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INFLUENCE OF SALT (NaCl) STRESS IN TWO GROUNDNUT (*ARACHIS HYPOGAEA* L.) VARIETIES

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ABSTRACT

Salinity is an environmental stress that limits growth and development in plants. Groundnut (*Arachis hypogaea* L.) is one of the oldest cultivated plants in the world and highly prized oil crop. The effect of salt stress on growth and carbohydrates were determined in two groundnut varieties (TMV-13 and TMV-14). Plants were subjected to salt stress of different concentrations (0, 40, 80 and 120 mM) as a basal dose and sampling was done in leaves on 20th Days After Treatment (DAT). Marked variation in plant height, leaf area, fresh and dry weight of the whole plant and chlorophyll content were observed between the two groundnut varieties subjected to salt stress. Amount of carbohydrates such as total sugar, reducing and non reducing sugar and starch varied in two groundnut varieties under salt affected conditions. The amount of total sugar, reducing and non-reducing decreased with increasing salinity concentrations while starch showed a reverse trend. Quantitative differences were also observed in the content of protein and proline under salinity stress conditions. The results revealed that TMV-13 exhibits higher growth rates with high content of chlorophyll, carbohydrates and proline compared toTMV-14 under salinity stress.

***Keywords*:** Carbohydrates, chlorophyll, groundnut, growth, nitrogen, proline, protein, salt

Introduction

Salinity is one of the major abiotic factors that limit the plant productivity and is a major problem in today's irrigation agriculture, as millions of tons of salt are annually dumped on to the soil from the irrigation water (Negroa *et al*., 2019; Al-huraby, 2022). According to the FAO, (2005), the total global area of salt-affected soils, including saline and sodic soils was 831 million ha (6% of world's total land area). Apart from natural sodicity, 1,500 million ha of land farmed by dry-land agriculture, 32 million ha (2%) are affected by secondary salinity to varying degrees. For crop plants, differences in salt resistance exist not only among different genera and species, but even within a species which may on the whole be considered salt sensitive.

In glycophytes, plant growth and development are generally limited by salinity (Greenway and Munns, 1980). Most of the world's crop species are glycophytes and they do not grow under high soil salinity. However, with increasing amounts of arable lands undergoing salinization and increasing food demand from the growing human population, the need to develop salt-tolerant crop varieties is unavoidable. To develop salt tolerant crops, it is necessary to identify the degree of salinity tolerance and its mechanisms in the crop plants. Investigations of physiological and biochemical based differences between closely related plants are particularly important because these studies provide necessary information to select traits for breeding of salt-tolerant crops (Kao *et al*., 2006). Salt stress limits plant growth by adversely affecting various physiological and biochemical processes, like, photosynthesis, antioxidant phenomena, nitrogen metabolism, carbohydrate metabolism and ion homeostasis (Dkhil and Denden, 2010). Agastian *et al.* (2000) have reported that soluble protein increases at low salinity and decreases at high salinity in mulberry cultivars. Salinity also affects the plant water status through its effect on soil osmotic potential-reducing water availability to the plant and thereby affecting leaf water potential, leaf growth and evapotranspiration (Yin *et al.,* 2010).

The peanut (*Arachis hypogaea* (L.)) also known as the groundnut, is a legume crop grown mainly for its edible seeds and highly prized oil crop. It is widely grown in the tropics and subtropics, being important to both small and large commercial producers. Salinity is a major factor limiting groundnut productivity and hence the profitability of the farmers in India. The main objective of the present study was to assess the growth and carbohydrate levels in two different groundnut varieties (TMV-13 and TMV-14) under salt-stressed conditions.

Materials and Methods

The certified groundnut (*Arachis hypogaea* (L.)) seeds (Varieties: TMV-13 and TMV-14) were procured from Oil Seed Research Station (TNAU-Extension Centre), Tindivanam. Seeds with uniform size were selected and the plants were raised in pots containing red and clay soil and pH of the soil was 7.2 with EC of 0.2 dsm^{-1}. After 20 days, seedlings were thinned and three plants of uniform vigor were maintained in each pot. Plants were grown under natural climatic conditions. Plants were watered for the first 20 days after germination. The seedlings were divided into four groups. One group of seedlings were maintained under non-salinized conditions which served as control plants. The watering solution for control plants consists of tap water and one-fourth strength of Hoagland nutrients (Hoagland and Arnon, 1950). Other three groups were salinized by irrigation daily to soil capacity (500 ml d^{-1}) with the nutrient medium containing 40 mM, 80 mM and 120 mM NaCl. Salt treatment was continued until each plant received the required mM NaCl. Care was taken for individual plants in each group received the pre-calculated concentrations of NaCl in full. Additional pots with plants were also maintained for control, as well as each salinity treatment for need of plant material. Young and fully matured leaves were taken from control and salinity treated plants on 20^{th} Days After Treatment (DAT), for all the experiments described below.

