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Differential responses of growth, chlorophyll content, lipid peroxidation and accumulation of compatible solutes to salt stress in peanut (*Arachis hypogaea* L.) cultivars

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The present study was aimed to compare differential responses of growth, chlorophyll content, lipid peroxidation and accumulation of compatible solutes in peanut (*Arachis hypogaea* L.) cultivars: Fleur 11 (salt-tolerant), Mbiah and PC 79-79 (moderately-tolerant) and Vanda (salt-sensitive) at the vegetative growth stage, under greenhouse conditions, in the presence of 0, 50, 100 or 200 mM NaCl. The root dry weight (RDW) and shoot dry weight (SDW) of cv. Vanda decreased significantly ($p< 0.05$) in salt-treated plants than those of cvs. Fleur 11, Mbiah and PC 79-79. The SDW reduction was notably noted at 100 mM NaCl in cvs. Mbiah and PC 79-79, while cv. Fleur 11 showed significantly ($P<0.05$) decrease in salt-treated plants only at 200 mM NaCl but had higher SDW accumulation than others. The leaf chlorophyll content increased in cvs. Fleur 11 and PC 79-79 and decreased in cv. Vanda with increasing NaCl levels. Proline (PRO) and glycine betaine (GB) contents significantly ($p<0.05$) increase, for all cultivars, in the stressed plants with the highest quantity in Fleur 11 and the lowest in Vanda. Malondialdehyde levels increased under salt stress in the leaves of cv. Vanda but decreased in cvsMbiah, PC 79-79 and Fleur 11 at 100 and 200 mM, respectively. Total phenolic content increased significantly ($p < 0.05$) at 200 mM NaCl in the leaves of cv. Vanda than others, while cv. Fleur 11 showed the lowest increase. The salt-tolerant cv. Fleur 11 exhibits a better protection mechanism against oxidative damage caused by salt stress maintaining a higher accumulation of PRO and GB than others. Higher PRO and GB accumulation in the leaves may be regarded as potential biochemical indicator for earlier selection of salt tolerant peanut and targets for improvement through transgenic approaches.

Keywords:*Arachis hypogaea*, glycine betaine, growth, lipid peroxidation, proline, salt stress

INTRODUCTION

Salinity stress is the most limiting factor in agricultural productivity in arid and semi-arid regions of the world (Horie and Schroeder, 2004). In saline environment excessive sodium affects plant growth of many sensitive species which comprised most of crops (Hossein and Fatemeh, 2012; Rameeh et al., 2012). The morphological,

physiological and biochemical attributes of plants is altered by salinity thus limiting its growth and development (Mudgal et al., 2010). Low external water potential, ion toxicity and interference with the uptake of nutrients are a range of mechanisms that inhibit plant growth due to salinity (Munns et al., 1995). The responses of plants to high soil salinity and the mechanisms of salt tolerance have been largely discussed (Ruan et al., 2010; Grigore et al., 2011). Compartmentation of ions in vacuoles and accumulation of compatible solutes in the cytoplasm are commonly proposing mechanisms of salt tolerance species (Munns, 2002). The basic mechanisms of salt tolerance in halophytes seem to be mostly dependent in their capacity to sequester toxic ions (Na^+, Cl^-) in the vacuoles and to accumulate compatible osmotica in the cytoplasm (Le Rudulier, 2005; Grigore et al., 2011). The compatible solutes accumulation that are mostly seen in plants are proline (PRO) and glycine betaine (GB), but other osmolytes can be stored at high concentrations in some species (Girija et al., 2002). An increase in salinity increases PRO as an adaptative change in metabolism pattern (Mudgal et al., 2010). Thylakoid and plasma membrane integrity are protected by GB after exposure to saline solutions (Rhodes et al., 1987).

