1.0 ppm) | 14.40 | 13.93 | 16.03 | 15.06 | 13.96 | 13.86 |
| SA (50 ppm) | 14.10 | 13.53 | 15.36 | 14.76 | 13.86 | 13.40 |
| SA (100 ppm) | 13.73 | 13.06 | 14.96 | 14.26 | 13.23 | 13.06 |
| Nutrient mixture | 13.26 | 12.90 | 14.33 | 13.90 | 12.93 | 12.90 |
| CD(0.05) | 0.14 | | 0.17 | | 0.16 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%) + Borax(0.2%) + Naphthalene Acetic Acid(20ppm) + SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

Both the groundnut varieties TMV7 and VRI3 with BR 1ppm treatment recorded significantly higher values of 16.0 and 15.0 mg g⁻¹ of calcium respectively than the control. The reduction in calcium content noticed at maturity stage revealed differential trend among the interaction treatments. Calcium plays a vital nutritional and physiological role in plant metabolism. Because Ca²⁺ ion readily displaced from its extra cellular binding sites by other cations, these functions may become seriously impaired by reduced Ca²⁺ availability. Root growth and function may be restricted by high Na⁺ / Ca²⁺. Osmotic effects are due to salt-induced decrease in the soil water potential. Salinity results in a reduction of K⁺ and Ca²⁺

content and an increased level of Na^+ , Cl^- and SO_4^{2-} , which forms its ionic effects (Mansour *et al.*, 2005).

Salinity also disturbs the level of Ca^{2+} and alters K^+/Na^+ selectivity (Melgar *et al.*, 2006). This finding was in accordance with the results of the present study, in which, the two groundnut varieties subjected to sodicity stress, showed a very low accumulation of calcium throughout the growth period and the effect was more pronounced in VRI3. Similarly David and Inayatullah (2000) recorded higher Na, lower K and Ca concentration and lower K/Na ratio in flag leaf sap of wheat grown at high sodicity. In the present study, brassinolide, an analog of auxins and gibberellins, showed its effectiveness in raising the calcium concentration in leaf by about 18 per cent in both the varieties, particularly at the time of pod formation. A considerable reduction in leaf Ca at maturity also indicates the efficient utilization of Ca for the development of reproductive structures.

4.5.7.6. Leaf magnesium content

Magnesium content of leaf revealed a trend similar to that of calcium content (Table 39). Main plot and sub plot treatments differed significantly at all growth stages. Between the main plots, TMV7 registered the highest magnesium content of 13.8 mg g^{-1} at pod formation stage, which was significantly higher than VRI3 (13.0 mg g^{-1}). Among the sub plot treatments, BR 1ppm registered significantly higher value of 13.8 mg g^{-1} followed by BR 0.5ppm (13.5 mg g^{-1}) at pod formation stage. All the other treatments also recorded significantly higher values than control. At maturity also BR 1ppm showed the highest value of magnesium in both the groundnut varieties.

Magnesium plays pivotal role on pigment synthesis, photosynthesis and as co factor for many metabolic reactions, therefore, the reduction of leaf Mg might lead to the reduction of above characters, thus poor crop stand and productivity. Though calcium strongly compete with Mg^+ under normal soil condition, increase in salinity, however, associated with reduction of both Ca and Mg concentration in a number of crops (Salama, 2001). In the present study, magnesium content of the leaf did not show significant variations among the interaction treatments at

flowering and maturity stages. However, at the time of pod formation, a slight decrease in magnesium content of the leaf was observed in both the varieties under sodicity conditions. At this stage, ameliorative chemicals also inhibited their adverse effectiveness in increasing the magnesium content of the leaves. BR 1 ppm, being the best treatment, resulted in 12 and 13 per cent increase in magnesium content in both the varieties TMV7 and VRI3 respectively over control.

