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Distribution of total sugars accumulation and protein content in peanut (*Arachis hypogaea* L.) local varieties under permanent salt stress condition during the growing stage

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ABSTRACT

This work investigates the adaptive responses in two local varieties of peanut (*Arachis hypogaea* L.) in order to predict future varietal improvement due to NaCl dynamics in agricultural soils. Thus, the seeds of the varieties Mbiah collected in Bandjoun (West region of Cameroon), and Ngondo collected in Douala (Littoral region of Cameroon) were germinated in the laboratory and transplanted onto sterilized sand contained in culture pots arranged in four randomized blocks and placed under shade. During 30 days of cultivation, salt stress was induced by using Wacquant (1974) nutrient solution enriched with 0, 50, 100, and 200 mM NaCl introduced into the pot. The results show that compared to the control, the length of stems and the total leaf area were significantly ($P<0.05$) reduced with increasing levels of NaCl. The relative water content also decreased significantly ($P< 0.05$) in each organ with increasing levels of NaCl but remained higher in the leaves in both varieties. Unlike the total chlorophylls and chlorophyll b content at 100 and 200 mM NaCl, the total protein content decreased significantly ($P< 0.05$) with increasing levels of NaCl in the roots and stems of the Ngondo variety from 100 mM NaCl and only at 200 mM NaCl in the Mbiah variety. It was the same observation for total sugars where the content decreased significantly ($P< 0.05$) from 50 mM NaCl in all parts of the Mbiah variety but from 100 mM NaCl in the leaves of the Ngondo variety. These responses suggest that the leaves and stems of the Mbiah variety and the leaves only of the Ngondo variety are preferred sites of interest to be investigated for a future improvement program.

Keywords: *Arachis hypogaea* L., salt stress, glycophyte, tolerance, yield.

RESUME

Les réponses adaptatives chez deux variétés locales d'arachide (*Arachis hypogaea* L.) afin de prévoir à cause de la dynamique du NaCl dans les sols agricoles une amélioration variétale future ont été investiguées dans ce travail. Ainsi, les graines des variétés Mbiah récoltée à Bandjoun (région de l'Ouest Cameroun) et Ngondo récoltée à Douala (région du Littoral Cameroun) ont été germées au laboratoire et repiquées sur du sable lavé contenu dans des pots de culture disposés en quatre blocs randomisés et placés sous ombrière. Durant 30 jours de cultures, le stress salin a été induit par l'apport de la solution nutritive Wacquant (1974) enrichie de 0, 50, 100 et 200 mM de NaCl introduite dans les réservoirs des pots. Les résultats montrent que comparativement au témoin, la longueur de tiges et la surface foliaire totale des plantes diminuent significativement ($P< 0.05$) avec les doses croissantes de NaCl. La teneur relative en eau également diminue significativement ($P<0.05$) dans chaque organe avec les doses croissantes de NaCl mais demeurent plus élevée dans les feuilles chez les deux variétés. Contrairement à la teneur en chlorophylles totales et b à 100 et 200 mM de NaCl, la teneur en protéines totales diminue significativement ($P<0.05$) avec les doses croissantes de NaCl dans les racines et les tiges de la variété Ngondo à partir de 100 mM de NaCl et uniquement à 200 mM de NaCl chez la variété Mbiah. Il en est de même pour les sucres totaux où la teneur diminue significativement ($P<0.05$) à partir de 50 mM de NaCl dans tous les organes chez la variété Mbiah mais uniquement à partir de 100 mM de NaCl dans les feuilles de la variété Ngondo. Ces réponses observées font préférentiellement des feuilles et des tiges chez la variété Mbiah, et des feuilles chez la variété Ngondo des sites d'intérêts à investiguer pour un programme futur d'amélioration.

Mots clés: *Arachis hypogaea* L., stress salin, glycophyte, tolérance, rendement.

1. INTRODUCTION

Soil salinization is currently the main factor limiting crop production (Flowers et al., 2010). On a global scale, around 800 million hectares of land are affected by soluble salts (Manchanda 2008), 80 % of which are of natural origin and 20 % of anthropogenic origin. In arid, semi-arid, and coastal regions, the physical and chemical properties of the soil are unfavorable to plant growth (Golla, 2021). Water scarcity is exacerbated by increased environmental salinity (Munns &Tester. 2008). This leads to a reduction in the osmotic potential of the soil. It also leads to nutrient imbalances and ionic toxicity. Plants react to variations in salinity in their environment either by disappearing or by triggering mechanisms that modify their morpho-physiological, metabolism, and mineral behavior (Amira & Abdul, 2011; Golla, 2021; HanumanthaRao et al., 2016). The improvement of plant tolerance to salinity is a major challenge for the improvement of individual plant growth (Meguekam et al., 2022; Khan et al., 2022) and plant productivity. Another solution to the problem of soil salinity is to research and select varieties that are tolerant to salt stress.