Peanut salt-tolerant cultivars accumulate the highest quantity of GB while moderately-tolerant cultivar stored intermediate amount and sensitive low quantity (Girija et al., 2002). Thus, the accumulation of osmo-protectants in tissues of plants growing in arid or semi-arid lands, may exhibit more tolerance to salt stress (Munns, 2002). Salt stress can lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of Reactive Oxygen Species (ROS) and induce oxidative stress (Parvaiz and Satyawati, 2008). Plants have developed a series of enzymatic and non-enzymatic detoxification systems to counteract ROS, and protect cells from oxidative damage (Sairam and Tyagi, 2004). The assessment of cell membrane stability is an appropriate technique to screen plants under saline condition (Munns et al., 2006). Salt stress increased lipid peroxidation or induced oxidative stress in plant tissues (Hernandez et al., 1993). Malondialdehyde (MDA) has been known as the end product of peroxidation of membrane lipids (Sajedi et al., 2011). Increase in the level of MDA, produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage (Azad et al., 2012). The salt stress was able to induce excessive generation of MDA in the root and leaf of maize seedlings (Azad et al., 2012). Phenolic compounds are a large group of secondary metabolites, which can play a role in any interaction that a plant can have with its environment (Waterman and Mole, 1994). These compounds have been implicated to stress resistance against biotic and abiotic factors (Bergmann et al., 1994). Total phenolic (TP) accumulation could be a cellular adaptive mechanism for scavenging oxygen free radicals during stress (Mohamed and Aly, 2008). The increased synthesis of PRO, TP and the antioxidant activity in dill seedlings exhibited a protective mechanism against the cellular structures from oxidative damage (Zahra et al., 2012).

Grain legumes provide large amounts of high quality proteins which contain relatively more of the essential amino acids not supplied by cereals in which the content of lysine and tryptophan are relatively small (Kay, 1979). Peanuts are essential sources of fat (34 to 54%) (Nyabyenda, 2005). Legumes intervene in crop rotation systems and participate in biological nitrogen fixation (Delgado et al., 1994). The selection of tolerant cultivars can be done efficiently in cultivated saline environments, and thus salt tolerance mechanisms potential can be identified within plant species which is becoming an increasing research priority in many countries. It is important to make a call to the ecophysiological approach which can constitute an attenuation of the effect of the soil's salinity on the cultivated plant performances (Mekhaldi et al., 2008). This would lead to the search of tolerant species or varieties of plant thus imposing a mastery of the knowledge of mechanisms to their adaptation to salinity.

The present study was aimed to compare differential responses of growth, physiological and biochemical characteristics in peanut cultivars differing in salt tolerance at the first vegetative growth stage and determine biochemical indicators which could serve as early selection criteria for tolerance of salt in peanut.

MATERIALS AND METHODS

Plant material, growth and stress conditions

Experiments were performed using seeds of four peanut (*Arachis hypogaea* L.) cultivars differing in salt tolerance; cv. Fleur 11 (salt-tolerant), cvs. Mbiah and PC 79-79 (moderately-tolerant) and cv. Vanda (salt-sensitive). Mbiah and Vanda were provided by the breeding program of the Agronomic Institute for Research and Development of Cameroon. Fleur 11 and Pc 79-79 were obtained from Senegalese Institute of Agronomic Research. Germination trials were conducted in 9 cm sterile Petri dishes lined with Whatman No.1 filter papers and moistened with 10 mL of distilled water. After seed surface sterilization with 70% (v/v) ethanol solution for 15 min, followed by rinsing with distilled water, seeds were sowed in Petri dishes and placed in seed germinator at 26°C for 5 d. After germination, the plants were sown in pots with sterilized sand in a greenhouse (26/23°C light/dark and 51 to 61%

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hygrometry), located at the Alexandru Ioan Cuza de Iaşi University, Romania, from June to September, 2011 and March to July, 2012. The pots were arranged in a complete randomized design with five replicates. One plant was grown in the middle of each pot. Each cultivar had 20 pots divided into four groups. Each group, with five replicates, were fertilized every two days with the nutrient solution (Boldor et al., 1983) containing 49.2% $Ca(CO_2)_2$, 13.6% KH_2PO_4, 6.0% $MgSO_4$, 7.5% KCl and 2.5% $FeCl_3$ added with one of the four NaCl concentration levels (0, 50, 100 and 200 mM) for one month. Plants were harvested for physiological and biochemical analysis at 35 days after sowing for a total of 20 plants from each treatment.

Plant growth parameters determination

Plant growth (root dry weight, shoot dry weight, stems diameter (SD) and total leaf area (TLA) was evaluated using twenty plants from each cultivar. All tissue parts (leaves, stems, and roots) were separated, and fresh weights of these tissue parts were measured. For the determination of dry weight, these tissue parts were dried at 65°C for 72 h. SD was measured every week on plants using a caliper. TLA was measured every week and calculated using the formula described by Kumar et al. (2002):

$TLA\ (cm^2) = L \times la \times 0.80 \times N \times 0.662$

Where L = length of leaf; la = width of leaf and N = total number of leaves.