Table 39

Leaf magnesium content (mg g⁻¹) in the two selected groundnut varieties (TMV7 and VRI3) at different stages of growth on treatment with selected plant growth regulators

| Treatments | Flowering | | Pod formation | | Maturity | |
|-------------------------|-------------|-------|---------------|-------|-------------|-------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 12.16 | 11.90 | 13.13 | 12.80 | 11.53 | 12.20 |
| BR (0.5 ppm) | 12.56 | 12.20 | 13.56 | 12.50 | 12.20 | 12.50 |
| BR (1.0 ppm) | 12.93 | 11.80 | 13.83 | 13.00 | 12.90 | 12.96 |
| SA (50 ppm) | 12.40 | 10.96 | 13.40 | 12.83 | 12.43 | 12.46 |
| SA (100 ppm) | 11.96 | 10.73 | 12.93 | 11.96 | 11.96 | 11.96 |
| Nutrient mixture | 11.90 | 10.16 | 12.80 | 11.80 | 11.80 | 11.60 |
| CD(0.05) | 0.18 | | 0.22 | | 0.16 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%) + Borax(0.2%) + Naphthalene Acetic Acid(20ppm) + SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

4.5.8. Yield Components

The data on yield components namely number of flowers per plant, number of pegs per plant, number of pods per plant, fertility coefficient, shelling percentage, 100 seed weight and harvest index were recorded at the time of harvest, besides pod yield. The data are presented in Table 40 and 41.

Table 40

Yield and yield components of the two selected groundnut varieties (TMV7 and VRI3) on treatment with selected plant growth regulators

| Treatments | No. of flowers per plant | | No. of pegs per plant | | No. of pods per plant | | Fertility coefficient (%) | |
|-------------------------|--------------------------|------|-----------------------|------|-----------------------|------|---------------------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 64 | 55 | 33 | 32 | 26 | 22 | 40 | 40 |
| BR (0.5 ppm) | 65 | 56 | 34 | 32 | 26 | 23 | 40 | 40 |
| BR (1.0 ppm) | 65 | 57 | 34 | 32 | 26 | 23 | 40 | 41 |
| SA (50 ppm) | 65 | 56 | 34 | 32 | 26 | 23 | 40 | 40 |
| SA (100 ppm) | 64 | 55 | 33 | 31 | 25 | 22 | 40 | 40 |
| Nutrient mixture | 64 | 54 | 33 | 31 | 25 | 22 | 39 | 40 |
| CD(0.05) | 0.20 | | 0.26 | | 0.18 | | 0.42 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture - Diammonium phosphate(1%)+Potassium nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

4.5.8.1. Number of flowers per plant

Number of flowers per plant differed significantly among main plot and sub plot treatments. Comparing the two main plots, the groundnut variety TMV7 recorded more number of flowers (65) than the variety VRI3 (57). In the sub plot treatment BR 0.5 ppm, BR 1ppm and SA 50 ppm were on par with each other registering a maximum number of 65 flowers per plant followed by other treatments (Table 40).

Among the two groundnut varieties employed in the present study, flowering was more in TMV7. Treatment with Brassinolide could effectively improve the flower production in both the groundnut varieties.

4.5.8.2. Number of pegs per plant

The two main plot treatments differed significantly with respect to number of pegs per plant. TMV7 variety produced more number of pegs (34) than VRI3 (31). The treatments BR 0.5ppm, BR 1ppm and SA 50 ppm were on par with each other in the peg formation (Table 40).

Number of pegs formed per plant also increased by BR application in both the varieties of groundnut. Peg and pod growth in groundnut is characterized by intensive cell proliferation and cell enlargement, which was much influenced by brassinolide, because BR actions are analogous to auxins (Zurek *et al.*, 1994). According to the results, both auxins and BR induce elongation but their kinetics is quite different. Auxin generally shows a very short lag time of 10 – 15 minutes between application and the on set of elongation, with maximum rate of elongation reached within 30-40 minutes. Conversely, BR has a lag time of at least 45 minutes with the elongation rates continuing to reach several hours. This result explains the better effect of BR than auxin elongation process.

4.5.8.3. Number of pods per plant

Number of pods per plant varied significantly between the two main plot treatments. TMV7 recorded 26 pods per plant, whereas VRI3 recorded 23 pods per plant. All the subplot treatments were effective in improving the pod number significantly over control in TMV7. The data has been depicted in Table 40.