Seed legumes, including peanuts (*Arachis hypogaea* L.), are an important part of people's diets, a source of cheap vegetable protein and oil, and their cultivation is a source of income for people in developing countries (FAO, 2008). Abiotic stresses, including salinity stress, are the main causes of the decline in legume yields, especially peanut yields and then, research into plant tolerance to salinity is important for the restoration of saline soils and increasing agricultural yields.

The causes of salinization of agricultural soils are variable and depend on the region under consideration; for example, in coastal regions, soil salinization is caused by the intrusion of seawater into groundwater, and soils in these regions can also be affected by the arrival of saline aerosols produced by violent wave activity during storms or strong winds on the sea. (Lapere et al., 2023) On the other hand, these salts have the ability to move within the soil, traveling considerable distances up channels to reach other regions; this is how salts can be found in groundwater in high concentrations without coming directly from the sea. The favorable saline balance within the root zone must be maintained by adequate leaching, which is almost non-existent due to insufficient rainfall, albeit spread out over time.

This is why, the aim of this study is to evaluate the distribution of the specific responses of two local peanut varieties grown in regions with different hydro-ecological characteristics but subject to the same saline constraints, with the preliminary aim of seeking associations of quantitative characteristics (continuous variables) used for varietal improvement. Two local Cameroonian varieties were used, one (Mbiah) from the western region and the other (Ngondo) from the coastal region.

2. MATERIAL ET METHODS

2.1. Plant Material

The study was conducted in the experimental field of the Higher Teacher Training College of Yaoundé between January and February 2018, under the shade between 03°51'N and 11°30' E, with mean temperature extremes of 28 -42 °C and relative humidity values of 42-61%. The biological material consisted of seeds of two local groundnut varieties: *Arachis hypogaea* L. namely Mbiah harvested in Bandjoun (West-Cameroon) and Ngondo harvested in Douala (Littoral-Cameroon). Mbiah variety exhibits protection mechanism against oxidative damage caused by salt stress (Meguekam et al., 2014), while Ngondo shows good growth and mineral uptake parameters up to 100 mM NaCl (Meguekam et al., 2010). Both varieties were identified at IRAD of Foumbot as ecotypes of ICGV86003 and Metchicha, respectively.

2.2. Methods

2.2.1. Setting up the experiment

Seeds of the same size of each variety, morphologically similar and apparently healthy were washed with tap water, then disinfected with 70 % V/V ethanol solution for 4 minutes, then rinsed abundantly with distilled water and placed in plastic trays between two layers of damp cotton to germinate and fed daily with distilled water until complete germination.

For each variety, two germinated seeds were transplanted into 10” wide×15” tall culture pots containing 250g of quartz sand previously washed with a 10 % V/V hydrogen peroxide solution, then rinsed thoroughly with distilled water. The pots were then placed on tanks containing 150 mL of distilled water until the seedlings had fully emerged.

2.2.2. Stress induction

After transplanting, the pots of each variety containing the emerged five-day-old plants were arranged in four completely randomized blocks of five pots each (El-iklil et al., 2002) and the distilled water in the tanks was replaced by the same volume of a nutrient solution containing 0.4 mM of KNO_3, 0.2 mM of KH_2PO_4,1.0 mM of Ca_2NO_3 and 0.4 mM of $MgSO_4$ (Wacquant, 1974) enriched with 0. 50. 100 and 200 mM of NaCl for the first, second, third, and fourth blocks respectively, this solution was renewed every five days, and the entire system was maintained in culture for thirty days.

2.2.3. Evaluation of parameters

2.2.3.1. Architectural traits

During this growth phase, the architectural parameters of each plant were measured before renewing the solutions: a centimeter scale tape was used to measure the length of plants from the collar to the collinear apex. The average leaf area of each plant is determined by measurements taken on the third leaf from the top by the formula $S(cm^2) = L \times l \times 0.80 \times N \times 0.662$, where L is the length of the leaf and l is the sum of the maximum widths of its leaflets and N is the number of spreading leaves (Kumar *et al.*, 2002).