Plant height, leaf area, fresh and dry weight of the whole plant were measured by standard procedure. The total chlorophyll content of the leaves was estimated according to Arnon, (1949). Carbohydrates: For alcoholic extraction, leaf sample was macerated in mortar and pestle, and 25 mg of the dried powder was boiled in water bath with 10 ml of ethyl alcohol (80%). The homogenate was centrifuged at 1500 g for 15 minutes. The supernatant was made upto 20 ml with 80% ethyl alcohol. This alcoholic extract was used for quantitative estimation of total sugars, reducing sugars. Residue was saved for starch estimation.

Total sugar content was estimated using the method of Dubois *et al.* (1956). Reducing sugar was estimated by following Somogy's method as modified by Nelson, (1944). Non-reducing sugars content was calculated by substracting the amount of reducing sugars from total sugar content. Starch content of the leaf was estimated according to Mc Cready *et al*. (1950). Total leaf protein content was estimated by Lowry's method (1951) using Folin-Ciocalteu reagent. Proline content was measured according to Bates *et al.* (1973).

For statistical analysis, five samples were taken for each treatment from five individual plants. Student’s t-test and Analysis of Variance (ANOVA) were applied for analyzing significant differences between the control and treated plants ($P<0.05$).

Results and Discussion

In recent years, salinization of soil is one of the challenging environmental concerns occurring all over the world (Arif *et al*., 2020; Alam *et al*., 2021). Soil salinity affects various plant physiological processes depending on salt type and concentrations, plant genotype, growth stage and environmental conditions (Ahmed and Jhon, 2005). Experiments on plant growth analysis with various concentrations of salinity clearly showed the overall effect of stress on specific plant growth components. Components of growth were measured in control and compared with varying concentration of salinity i.e., 40 mM, 80 mM, 120 mM, in two groundnut varieties TMV-13 and TMV-14. The growth parameters were taken at 20 days after salinity treatments and the data indicate that salinity stress has significant effect on groundnut growth and its physiology in both varieties. The reduction in plant growth under salinity is a consequence of several physiological responses including modification of water status, photosynthetic efficiency, carbon allocation and utilization (Desingh and Kanagaraj, 2007). In our study, it was observed that in TMV-13, the total plant height was reduced to the tune of 28% at 120mM salinity while in TMV-14, it was 58% when compared to respective control plants (Table 1). Salt accumulation in leaves might first inhibit photosynthesis by decreasing stomatal and mesophyll conductance to CO_2 diffusion and is known impair RuBP carboxylase which leads to reduction in height (Querghi *et al*., 2000).

Salinity stress causes a number of changes in plant metabolism and growth (Abdin *et al*., 2002). It inhibits protein synthesis and causes a decrease in fresh and dry weight of plants (Kaya *et al*., 2002). Total fresh and dry weight of the salinity treated plant was positively related to the plant height, which was significantly decreased under salinity stressed conditions in both the groundnut varieties TMV-13 and TMV-14 (Table 1). The reduction in plant dry weight, can be attributed to the reduced photosynthetic capacity of the leaves under salinity stressed conditions (Querghi *et al.,* 2000). In the current study, TMV-14 showed more reduction of fresh (53%) and dry (50%) weight of the whole plant at 120 mM salinity treatment compared to TMV-13. The result however has strong for relation between the dry matter accumulation and the plant growth rates. It is thus possible to predict that decreased photosynthetic rates under salinity condition could have reduced the shoot growth and development, thus finally yielding lower biomass production compared to control plants. The reduced fresh and dry weight of the whole plant as shown in the experiments directly reflects the reduced leaf area (Table 1), which finally manifested the overall leaf growth and development.