As the number of pods per plant is one of the important yield determining factors in groundnut, the reduction in pod number would have a direct reflection on total pod yield. Salinity as well as sodicity, in general, affects the yield components related to grain yield or seed yield of crop plants. As observed in the present study, pods number per plant was drastically reduced in both the varieties under sodicity condition, with a higher effect in the variety VRI3. Sharma *et al.* (2003) noticed a remarkable reduction in the number of kernels per plant as EC of water increased from 2.5 to 7.5 dSm⁻¹. Exogenous application of plant growth regulating chemicals can have a promotive effect through alleviating the adverse effects of stresses. Application of BR in the present study exhibited its greater effect on enhancing the

Pods number in both the varieties. A lesser response to other chemicals and nutrients was observed in both the varieties.

4.5.8.4. Fertility coefficient

The fertility coefficient differed significantly between the two main plot treatments and the groundnut variety VRI3 recorded significantly higher value of 41 per cent, which was significantly higher than the variety TMV7. Among the treatments, BR 1ppm recorded the highest fertility coefficient of 41 per cent (Table 40).

Fertility coefficient, a measure of number of pods produced to the total number of flowers formed, was greatly influenced by plant growth regulating chemicals, as observed in the present study. Brassinolide at 1 ppm concentration had shown greater improvement on fertility coefficient in the variety VRI3 over control. The possible reason for this better fertility status of the crop might be the maintenance of high auxin content at the time of pegging. Since auxin and abscissic acid interaction decides the flower drop, maintenance of high level of auxin due to BR treatment might have resulted in high fertility coefficient of the crop. Sheela and Gehlot (2000) pointed out the specific role of BR that BR acts to influence a diverse array of plant developmental processes just like the action of auxins, gibberellins and cytokinins.

4.5.8.5. Pod yield per plant

Pod yield varied significantly among main plot and sub plot treatments. The groundnut variety TMV7 recorded significantly higher mean pod yield of 1712 kg ha⁻¹, whereas VRI3 recorded a mean yield of 1601 kg ha⁻¹. All the subplot treatments significantly influenced the pod yield of the two varieties. Among them, BR 1ppm registered the highest mean yield of 1712 kg ha⁻¹, followed by BR 0.5 ppm with the mean yield of 1708 kg ha⁻¹. These two elite treatments were followed by SA 50ppm (1707 kg ha⁻¹) and SA 100ppm (1702 kg ha⁻¹). The data for the same has been depicted in Table 41.

Pod yield of groundnut, contributed by number of pods and pod size, greatly affected by sodicity. VRI3 showed greater reduction than TMV7. The study of Sharma *et al.* (2003) revealed a pod yield reduction of 18, 51, 60 and 70 percent at

EC 3.75, 5.0, 6.25 and 7.5 dSm⁻¹ respectively. The inhibiting effect of salinity stress could be reversed by application brassinolide in the form of 24 epibrassinolide or 28 homobrassinolide, as reported by many researchers. They also further stated that BR not only removed the inhibitory effect of salinity, but also promoted the growth and yield of crops. In the present study also, the beneficial role of BR could be revealed through yield improvement of two groundnut varieties under sodicity condition. The possible reason for the effective role of BR on yield improvement was explained by Fujii and Saka (2001). According to them BR is assured to increase the sink capacity by promoting translocation and accumulation of starch within the reproductive parts, resulting in promotion of maturation of the kernels.

Table 41

Yield and yield components of the two selected groundnut varieties (TMV7 and VRI3) on treatment with selected plant growth regulators

| Treatments | Pod Yield (kg ⁻¹) | | Shelling Percentage (%) | | 100 Seed Weight (g) | | Harvest Index (%) | |
|-------------------------|-------------------------------|------|-------------------------|------|---------------------|------|-------------------|------|
| | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 | TMV7 | VRI3 |
| Control | 1700 | 1589 | 64.7 | 41.9 | 51.3 | 41.9 | 35.2 | 30.9 |
| BR (0.5 ppm) | 1708 | 1596 | 65.3 | 42.3 | 51.8 | 41.9 | 35.4 | 31.1 |
| BR (1.0 ppm) | 1712 | 1601 | 67.3 | 42.9 | 51.9 | 42.6 | 36.0 | 31.8 |
| SA (50 ppm) | 1707 | 1600 | 66.6 | 42.4 | 51.4 | 42.1 | 35.8 | 31.1 |
| SA (100 ppm) | 1702 | 1597 | 66.3 | 41.9 | 51.0 | 41.9 | 35.5 | 31.0 |
| Nutrient mixture | 1701 | 1594 | 66.0 | 41.6 | 51.0 | 41.5 | 35.1 | 30.8 |
| CD(0.05) | 3.18 | | 0.62 | | 0.46 | | 0.038 | |