2.2.3.2. Physiological Parameters

Thirty days after transplanting, the plants of each variety are harvested and separated into leaves, stems, and roots. The first part of the samples from each plant organ is used immediately to determine the relative water and chlorophyll content, the second part is stored in a freezer at -20 °C for protein determination and the third part is stored in an oven at 70 °C for total sugar analysis.

2.2.3.3. Relative water content (RWC) distribution

The relative water content of different plant organs was determined using the following formula RWC (%) = (FW-DW) / (TW-DW) × 100 where FW is the fresh weight, TW is the turgid weight measured after 24 hours of saturation on deionized water in dark place (Creus et al., 1998) and DW is the dry weight determined after 48 hours in the oven at 85 °C (Taffouo et al., 2009).

2.2.3.4. Leaf chlorophyll content

The chlorophyll content of the leaf samples was estimated immediately after harvest on 20 mg of fresh and crushed plant material, following the procedure described by Holm-Hansen et al. (1965). Chlorophyll present in the samples was extracted with 80 % (V/V) alkaline acetone. Complete extraction of the chlorophyll was obtained when the sample was decolorized. The absorbance of the extracts was measured at 663 and 645 nm using a Beckman DU 68 spectrophotometer and the Chlorophyll content given by (mg. g^{-1} FW) was first calculated using the following equations: $[\text{Chlorophyll a}] = (0{,}0127 \times O.D_{663}) - (0{,}0029 \times O.D_{645})$

$$[\text{Chlorophyll b}] = (0{,}0229 \times O.D_{645}) - (0{,}00468 \times O.D_{663})$$

$$[\text{Chlorophyll Total}] = (0{,}0202 \times O.D_{645}) + (0{,}00802 \times O.D_{663})$$ (Arnon, 1949)

2.2.3.5. Total sugars distribution

Total sugars (TS) were determined by the modified method of Dubois et al. (1956) whose principle is as follows: The thermal effect of concentrated sulfuric acid causes the departure of several water molecules from the sugar monomers. Root, stem, and leaf samples were dried at 70 °C, homogenized in 80 % (V/V) ethanol solution, and placed in a water bath at 80 °C for 30 min after centrifugation at 3000 g for 5 min. The samples were washed twice with H_2O. Each sample was resuspended in 3 ml of H_2O and boiled for 2 hours. The total sugars content was then measured by the Nelson-Somogyi method described by Oser (1979).

2.2.3.6. Total protein content distribution

Total protein (Pr) content was determined by the modified method of Bradford, 1976 on different plant organs. The standard curve was obtained using BSA 1 mg/ml. Bovine serum albumin (BSA) was used as protein standard 2.5 ml protein extracts from different crude samples were homogenized with 3 ml of an already prepared sodium-phosphate buffer, pH 7. The mixture was centrifuged for 5 min at 13000 rpm and 4 °C. Approximately 1 ml of the supernatant was poured into a tube containing 1.5 ml of the Bradford reagent and this mixture was shaken and incubated in a dark environment for 15 min. The absorbance of the resulting blue complex was read at 595 nm using the UV spectrophotometer (Jenway 6305) against a blank in which the extract was replaced by the extraction buffer.

2.3. Statistical analysis

The results were subjected to a descriptive statistical analysis with one factor for physiological parameters and two factors for the others. The histograms and curves presented show the average values of five repetitions framed by their standard deviations. Differences were made using the NEWMAN- KEULS test (1999), based on the smallest significant value, using XLSTAT software (STATCON, NY, United States). They were considered significant if $P<0.05$.

3. RESULTS AND DISCUSSION

3.1. Effect of permanent salt stress on the architectural traits of *Arachis hypogaea* L.

3.1.1. Stem length

Maintaining peanut plants under conditions of permanent salt stress results in visible changes in architectural traits (Fig. 1). In the Mbiah variety (Fig. 1A), increasing levels of NaCl significantly ($p< 0.05$) inhibited stem elongation from the second week. The same was true for the Ngondo variety (Fig. 1B) from the second week, compared to the control.