Biochemical parameters determination

Proline (PRO) content was extracted from fresh leaves according to the method of Bates et al. (1973). Leaves samples (0.5 g) were homogenized in 10 mL of 3% (w/v) aqueous sulfosalicylic acid to precipitate protein. Samples were centrifuged at 18,000 *g* for 10 min and supernatant was used for estimation of PRO content. The reaction mixture consisted of 1 mL acid ninhydrin and 1 mL of glacial acetic acid, which was boiled at 100°C for 1 h. After tubes cooling in the ice, the products were extracted with 2 mL of toluene by vortex mixing and the upper (toluene) phase decanted into a glass basin. The absorbance was recorded at 520 nm and the PRO concentration was determined as µg g^{-1}FW using a standard curve.

Glycine betaine (GB) content was measured in leaves tissue extracts as described by Grieve and Grattan (1983). The plant tissue was finely ground, mechanically shaken with 20 mL deionised water for 24 h at 25°C. The samples were then filtered through Miracloth and filtrates were diluted to 1:1 with 2 N H_2SO_4. Aliquots were kept in centrifuge tubes and cooled in ice water for 1 h. Cold KI-I_2 reagent was added and the reactants were gently stirred with a vortex mixture. The tubes were stored at 4°C for 16 h and then centrifuged at 10,000 *g* for 15 min at 0°C. The supernatant was aspirated with a fine glass tube. The periodide crystals were dissolved in 9 mL of 1, 2-dichloroethane. After 2 h, the absorbance was measured at 365 nm using GB as standard. The GB concentration was expressed as µmol g^{-1}DW.

Malondialdehyde (MDA) content was determined in leaves using a modified thiobarbituric acid (TBA) assay (Hodges et al., 1999). Samples were homogenized with inert sand in 1:25 (g FW:mL) 80:20 (v/v) ethanol: water, followed by centrifugation at 3,000 g for 10 min. One millilitre of the sample was added to a test tube with 1 mL of either (1) TBA solution comprised of 20% (w/v) trichloroacetic acid and 0.01% butylated hydroxytoluene or (2) +TBA solution containing the above and 0.65% (w/v) thiobarbituric acid. Samples were then mixed vigorously, heated at 95°C for 25 min, cooled and centrifuged at 3,000 g for 10 min. Absorbance values were recorded at 440, 532 and 600 nm. MDA equivalents were calculated in the following manner:

1. [(Abs532+TBA-Abs600+TBA). (Abs532-TBA- Abs600-TBA)] = A
2. [(Abs440+TBA-Abs600+TBA) 0.0571] = B
3. MDA equivalents ($nmolmL^{-1}$) = $(A-B/157,000)10^6$

Results were expressed as MDA equivalents (nmol g^{-1}FW) and represent the mean of five samples.

Total phenolic (TP) content was assayed according to method of Marigo (1973) using the Folin-Ciocalteu reagent. 1 g fresh tissues leaf was ground at 4°C in 3 mL of 0.1 N HCl for 20 min. After incubation the homogenate was centrifuged at 6,000 *g* for 40 min. The supernatant was collected, the pellet re-suspended in 3 mL of 0.1 N HCl and centrifuged as previously. The two supernatant are mixed and constitute the crude extract of soluble phenol. The reaction mixture containing 15 µL of extract, 100 µL Folin-Ciocalteu reagents, 0.5 mL of 20% Na_2CO_3 was incubated at 40°C for 20 min and absorbance read at 720 nm with a BECKMAN DU 68 spectrophotometer. A standard curve was established using chlorogenic acid. TP content was expressed as µg g^{-1} FW.

Measurements of the chlorophyll content

Chlorophyll (CHL) content in leaves was estimated after extracting 20 mg of the ground material, following the procedure described by Arnon (1949). Chlorophyll of samples was extracted with 80% alkaline acetone (v/v). Full extraction of chlorophyll was achieved when the sample was discoloured. The absorption of the extracts was measured at 663 and 645 nm with a BECKMAN DU 68 spectrophotometer and CHL was calculated using the following formula:

Total chlorophyll = (20.2 x D645 + 8.02 x D663) x (50/1000) x (100/5) x 1/2

Where, D = absorbance and expressed as mg g^{-1}FW.

Statistical analysis

Results obtained from all the manipulations are expressed as mean ±standard deviation and analyzed using SPSS software. Statistical differences between treatment means were established using the Fisher LSD test at p values < 0.05. Multifactorial ANOVA was used to estimate whether cultivar, salinity level, alone or in interaction, had a significant influence on the measured parameters.