BR - Brassinolides

SA - Salicylic Acid

Nutrient Mixture- Diammonium phosphate(1%)+Potassium

nitrate(0.5%)+Borax(0.2%)+Naphthalene Acetic Acid(20ppm)+SA(50ppm)+BR(1ppm)

Values presented are mean of three replicates in each variety

4.5.8.6. Shelling percentage

The data on shelling percentage indicated a significant variation between the two main plots (Table 41). TMV7 variety recorded significantly increased shelling percentage of 67.3, whereas in VRI3 the shelling percentage is 42.9 only.

TMV7 had 50 per cent higher shelling percentage than VRI3 and also responded more to BR 1 ppm spray. Salicylic acid was also effective in TMV7, increasing the shelling percentage, whereas VRI3 recorded significantly reduced shelling percent over the control. TMV7 in general, produced larger kernels than VRI3. BR 1 ppm increased the kernel weight compared to the control.

4.5.8.7. Hundred seed weight

Two main plot treatments significantly differed with respect to 100 seed weight. TMV7 considered to be the best variety, registered a considerably higher 100 seed weight of 51.9, whereas the other variety VRI3 had a weight of 42.6. All the subplot treatments except nutrient mixture influenced the 100 seed weight in both the main plots. BR 1ppm, being an effective treatment, registered the significantly highest value of 51.9 compared to the control (Table 41). However the effect of BR 1 ppm on seed weight of VRI3 was lesser than TMV7.

4.5.8.8. Harvest index

A significant varietal variation was observed between the two main plot treatments. Harvest index of the groundnut variety TMV7 was recorded to be 36 per cent, whereas for the other variety VRI3, it was 32 per cent. All the subplot treatments significantly improved the harvest index. The treatments BR 0.5ppm and nutrient mixture were on par with each other and with the control (Table 41).

Hossain *et al.* (2008) reported that the harvest index of mungbean decreased with increase in salinity level. Harvest index, the ratio of grain yield to biological yield, provides an estimate of the conversion efficient of dry matter to grain yield. Commonly, harvest index is sensitive to environmental stresses .A given cultivar on a given climate may result in different harvest index values (Ranjbar, 2010). Yield is a very complex character which comprise of many components and these yield components are related to final grain yield which are

also severely affected by salinity (Shereen *et al.*, 2005). The magnitude of salt induced yield losses could not be attributed to single factor. Different physiological, biochemical factors at different stages of plants may be involved. One factor may be the over all control mechanism (before flowering) of sodium uptake through root properties and its subsequent distribution in different vegetative and floral parts especially in leaves where it causes leaf mortality thereby reduces transportation of total assimilates to the growing region (Munns, 2002).

The present findings clearly demonstrate the ability of brassinosteroids to alleviate the inhibitory effects of salinity on germination, seedling growth and plant yield. However further studies are required to unravel the tolerance mechanism conferred by brassinosteroids in the case of salinity stress.

4.6. Analysis of Heat Shock Protein (hsp 70) Gene Expression in the tolerant variety of Groundnut identified in the field study

Drought and salinity are becoming particularly widespread in many regions and may cause serious salinization of more than 50% of all arable lands by the year 2050. Oxidative stress, which frequently accompanies high temperature, salinity, or drought stress, may cause denaturation of functional and structural proteins. As a consequence, these diverse environmental stresses often activate similar cell signaling pathways and cellular responses, such as stress proteins production, upregulation of anti-oxidants and accumulation of compatible solutes. The ongoing elucidation of the molecular control mechanisms of abiotic stress tolerance, which may result in the use of molecular tools for engineering more tolerant plants, is based on the expression of specific stress-related genes. These genes include three major categories: (i) those that are involved in signaling cascades and in transcriptional control, such as Myelocytomatosis (MyC), Mitogen Activated Protein (MAP) kinases and Salt Overly Sensitive (SOS) kinase, phospholipases and transcriptional factors such as Heat shock Factor (HSF), and the C-repeat-binding factor / dehydration-responsive element binding protein (CBF/DREB) and ABA responsive element binding factor / ABA responsive element (ABF/ ABAE) families (ii) those that function directly in the protection of membranes and proteins, such as heatshock proteins (Hsps) and chaperones, late embryogenesis abundant (LEA) proteins, osmoprotectants and free-radical