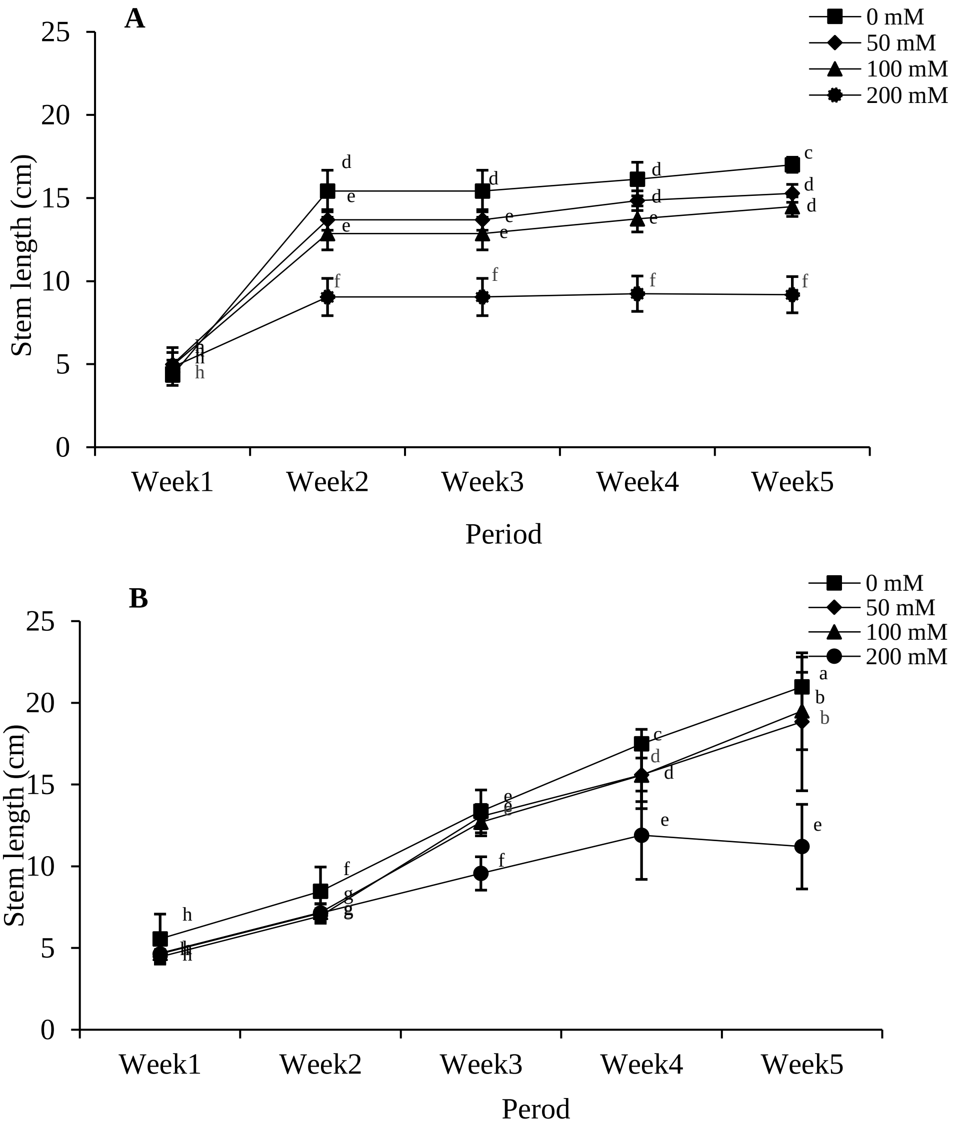

Figure 1: Effect of the permanent salt stress on variation in stem length (cm) of *Arachis hypogaea* L. Each point is an average of 5 repetitions. Values with different letters indicate significant differences per $p<0.05$ according to the control. A: Mbiah, B: Ngondo

3.1.2. Leaf area

Likewise, this continuous NaCl stress leads to a significant reduction ($p<0.05$) in the leaf area in the two peanut varieties studied (Fig.2) compared to the low concentration (50 mM NaCl). However, in the Mbiah variety (Fig.2A), the blocks of plants fed with 50 mM NaCl solutions have leaf areas as large as those of the control plants.

In the Ngondo variety (Fig.2B), increasing the NaCl level resulted in a significant ($p<0.05$) reduction in leaf area from 50 mM NaCl compared to the control in the third week of treatment.