RESULTS

Growth parameters

Peanut growth was estimated by measuring root dry weight (RDW), shoot dry weight (SDW), total leaf area (TLA) and stem diameter (SD) (Table 1). A significant two-way interaction between the factors, salinity level and cultivars, was observed for SDW and TLA (Table 1). The RDW and SDW of cv. Vanda decreased significantly ($p< 0.05$) in salt-treated plants, when compared with control plants than those of cvs. Fleur 11, Mbiah and PC 79-79. The SDW inhibition effect of salt was notably noted at 100 mM NaCl in cvs. Mbiah and PC 79-79, while cv. Fleur 11 showed significantly ($p<0.05$) decrease in salt-treated plants only at 200 mM NaCl but had higher SDW accumulation than others (Table 1). TLA of all cultivars was negatively affected with increasing levels of salinity (Table 1). At the highest salt concentration (200 mM

Table 1. Peanut cultivars growth parameters at different salinity levels. Data are mean ± standard error (n =5).

Cultivar	Salinity level (mMNaCl)	Plant dry weight (g plant^{-1})		Stem diameter (cm)	Total leaf area (cm^2 plant^{-1})
		Shoot	Root		
Fleur 11	0	0.74±0.04^{b}	0.11±0.01^{b}	0.27±0.01^{a}	39.83±2.33^{e}
	50	0.78±0.02^{a}	0.14±0.02^{a}	0.27±0.02^{a}	36.91±2.19^{f}
	100	0.75±0.03^{b}	0.14±0.02^{a}	0.26±0.03^{a}	29.74±2.11^{h}
	200	0.50±0.02^{f}	0.12±0.01^{ab}	0.25±0.02^{a}	28.79±1.96^{h}
Mbiah	0	0.65±0.02^{c}	0.10±0.03^{b}	0.27±0.02^{a}	64.89±1.55^{a}
	50	0.68±0.02^{b}	0.12±0.03^{ab}	0.27±0.02^{a}	58.23±2.67^{b}
	100	0.58±0.02^{e}	0.14±0.02^{a}	0.26±0.03^{a}	51.96±3.02^{c}
	200	0.43±0.01^{g}	0.07±0.03^{c}	0.25±0.01^{a}	44.32±2.23^{d}
PC 79-79	0	0.70±0.04^{c}	0.15±0.05^{a}	0.27±0.01^{a}	46.76±1.70^{d}
	50	0.67±0.05^{c}	0.14±0.04^{a}	0.27±0.03^{a}	41,69±1.02^{e}
	100	0.43±0.03^{g}	0.14±0.02^{a}	0.26±0.04^{a}	35.67±2.09^{f}
	200	0.34±0.03^{h}	0.12±0.01^{ab}	0.24±0.02^{ab}	32.58±1.33^{g}
Vanda	0	0.74±0.01^{b}	015±0.03^{a}	0.26±0.01^{a}	40.98±2.98^{e}
	50	0.63±0.03^{cd}	0.06±0.01^{c}	0.25±0.02^{ab}	32.60±1.88^{g}
	100	0.37±0.01^{h}	0.05±0.01^{c}	0.24±0.02^{ab}	26.47±1.44^{i}
	200	0.26±0.01^{i}	0.03±0.00^{cd}	0.23±0.01^{b}	23.42±1.12^{j}
Cultivar (C)		*	*	ns	*
Salinity level (S)		***	**	*	*
C x S		*	ns	ns	*

Within columns, means followed by the same letter are not significantly different (p< 0.05) by Fisher LSD test; *, **, ***, Significant at p < 0.05, p < 0.01 and p < 0.001 respectively, ns not significant.

NaCl), after four weeks of salt treatment, TLA was strongly reduced in cv. Vanda compared to control plants than those of cvs. Fleur 11, Mbiah and Pc 79-79. SD of all cultivars was not affected by salinity levels except for Pc 79-79 and Vanda at high salinity level (200 mM) after four weeks of salt treatment (Table 1). In general, plant growth was influenced by NaCl treatment except for SD and the magnitude of responses varied according to cultivars differing in salt-tolerance (Table 1).

Biochemical characteristics

Proline (PRO) content in leaf of control and NaCl stressed plants of all peanut cultivars were found at 35 DAS and results are presented in Figure 1a. The PRO content was significantly increased in the stressed plants compared to control plants of all cultivars at all salinity levels but differences in PRO accumulation have been noticed during plant growth between peanut cultivars. The highest increased was observed in cv. Fleur 11 while the lowest was found in cv. Vanda; cv. Mbiah maintained higher increase of PRO content than the cv. PC 79-79.