scavengers; (iii) those that are involved in water and ion uptake and transport such as aquaporins and ion transporters (Wang *et al.*, 2003; Wang *et al.*, 2001).

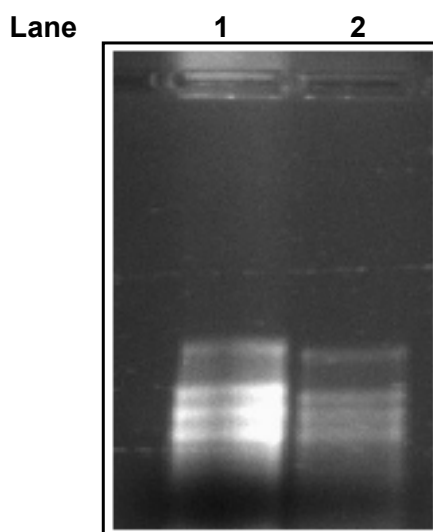
Molecular chaperones are key components contributing to cellular homeostasis in cells under both optimal and adverse growth conditions. They are responsible for protein folding, assembly, translocation and degradation in a broad array of normal cellular processes; they also function in the stabilization of proteins and membranes and can assist in protein refolding under stress conditions. A wide range of proteins has been reported to have chaperone activity. Moreover, many molecular chaperones are stress proteins and many of them were originally identified as heat-shock proteins (Hsps). Thus, abiotic stresses, such as drought, salinity, extreme temperatures, chemical toxicity and oxidative stress are serious threats to agriculture and result in the deterioration of the environment. Abiotic stress is the primary cause of crop loss worldwide, reducing average yields for most major crop plants by more than 50% (Wang *et al.*, 2003). It is most likely, being supported by experimental data in plants and other organisms, that Hsps/chaperones play a crucial role in protecting plants against stress and in the reestablishment of cellular homeostasis (Wang *et al.*, 2004).

In real-time PCR (RT-PCR), a fluorogenic dye is used to continuously monitor product accumulation. With this method, the initial concentration of template DNA is assessed by using the number of PCR cycles at which PCR amplification enters the exponential phase. Other methods such as Northern hybridization, RNase protection assay and in situ hybridization have been used for the quantification of gene expression. However, RT-PCR has many advantages over these methods, including time, sensitivity, specificity, ease of use and reproducibility (Radonic *et al.*, 2004; Fronhoffs *et al.*, 2002; Klein, 2002; Liu and Saint, 2002). The quantification is performed by comparing the expression of internal standards, mainly housekeeping genes, expression of which occurs in all nucleated cell types being necessary for cell survival (Giulietti *et al.*, 2001; Bustin, 2000). Although such synthesis often shows very little fluctuation, their use as internal standards should be carefully examined in relation to cell types and cell metabolism. The internal standard selected for the present study was the

housekeeping gene tubulin (*tub*). In higher plants, as in higher animals, it is now accepted that tubulins are generally encoded by multigene families. Even in *Arabidopsis*, with its 'minimal' genome, families with at least six different α and nine β tubulin members have been found (Kopczak *et al.*, 1992; Snustad *et al.*, 1992).

In order to study the expression pattern of hsp 70 protein if any in the groundnut variety TMV7, which was identified as the tolerant one in the pot and field study, the RNA was isolated and RT-PCR was run with tubulin as endogenous control. The isolated RNA (Plate 10) was treated with DNase for cDNA synthesis which was done using Revert Aid H Minus First Strand cDNA Synthesis Kit .