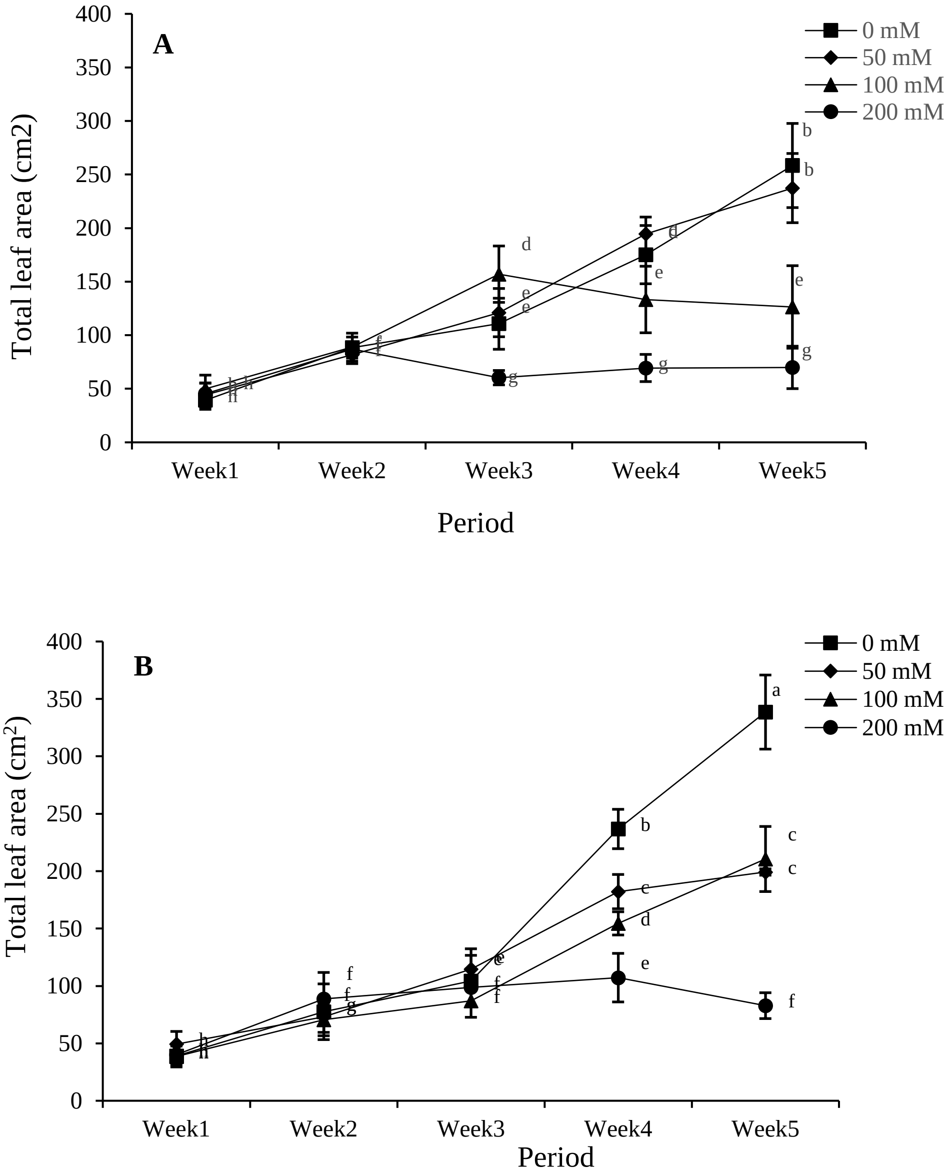

Figure 2: Effect of the permanent salt stress on the total leaf area ($cm^2.plant^{-1}$) of *Arachis hypogaea* L. Each point is an average of 5 repetitions. Values with different letters indicate significant differences per $p<0.05$ according to the control. A: Mbiah, B: Ngondo

3.2. Effect of permanent salt stress on the physiological traits of *Arachis hypogaea* L.

3.2.1. Relative water content (RWC)distribution

The distribution of water between the different plant organs of the two peanut varieties studied is presented in Table 1. The analysis of this table shows that: the increased doses of NaCl in the medium led to a significant decrease ($p < 0.05$) of the relative water content contained in each part of the plant compared to the control. At each NaCl concentration level, the root had the highest content, followed by the stems and finally the leaves. Furthermore, under optimal and stress conditions, the root, followed by the stem, were the most hydrated organs compared to the leaves.