Although the effects of various stresses on plant pigment have been known, little is understood about the effects of salinity on the pigment content in groundnut varieties. Our data indicate that photosynthetic pigments were affected under salt stress conditions in two groundnut varieties (Table 2). However, TMV-13 recorded high total chlorophyll value (1.18 mg /gfw) even under higher salinity compared to TMV-14 (0.70 mg/gfw). Same trend was observed in the content of chlorophyll a and b in both groundnut varieties (Table 2). The leaf chlorophyll content and fluorescence were reduced with salinity (Neocleous *et al*., 2007). One of the notable effects of salinity is the degradation of the membranes of cell organelles (Mitsuya *et al*., 2000), particularly, the thylakoids of the chloroplasts. It is well known that the harmful effects of high soil alkalinity are related to the non-availability of nutrients, particularly iron (Yousfi *et al*., 2007). It is evident from our data that chloroplast integrity has been damaged under stressful conditions in groundnut varieties.

Soluble carbohydrates have been mentioned as important compounds in osmoregulation in plants under water and salt stresses (Silva *et al*., 2008) and also needs for cell growth, are supplied mainly through the process of photosynthesis and photosynthesis rates are usually lower in plants exposed to salinity especially to NaCl (Ahmad *et al*., 2017). As already discussed, salinity causes reduced CO_2 assimilation rates which intent might affect the total carbohydrate content in the leaves. It was hypothesized that due to limitation supply of structural and non structural carbohydrate in groundnut leaves, plant growth will be significantly reduced due to limited supply of energy and carbon skeletons during various stages of growth. Sugars are thought to help control key metabolic processes such as photosynthesis (Krapp *et al.,* 1993) and starch synthesis and breakdown (Koch, 1996). In the present study, high content of total sugar was noticed in TMV-13 (14.62 mg/gdw) than TMV-14 (8.28 mg/gdw) even under 120 mM salinity (Table 3). The sink systems of the plant compete for the limited carbon supplies under salinity which affect the overall plant growth and yield (Daie, 1996).

It was observed that starch content was inversely related to salinity in both groundnut varieties (Table 3). The reducing levels of starch in control and low salinity concentration (40 mM) treated plants indicate that the export of carbohydrates to various organs is at a faster rate compared to those with high salinity treated plants. The study also suggests that salinity stress causes a significant accumulation of starch in the leaves which might ultimately reduce the CO_2 assimilation patterns in the intact leaves. Salinity stress might alter the export of photoassimilates to the growing regions, thus affecting the overall growth and development of groundnut. The results also show that starch accumulation in leaves under salinity stressed conditions of both groundnut varities TMV-13 and TMV-14, might be due to decreased capacity to metabolize starch, producing during photosynthesis. The regulation of carbon allocation and partitioning would have an important influence in the maintenance of growth rate and yield (Balibrea *et al*., 1999). Our study on carbohydrates clearly indicates that TMV-13 had an effective carbohydrate partitioning mechanism than TMV-14 which might contribute for efficient photosynthesis.

Soluble protein contents of leaves decrease in response to salinity stress (Parida and Das, 2005). The leaf protein content was significantly low in the leaves of both groundnut varieties after 20 days of treatments (Table 3). However, high protein content was recorded in TMV-13 even at 120 mM salinity (47.18 mg/gfw) than TMV-14 (26.48 mg/gfw). Hsiao, (1973) reported that water stress affects polysomes and protein synthesis. Proteins can akso help in osmotic adjustments under salt stress (Zhang *et al;,* 2013). Accumulation of proline is a wide spread response to environmental stress and acts as a 'compatible' solutes (Kavikishore *et al*., 1995). Quantitative estimation of proline in TMV-13 and TMV-14 showed increased level under salinity stressed conditions (Table 3). For example, at 120 mM salinity, TMV-14 showed 25% (4.24 mg/gfw) increase of proline content compared to control plants (3.17 mg/gfw) while 59% (8.52 mg/gfw) increase of proline was observed in TMV-13 compared to control plants (3.47 mg/gfw). Proline may protect proteins and membranes from damage by inactivating hydroxyl radicals or other highly relative chemical species that accumulate when stress inhibits electron-transfer processes (Rantein *et al*., 2002). Proline suppresses nuclear deformation and chromatin condensation and improves membrane integrity under salt stress (Banu *et al*., 2008).

Conclusion

Although salt stress elicited considerable variations between the two varieties of groundnut, the varieties that show less susceptibility to salt stress is expected to improve the groundnut crop production in semi-arid areas. The present study clearly shows that variety TMV-13 is superior in maintaining growth with efficient carbohydrate allocation under salt stressed conditions. Such studies can be useful in groundnut breeding programs or transgenic groundnut research to generate plants with high yielding even under salt stressed regimes.