Glycine betaine (GB) content was determined in the absence (non-saline control) as well as in presence of NaCl in leaves of all the peanut cultivars after 4 weeks of salinity treatment (Figure 1b). GB content significantly ($p<0.05$) increased in presence of NaCl in all the cultivars compared to control plants. This increase was more pronounced in cv. Fleur 11 than others, while cv. Vanda showed the lowest increase.

NaCl salinity induced total phenolic (TP) accumulation in leaves in all cultivars compared to control plants (Figure 1c) with higher values increasing salt concentrations. TP increased significantly ($p < 0.05$) at higher salt level (200 mM NaCl) in cv. Vanda than others, while cv. Fleur 11 showed the lowest increase. A rise in malondialdehyde (MDA) content, an indicator of membrane damage was observed under NaCl salinity. MDA levels in the leaves increased with increasing salinity concentrations in cv. Vanda (Figure 1d). Results showed that MDA of the cvs. Mbiah and PC 79-79 increased at 50 mM also, but decreased at 100 and 200 mM NaCl. MDA levels in the leaves showed no change in cv. Fleur 11 at 50 and 100 mM NaCl but decreased at 200 mM NaCl.

Changes in the chlorophyll content

Leaf total chlorophyll content (CHL) was substantially increased in cvs. Fleur 11 and PC 79-79 with increasing