Table 1: Relative water content (%) distribution in plant's organ of peanut (*Arachis hypogaea* L.) on the permanent salt stress condition

		NaCl solution (mM)			
Variety	**Plant's organ**	**0**	**50**	**100**	**200**
Mbiah	Leaf	59.1± 0.05^{b}	56.4 ± 0.026^{c}	49.3 ± 0.025^{c}	17.2 ± 0.010^{d}
	Stem	83.3 ± 0.08^{a}	77.3 ± 0.077^{b}	71.5 ± 0.043^{b}	43.9 ± 0.072^{c}
	Root	85.0 ± 0.03^{a}	82.2 ± 0.085^{a}	79.8 ± 0.085^{a}	52.4 ± 0.053^{b}
Ngondo	Leaf	59.1 ± 0.059^{a}	46.3 ± 0.056^{b}	25.3 ± 0.049^{b}	10.2 ± 0.012^{c}
	Stem	83.3 ± 0.083^{a}	77.3 ± 0.077^{b}	53.7 ± 0.071^{c}	51.4 ± 0.025^{c}
	Root	89.7 ± 0.085^{a}	87.8 ± 0.082^{b}	61.5 ± 0.080^{a}	53.9 ± 0.014^{c}

Each value is an average of five repetitions. Values with different letters are significantly different by $p < 0.05$ according to the control for each variety.

3.2.2. Total sugars content distribution

Maintaining peanut plants under salt stress leads to variations in the accumulation of total sugars in the leaf, stem, and root (Fig.3). In the Mbiah variety, the total sugars content decreased very significantly ($p < 0.05$) with increasing levels of NaCl in leaves, stem, and root from 50 mM NaCl. However, the highest levels were found in leaves at each level of salt treatment and the lowest in the root of plants stressed at 200 mM NaCl. On the other hand, in the Ngondo variety, the total sugars content decreased very significantly from 50 mM NaCl in the stem and 100 mM NaCl in the leaf. On the other hand, in the root, this content decreases from 50 mM NaCl, but remains constant up to 200 mM NaCl.

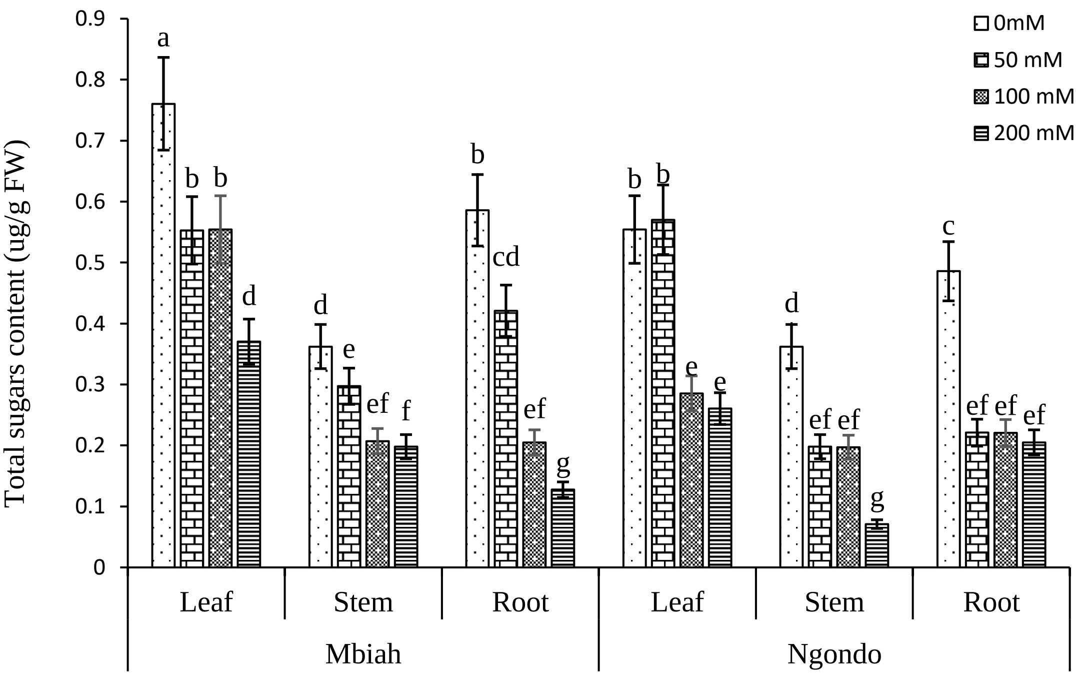

Figure 3: Effect of the permanent salt stress on distribution of total sugars content (ug/g DW) of *Arachis hypogaea* L. Each point is an average of 5 repetitions. Values with different letters indicate significant differences per $p<0.05$ according to the control for each variety.