Table 1 : Effect of salt stress on Plant Height, Leaf Area, Fresh and Dry weight of the whole plant in two Groundnut varieties.

Variety and parameters	Salinity treatments (mM)			
	control	40	80	120
Plant height (cm)				
TMV-13	46.11 ±4.26	40.24 ±4.10	35.22 ±3.88	33.15 ±3.27
TMV-14	43.20 ±4.15	34.72 ±3.76	21.60 ±2.26	18.30 ±1.91
Leaf area (cm^2)				
TMV-13	23.10 ±2.67	20.19 ±2.25	18.24 ±1.78	17.09 ±1.58
TMV-14	20.21 ±2.32	16.44 ±1.06	11.65 ±1.41	8.28 ±0.85
Fresh weight of the whole plant (g)				
TMV-13	25.98 ±2.51	23.72 ±2.31	20.22 ±2.21	19.02 ±1.19
TMV-14	20.72 ±2.12	17.62 ±1.11	14.32 ±1.09	8.90 ±0.81
Dry weight of the whole plant (g)				
TMV-13	10.76 ±0.99	9.28 ±0.96	6.98 ±0.65	7.32 ±0.76
TMV-14	6.63 ±0.64	4.69 ±0.41	3.34 ±0.37	2.62 ±0.28

The data are expressed as mean ±s.e. for five independent determinations (P<0.05).

Table 2 : Influence of salinity stress on Total Chlorophyll, Chlorophyll-a, and Chlorophyll-b in two Groundnut varieties.

Variety and Parameters	Salinity treatments (mM)			
	control	40	80	120
Total chlorophyll (mg/gfw)				
TMV-13	1.54 ±0.045	1.39 ±6.030	1.30 ±0.02	1.18 ±0.021
TMV-14	1.47 ±0.041	1.06 ±0.022	0.94 ±0.01	0.70 ±0.012
Chlorophyll a (mg/gfw)				
TMV-13	0.71 ±0.015	0.65 ±0.013	0.60 ±0.01	0.52 ±0.010
TMV-14	0.65 ±0.014	0.43 ±0.012	0.34 ±0.01	0.26 ±0.010
Chlorophyll b (mg/gfw)				
TMV-13	0.83 ±0.019	0.74 ±0.017	0.70 ±0.01	0.66 ±0.015
TMV-14	0.82 ±0.018	0.63 ±0.015	0.60 ±0.01	0.44 ±0.011

The data are expressed as mean ±s.e. for five independent determinations (P<0.05).

Table 3 : Influence of salinity stress on the content of Total Sugar, Reducing Sugar, Non-Reducing Sugar, Starch, Protein and Proline in two Groundnut varieties.

Variety and parameters	Salinity treatments (mM)			
	control	40	80	120
Total sugar (mg/gdw)				
TMV-13	20.03 ±2.42	18.02 ±2.26	15.83 ±1.20	14.82 ±1.17
TMV-14	18.92 ±2.27	15.20 ±1.27	11.23 ±1.15	8.28 ±1.10
Reducing sugar (mg/gdw)				
TMV-13	15.40 ±1.19	13.52 ±1.12	11.92 ±1.08	11.25 ±1.02
TMV-14	13.96 ±1.15	11.35 ±1.12	7.87 ±0.08	6.19 ±0.92
Non-reducing sugar (mg/gdw)				
TMV-13	4.63 ±0.19	4.50 ±0.17	3.91 ±0.12	3.57 ±0.15
TMV-14	4.96 ±0.22	3.85 ±0.16	2.36 ±0.13	2.09 ±0.052
Starch (mg/gdw)				
TMV-13	26.81 ±2.76	29.58 ±2.85	33.87 ±3.45	37.53 ±3.49
TMV-14	28.81 ±2.80	36.53 ±3.22	48.79 ±3.33	60.48 ±3.80
Protein (mg/gfw)				
TMV-13	61.51 ±5.69	57.97 ±5.11	50.47 ±4.76	47.18 ±4.32
TMV-14	60.13 ±5.34	52.88 ±4.99	38.37 ±4.25	26.48 ±3.79
Proline (mg/gfw)				
TMV-13	3.47 ±0.32	4.38 ±0.47	5.20 ±0.49	8.52 ±0.59
TMV-14	3.17 ±0.28	3.33 ±0.31	3.49 ±0.34	4.24 ±0.43

The data are expressed as mean ±s.e. for five independent determinations ($P<0.05$).

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