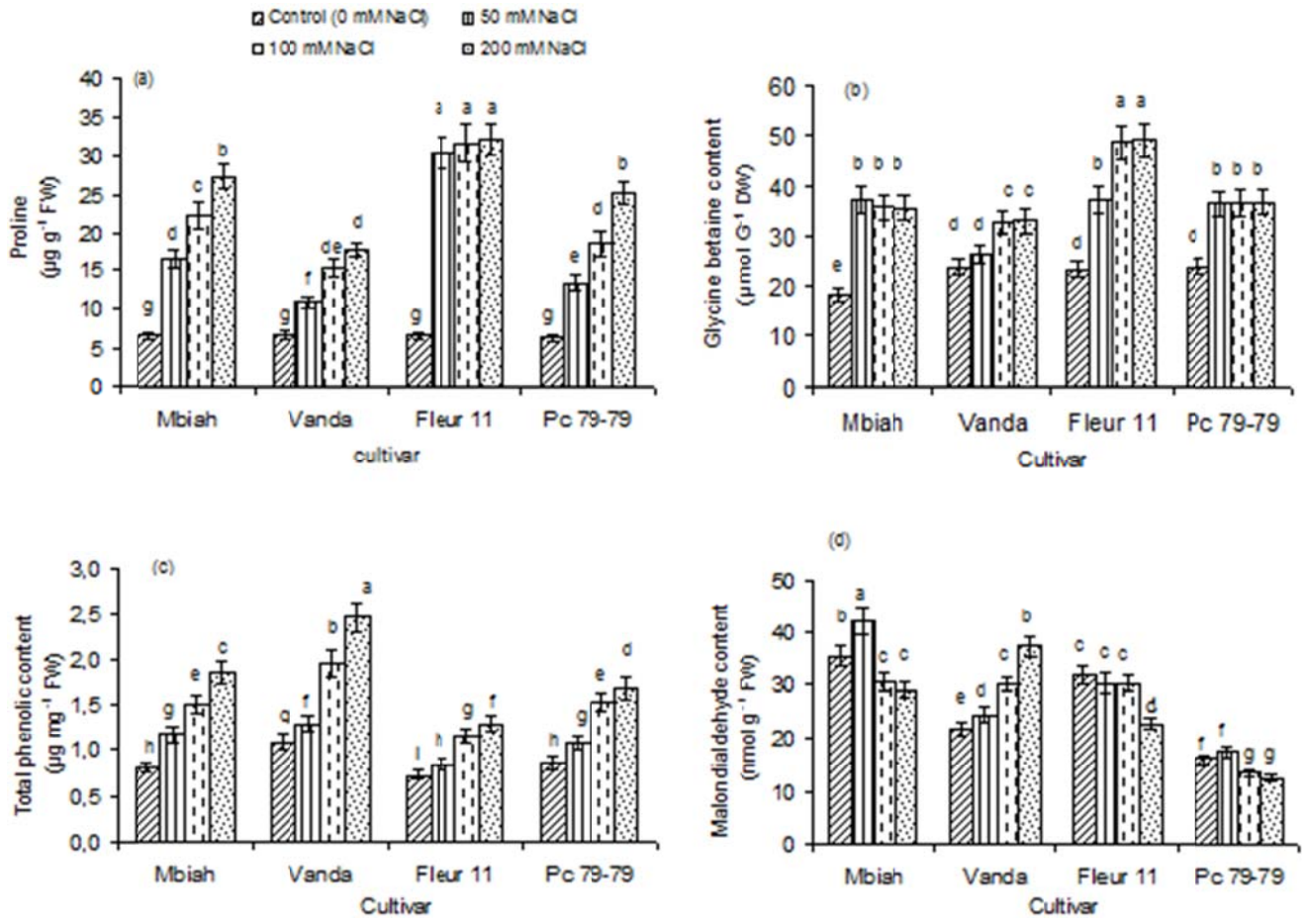

Figure 1. (a) Proline, (b) Glycine betaine, (c) Total phenol and (d) Malondialdehyde contents in leaves of peanut cultivars in response to NaCl concentration levels. Data are mean ± standard error (n = 5). Means followed by the same letter are not significantly different ($p < 0.05$) as determined by Fisher LSD test. Bars indicate standard error.

NaCl concentrations (Figure 2). Salt stress caused gradual reduction in CHL content of cv. Vanda but showed no change and decreased in cv. Mbiah at 50 and 100 mM NaCl, respectively, compared to control plants. Under stressed conditions, CHL content in cv. Vanda was lower than that in other cultivars.

DISCUSSION

Plant growth

Depletion in plant growth (RDW and SDW) under saline stress in cv. Vanda than cvs. Fleur 11, Mbiah and PC 79-79 after 4 weeks of salt treatment (Table 1) is attributed to decreased water uptake followed by limited hydrolysis of food reserves from storage tissue, as well as due to impaired translocation of food reserves from storage tissue to the developing embryo axis (Meneguzzo et al., 1999). Numerous studies have reported the reduction of RDW and SDW stimulated by salinity (Taffouo et al., 2009; Zahra et al., 2012; Navarro et al., 2014). The reduction in growth parameters is a consequence of several physiological responses including modification of ion balance, mineral nutrition, stomatal behaviour and photosynthetic efficiency also (Rajest et al., 1998; Dadkhah, 2011). This is consistent with the reports that NaCl reduces the ability of the plant to take up water, and this leads to slow growth and then, when excessive amounts of salt entering the transpiration stream will eventually injure cells in the transpiring leaves and this may further reduce growth (Munns et al., 2006). In the present study, the growth inhibition effect of salt in growth parameters studied was significantly noted at 50 mM NaCl in cv. Vanda than others, while the growth of cv. Fleur 11 was significantly ($p < 0.05$) affected only at 200 mM NaCl. These results demonstrate that cv. Vanda, in common with certain other bambara groundnut cultivars (black seed coat and light red seed coat), is highly sensitive to salt with severe effects at 50 mM NaCl (Levitt, 1980). Under salt stress cv. Fleur 11 was observed to have relatively higher tolerance on average