3.2.3. Chlorophyll distribution

The distributions of chlorophylls a, b, and total in the leaves of peanut varieties subjected to permanent stress are given in Table 2. The analysis of this table shows that: in the Mbiah and Ngondo varieties, increasing doses of

NaCl in the medium led to a significant decrease ($p<0.05$) in the chlorophyll a content from 100 mM NaCl compared to the control. On the other hand, in both peanut varieties, the chlorophyll b and total content increased significantly ($p<0.05$) from 50 mM NaCl, but the content of chlorophyll b remained constant in media enriched with 100 and 200 mM of NaCl.

Table 2: Chlorophyll content 10^{-4} ($mg.g^{-1}$ FW) distribution in leaves of peanut (*Arachis hypogaea* L.) under permanent salt stress condition

		NaCl solution (Mm)			
Variety	**Chl. Content ($mg.g^{-1}$ FW)**	**0**	**50**	**100**	**200**
Mbiah	Chlorophyll a	12.66 ± 0.28^{a}	12.29 ± 1.30^{a}	8.55 ± 1.89^{b}	6.45 ± 2.26^{c}
	Chlorophyll b	11.86 ± 0.34^{a}	12.11 ± 0.21^{a}	12.67 ± 0.31^{a}	12.02 ± 0.19^{a}
	Chlorophyll total	34.25 ± 1.48^{a}	36.38 ± 2.23^{a}	35.88 ± 2.10^{a}	37.06 ± 1.49^{a}
Ngondo	Chlorophyll a	14.03 ± 0.07^{a}	13.93 ± 1.13^{a}	10.29 ± 0.47^{b}	10.61 ± 0.43^{b}
	Chlorophyll b	10.63 ± 0.56^{b}	12.59 ± 0.33^{a}	12.56 ± 0.57^{a}	12.22 ± 0.87^{a}
	Chlorophyll total	25.63 ± 0.60^{a}	24.81 ± 2.69^{a}	27.03 ± 3.23^{a}	28.21 ± 0.3^{a}

Each value is an average of 5 repetitions. Values with different letters indicate significant differences per $p < 0.05$ depending on the control for each variety.

3.3. Effect of permanent salt stress on total protein content distribution of *Arachis hypogaea* L.

The distribution of protein accumulated in *Arachis hypogaea* L. plants subjected to permanent salt stress is presented in Figure 4. The analysis of this figure shows that in the Mbiah variety, increasing levels of NaCl in the culture medium led to a resulted in significant accumulation ($p< 0.05$) of total protein at 50 mM NaCl in the leaves and then 50 and 100 mM NaCl in the stems compared to the control. In contrast, in the Ngondo variety, the total protein content increased significantly in leaves from 50 mM NaCl and decreased in the stems and roots under these conditions. However, the highest levels were observed in the stems. These two varieties presented this similarity: increasing levels of NaCl in the growing substrate medium led to a significant decrease ($p< 0.05$) of this parameter at the plant root.