Plate 10
RNA bands from the salt tolerant groundnut variety TMV7
obtained in 1 % denaturing agarose gel
electrophoresis



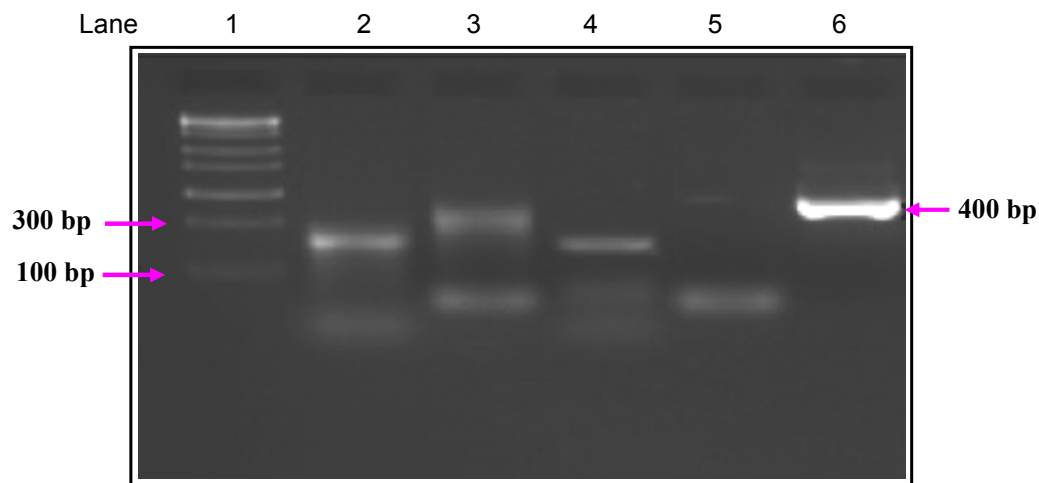
Lane 1 - Control Lane 2 – 100mM NaCl treated TMV7 groundnut variety

Hsp70 and tubulin genes were amplified from prepared cDNA using gene specific primers for optimization of cycling conditions and primer to template ratio which has been depicted in Figure 25 and 26. From the result arrived, it was obvious that there is expression of hsp 70 gene in the groundnut variety TMV7 which might have played the key role in tolerating the sodium chloride stress during its growth period.

Immediately after exposure to high stresses changes occur at the molecular level altering the expression of genes and accumulation of transcripts, thereby leading to the synthesis of stress related proteins as a stress tolerance strategy (Iba, 2002). The synthesis of hsp's allows the plants to cope with and adapt to severe abiotic stress. This molecular mechanism is implicated in the processes of adaptation by triggering the synthesis of a specific range of protein involved in the tolerance to abiotic stresses and increasing the synthesis of normal structural and functionally proteins (Lee and Vierling, 2000; Essemine *et al.*, 2010).

Plate 11

Isolated bands in 2 % agarose gel showing amplification of genes from cDNA



Lane 1 : DNA ladder (10000 bp- 100 bp)

Lane 2 : Amplicon of 250 bp amplified with tubulin gene specific primer from Sample -1 (Groundnut varietyTMV7, treated with 100mM NaCl)

Lane 3 : Amplicon of 350 bp amplified with hsp-70 gene specific primer from Sample -1 (Groundnut varietyTMV7, treated with 100mM NaCl)

Lane 4 : Amplicon of 250 bp amplified with tubulin gene specific primer from Sample -2 (Groundnut varietyTMV7, untreated)