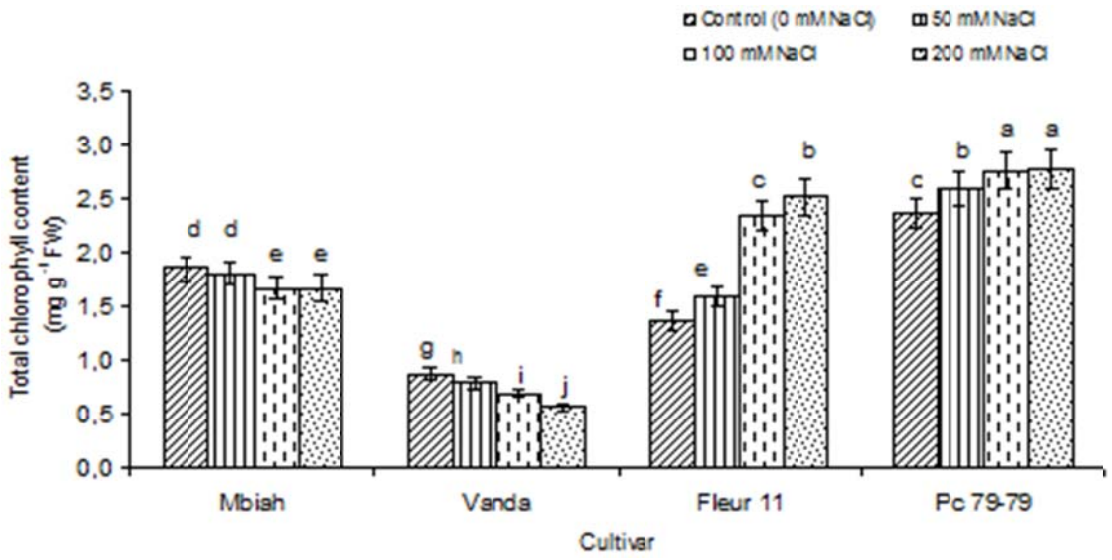

Figure 2. Total chlorophyll content in leaves of peanut cultivars in response to NaCl concentration levels. Data are mean ± standard error (n =5). Means followed by the same letter are not significantly different ($p < 0.05$) as determined by Fisher LSD test. Bars indicate standard error.

of all growth parameters than others. Similar observations for plant growth were reported in *Ceriops roxburghiana* (Rajest et al., 1998) and *Vigna subterranea* cv. white seed coat (Taffouo et al., 2010a) described as salt-tolerant plant species. In the first phase of a biphasic model of growth response to salinity, the vegetative growth is reduced by a decrease in a soil water potential due to water stress effect and may be regulated by inhibitory signals from the roots (Munns et al., 1995).

Biochemical characteristics

The result of the present study shows that the proline (PRO) content was significantly ($p < 0.05$) increased in the stressed plants compared to control plants of all cultivars at all salinity levels but differences in PRO accumulation have been noticed during plant growth between peanut cultivars (Figure 1a). The highest increase, which was observed at 200 mM NaCl in cv. Fleur 11, is consistent with numerous studies which found accumulation of PRO in salt tolerant plants exposed to salt stress (Sivaramakrishnan et al., 1988; Meloni et al., 2004). A positive correlation between magnitude of PRO accumulation and salt tolerance has been suggested as an index to determine salt tolerance potentials between cultivars (Ramanjulu and Sudhakar, 2001; Giridara Kumar et al., 2003; Grigore et al., 2011). By contrast, it has been also reported that the salt sensitive cultivars accumulated significantly higher levels of PRO compared to tolerant ones (Lutts et al., 1999; Vaidyanathan et al., 2003). In this study, we report a significant difference in PRO accumulation between salt-tolerant cv. Fleur 11 and salt-sensitive cv. Vanda during plant growth. The PRO increases with increasing salinity are an adaptative change in metabolism pattern (Mudgal et al., 2010). Previously, it has been reported that Na^+ and Cl^- are efficient osmolytes for osmotic adjustment and sequestered in the vacuole of a cell in salt-tolerant species, the osmotic balance of the cytoplasm is ensured by an active synthesis of the organic and soluble compounds (Le Rudulier, 2005; Grigore et al., 2011). This synthesis of the PRO is a mechanism of stress resistance (Greenway and Munns, 1980), because its accumulation contributes to the acquisition of resistance by maintaining the cell turgor in many species which is responsible for the osmotic adjustment in susceptible plants under stress (Meloni et al., 2004). The species growing in arid or semi-arid lands, may exhibit more tolerance to salt stress, because of the accumulation of osmo-protectants in their tissues (Munns, 2002).

In the present investigation, it has been noticed that the highest accumulation of glycine betaine (GB) was found in salt-tolerant cv. Fleur 11 than others, while the lowest was observed in salt-sensitive cv. Vanda (Figure 1b). GB is one of many nitrogenous osmolytes accumulated under osmotic stress conditions in salt-tolerant plants (Girija et al., 2002). Numerous studies reported that the accumulation of GB was found to be high in salt-tolerant cultivar (Rhodes et al., 1987; Giridara Kumar et al., 2003), while salt-sensitive cultivar exhibited a low magnitude of GB accumulation (Hitz and Hanson, 1980). GB preserves thylakoid and plasma membrane integrity of *Zea mays* after exposure to saline solutions (Rhodes et al., 1987). Thus, the accumulation of osmo-protectants in tissues of plants growing in arid or semi-arid lands, may

exhibit more tolerance to salt stress (Munns, 2002). Salt stress can lead to stomatal closure, which reduces CO_2 availability in the leaves and inhibits carbon fixation, exposing chloroplasts to excessive excitation energy, which in turn could increase the generation of Reactive Oxygen Species (ROS) and induce oxidative stress (Parvaiz and Satyawati, 2008). The osmo-protectants that accumulate most commonly are PRO and GB, although other molecules can accumulate to high concentrations in certain species (Girija et al., 2002).