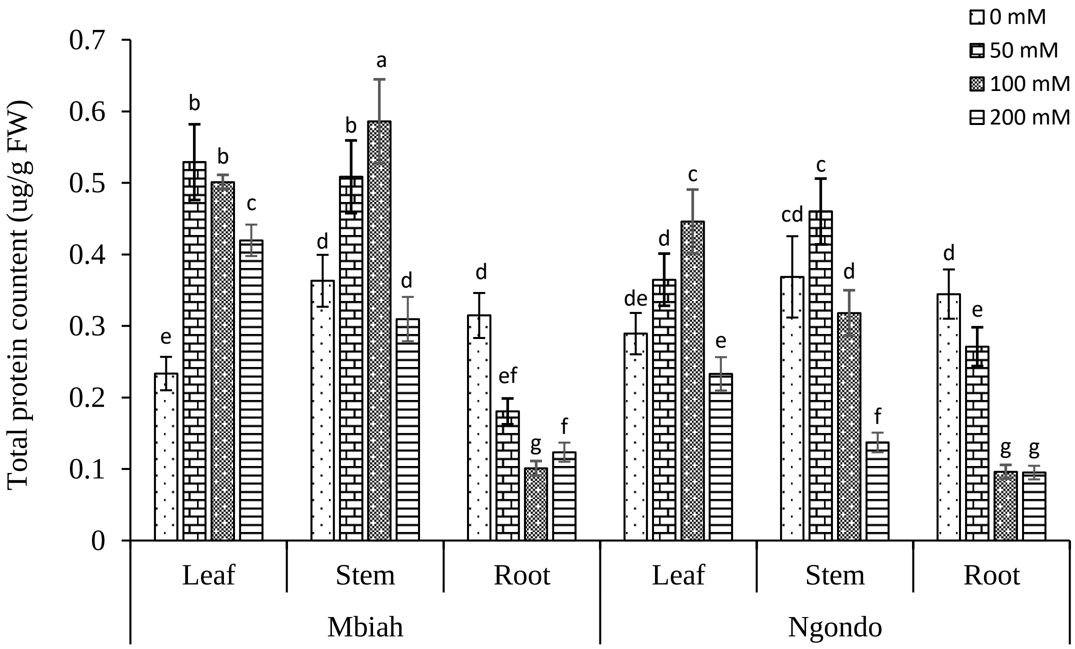

Figure 4: Effect of the permanent salt stress on the total protein content (ug/g FW) of *Arachis hypogaea* L. Each point is an average of 5 repetitions. Values with different letters indicate significant differences per $p <0.05$ according to the control for each variety.

4. DISCUSSION

The reduction in leaf area observed in these two varieties of peanut would be the expression of an adaptive trait in response to the unavailability of water caused by the accumulation of NaCl in the growing substrate (Chourasia *et al*. 2021; Meguekam *et al*. 2022). The morpho-anatomical traits of species are affected by abiotic stress. The same goes for the reduction in plant height in response to stress, in order to minimize the energetic cost imposed by the rise of water in the conductive tissues, which would be redirected towards the metabolic synthesis of resistance of the plant (Chourasia *et al*. 2021).

Water deficit resulting from high accumulation of Na^+ and Cl^- salts leads to biochemical changes as well as chlorophylls and total sugars; This water deficit proves the existence of an antagonistic relationship between the concentration of NaCl and the osmotic potential it generates, resulting from a strong accumulation of Na^+ and Cl^- ions in the growth medium leading to biochemical modifications as well as chlorophylls and total sugars. Thus, these varieties, although tolerant (Meguekam *et al*. 2014) always undergo stress in their leaves, which are less hydrated than their roots. Similar results were obtained by Dadach et al. (2015) in *Medicago arborea* even with much lower reduction rates.

Salinity significantly reduces the chlorophyll a content of the leaves of stressed plants. The accumulation of total chlorophyll and chlorophyll b in tolerant varieties has been observed in plants in general and in legumes in particular (Meguekam *et al*. 2022, Dadach *et al*. 2015). It should be noted that this increase is accompanied at the same time by an increase in total protein synthesis, which could be explained by the fact that soluble sugars play a determining role in osmotic adjustment, as well as in the level of stabilization of certain proteins including resistance proteins (HanumanthaRao *et al*. 2016).

The increase in the total sugar content accompanied by an increase in the synthesis of total proteins in the leaves and stems of these varieties would justify the role of sugars in osmotic adjustment, as well as in the stabilization of certain proteins including salt stress resistance proteins (Meguekam *et al*., 2014).

5. CONCLUSION

The result of this study, which aimed to study the responses variability of two local peanut varieties under salt stress conditions with the aim of targeting sites of interest, was a variability of responses with more or less specificities: The relative water content remains higher in the stem and more in the roots with an increase in NaCl followed by the reduction on the one hand of the transpiration surfaces and on the other hand of the points of energy expenditure by the transfer of water and nutrients, namely the stems. Furthermore, the site of preferential accumulation of total sugars as well as total protein content are the leaves and stems of stressed plants. Total chlorophylls and the chlorophyll b appear to be the markers of salt stress in the two peanut varieties Mbiah and Ngondo due to their accumulation under these conditions. Only chlorophyll a decreased in the leaves of both peanut varieties in response to the increased NaCl content in the growth medium.

These responses to salt stress in general, and the biochemical responses in particular, make both the leaves as well as stems of these two varieties interesting for future varietal improvement analyses.

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