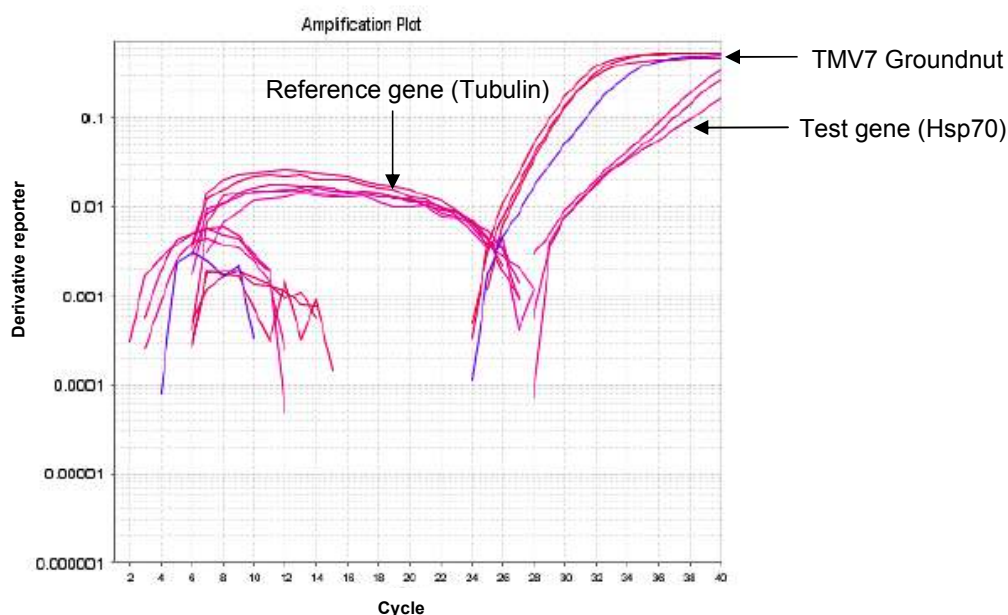
Lane 5 : Amplicon of 350 bp amplified with hsp-70 gene specific primer from Sample -2 (Groundnut varietyTMV7, untreated)

Lane 6 : Amplicon of 400 bp amplified using control sample.

In the present assay, samples 1 and 2 (Groundnut variety, TMV7 treated with 100mM NaCl and control, untreated) were relatively quantified using real time PCR with hsp70 (Heat Shock Protein-70) as test gene and TUB (Tubulin) gene as reference gene. Real Time PCR reaction for both genes of both the samples was performed in triplicate using Quantifast SYBR GREEN Real Time PCR Master mix.

Figure 25

Amplification plot of the TMV7 groundnut along with test and reference genes in relative quantification assay



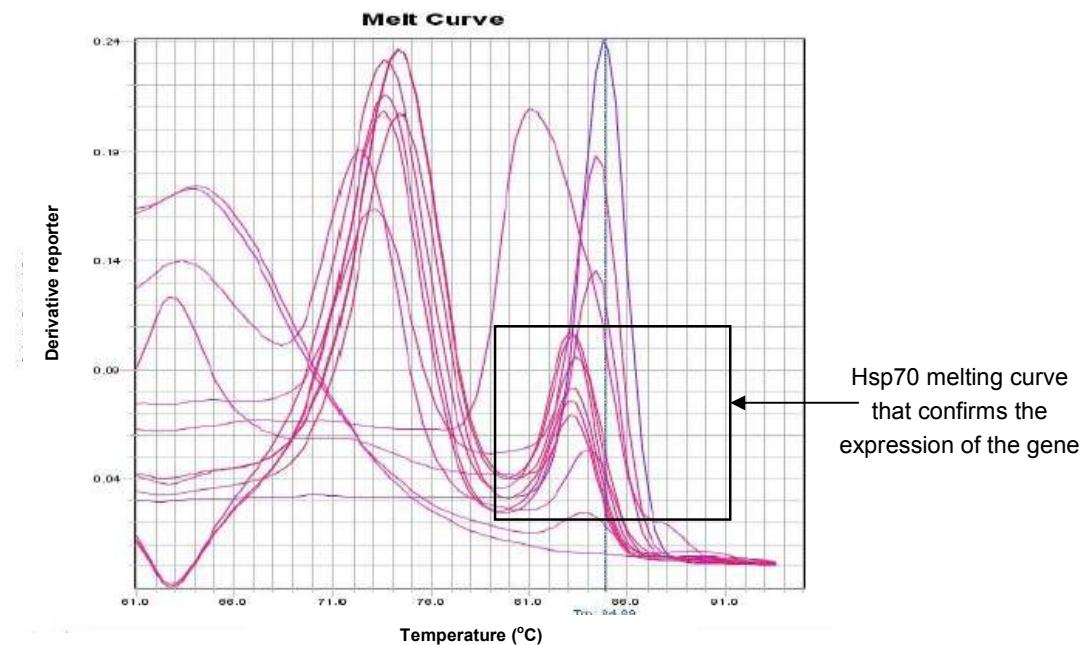
During amplification, multiple peaks were observed, which could be due to the primer amplifying multiple bands. As a result of this phenomenon, relative quantification of the product could not be arrived at.