NaCl salinity induced total phenolic content (TP) accumulation in leaves in all cultivars compared to control plants (Figure 1c). TP accumulation in leaves under salt stress could be a cellular adaptative mechanism for scavenging oxygen free radicals during stress conditions (Mohamed and Aly, 2008). Numerous studies have reported that TP production is stimulated by NaCl (Hanen et al., 2008; Zahra et al., 2012). Antioxidants prevent lipid oxidation and can act in different ways, including decreasing oxygen concentrations, scavenging initiating radicals, and binding metal ions to prevent initiating radical formation (Dorman et al., 2003).

In this study, we present the evidence that salt stress is able to produce excessive quantity of malondialdehyde (MDA) in the leaf of cv. Vanda plants than others cultivars (Figure 1d). Increase in the level of MDA, produced during peroxidation of membrane lipids, is often used as an indicator of oxidative damage (Azad et al., 2012). Free radical-induced peroxidation of lipid membranes is a reflection of stress-induced damage at the cellular level (Nagest and Devaraj, 2008). As a sequel, lipid peroxidation products such as MDA will accumulate and severe membrane damage will inevitably occur (Azad et al., 2012). Previously, it has been reported that there was an improvement in MDA content in leaves of salt-sensitive *Vigna radiata* and *Plantago media*, but decreased at 200 mM in salt-tolerant *Plantago maritime* under salinity (Sekmen et al., 2007; Saha et al., 2010). MDA level and cell membrane damage increased under salt stress condition in salt-sensitive cv. Vanda because of elevating of ROS production (Azad et al., 2012). MDA concentration decreased at 200 mM in salt-tolerantcv. Fleur 11. These results suggested that the cv. Fleur 11 showed a better protection mechanism against oxidative damage caused by salt stress by its higher induced activities of antioxidant enzymes than the salt-sensitive cv. Vanda (Sekmen et al., 2007).

Changes in the chlorophyll content

Salinity decreased the chlorophyll (CHL) content in salt-sensitive cv. Vanda (Figure 2) leaves, which is in accordance with Taffouo et al. (2010a; b), and Giannakoula et al. (2012). This effect of salt was attributed to a salt-induced weakening of protein-pigment-lipid complex (Strogonov et al., 1970) or increased chlorophyllase enzyme activity (Stivesev et al., 1973). By contrast, the CHL content was substantially increased in Fleur 11 and PC 79-79 with increasing NaCl concentrations. Similar observations have been reported by Robinson et al. (1983) and Morales et al. (1992) in salt-stressed spinach and barley, respectively. Moreover, the cultivars Fleur 11 and PC 79-79 screened for their salt tolerance in this study were grown under natural field conditions of Bambey (Senegalese) and were probably exposed to different environmental constraints. The interaction between these stresses could be taken as a part of the adaptive mechanisms of plants to survive under saline conditions and high temperatures (Giannakoula et al., 2012).

Conclusion

In general, the results of this study showed that salt stress caused a serious decrease in plant growth by means of reduced RDW, SDW and TLA due to ionic toxicity and decrease osmotic potential in all peanut cultivars but the magnitude of responses varied according to cultivars. Higher osmolyte accumulation, especially proline and glycine betaine was found in the salt tolerant cultivar (Fleur 11) whereas the lower in salt-sensitive one (Vanda). The salt stress was able to excessively generate MDA in the leaves of Vanda plants, that is, an indicator of oxidative damage. MDA levels increased under salt stress in the leaves of cv. Vanda but decreased in cvs Mbiah, PC 79-79 and Fleur 11 at 100 and 200 mM, respectively. These results suggest that Fleur 11 exhibits a better protection mechanism against oxidative damage caused by salt stress due to its higher induced activities of antioxidant enzymes and osmolyte accumulation than Mbiah, PC 79-79 and Vanda. Fleur 11 can tolerate moderate saline conditions owing to better antioxidant system. It seems that the evaluation of osmolyte accumulation and antioxidant system is useful for assessment of salinity tolerance of peanut cultivars.

Conflict of Interests

The author(s) have not declared any conflict of interests.

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