Complex traits of abiotic stress phenomena in plants make genetic modification for efficient stress tolerance difficult to achieve. The discovery and use of new stress-tolerance associated genes, as well as heterologous genes, to confer plant stress tolerance (including those unique to extreme-growth-environment organisms e.g. halophytes, thermophilic organisms), has been the subject of ongoing efforts to obtain tolerant plants. Due to noise during amplification the

differential expression of hsp 70 was not quantified. However, the presence 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.

Figure 26

**Spectra of salt tolerant groundnut variety TMV7 under salt stress
by RT-PCR melt curve analysis in relative
quantification assay**



An ideal genetically modified crop should possess a highly regulated stress-response capability that will not affect the crop performance in the absence of stress. In this respect, conventional breeding and selection techniques will continue to make a contribution. As opined by Dunwell (2000), certain transgenic crops have already been moved from the laboratory, only a few stress-resistant transgenic crops have been evaluated in field trials under real stress conditions. When facing the deleterious effects of drought and salinity, it is imperative that more crops, which are genetically resistant to abiotic stress, be designed, tested and eventually released for application as new commercial varieties.

4.7. Sequence Similarity Search and Identification of Conserved Regions of Heat Shock Protein using *In silico* Methods

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 DOMPRED 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.

The conserved domains of the heat shock protein identified using DOMPRED has been depicted in Figure 27 and 28. All these results paved way for further research in detail about the heat shock protein present in groundnut plants under abiotic stresses.

Figure 27

Alignment score sheet of the heat shock protein 70 with other sequence matching species

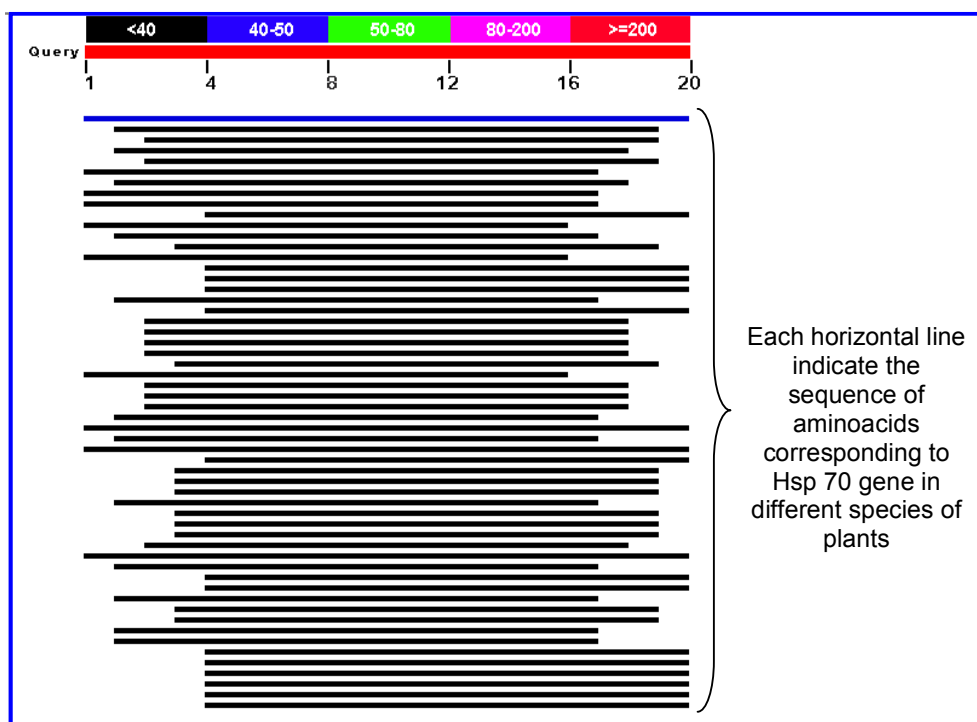


Figure 28

Conserved domains of hsp70 gene identified in various species of plants

