

Changes in plant growth, leaf relative water content and physiological traits in response to salt stress in peanut (*Arachis hypogaea* L.) varieties

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Abstract

Salinity is the main environmental factor accountable for decreasing crop productivity worldwide. The effects of NaCl salinity on plant growth (leaf relative water content (RWC), leaf dry weight (LDW), shoot length (SL), number of leaves (NL), number of branches (NB) and total leaf area (TLA) and physiological characteristics (stomatal conductance (g), transpiration rate (TR), net photosynthetic (P_n), yield of photosystem II (ΦP_sII) and the intercellular CO₂ concentration (CO_{2int}) in peanut (*Arachis hypogaea* L.) varieties ('Vanda', 'P244601' and 'P1184948', widely used in Cameroon, Tanzania and Ghana, respectively, were investigated under hydroponic condition. Plants were subjected to four levels of NaCl (0, 40, 80 and 120 mM) at early seedling growth stage of plant development. Application of NaCl treatment led to a significant decrease in LDW, SL, NL, TLA, P_n, g, TR and CO_{2int} concentration of 'Vanda' and 'P244601' compared to untreated plants while the plant growth inhibition was notably noted at 120 mM NaCl in 'P1184948' for LDW, SL and NB. The highest depressive effect was detected in g of salt-sensitive 'Vanda' while the lowest were recorded in g of salt-tolerant 'P1184948' at high salinity level. Enhanced NaCl concentrations led to a significant increase in ΦP_sII of 'P1184948' compared to 'Vanda', 'P244601' and untreated plants. Leaf CHL content was significantly increased in moderately-tolerant 'P244601' and salt-tolerant 'P1184948' at 80 mM NaCl compared to salt sensitive 'Vanda' and untreated plants. The depressive effect of salt on RWC was recorded at 120 mM NaCl in peanut leaves of all varieties. Under salt stress 'P1184948' was observed to have relatively higher tolerance on average of all growth and physiological traits than 'Vanda' and P244601' suggesting that it could be grown in salt-affected soils.

Keywords: hydroponic condition; plant growth; physiological traits; peanut; salinity

Abbreviations: calcium-Ca; chloride-Cl; chlorophyll-CHL; days after sowing-DAS; intercellular CO₂ concentration-CO_{2int}; leaf dry weight-LDW; magnesium-Mg; net photosynthetic-P_n; nitrogen-N; number of leaves-NL; number of branches-NB; phosphorus-P; potassium-K; relative water content-RWC; shoot length-

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SL; sodium-Na; stomatal conductance-g, total leaf area-TLA; transpiration rate-TR; yield of photosystem II- Φ PsII.

Introduction

Salinity is the main environmental factor accountable for decreasing crop productivity in many areas of the world especially in arid and semi-arid regions (Turan *et al.*, 2007; Mirza *et al.*, 2019). Salinity reduces growth and yield of the non-halophytes plants by decreasing the availability of water to the roots due to the osmotic effect of external salt and by toxic effects of excessive salt accumulation within the plant (Munns *et al.*, 2006; Munns and Gilliham, 2015). The detrimental effects of salt on plants are the consequence of both a water deficit that results from the relatively high solute concentrations in the soil as well as a stress specific to Cl and Na, resulting in a wide variety of physiological and biochemical changes that inhibit plant growth and development and disturb photosynthesis, respiration, protein synthesis and nucleic-acid metabolism (Sairam *et al.*, 2002; Negrão *et al.*, 2017). P_n , TR, g_s and CO_{2int} concentration are all affected strongly by saline conditions (Sobrado, 1999; Turan *et al.*, 2007; Mekhaldi *et al.*, 2008) and the changes in P_n may result from the closure of stomata induced by osmotic stress, or from salt damaging of photosynthetic apparatus or other metabolic processes (Negrão *et al.*, 2017). Φ PsII is also reflect the damages to photosynthetic apparatus under the salt stress (Ashraf and Harris, 2013; Chen *et al.*, 2015).

The responses of plants to high soil salinity and the mechanisms of salt tolerance have been discussed in many works published (Meguekam *et al.*, 2014; Nouck *et al.*, 2016; Taffouo *et al.*, 2017; Hniličková *et al.*, 20017). Under salt stress, plants have evolved complex mechanisms allowing for adaptation to osmotic and ionic stress caused by high salinity. These mechanisms include osmotic adjustment by accumulation of compatible solutes such as soluble sugar, proline, glycine betaine, and soluble protein (Turan *et al.*, 2007; Xu *et al.*, 2009; Meguekam *et al.*, 2014). However, an active antioxidative defense system comprising enzymatic such as peroxidase and superoxide dismutase (Waterman and Mole, 1994; Taffouo *et al.*, 2017) and non-enzymatic antioxidants as well as flavonoids and phenolic compounds reduce the level of oxidative stress in plant cells by scavenging free radicals (Ashraf, 2009; Azooz *et al.*, 2009; Abogadallah *et al.*, 2010). In case of high salinity, oxidative stress occurs due to closure of stomata, interruption of photosynthetic electron transport and disruption of cellular membrane integrity and antioxidative defence systems of plants start work against oxidative damage (Sairam *et al.*, 2002; Mehr *et al.*, 2012). Water deficit causes a leaf turgor decrease, further causing stomata closure and decreases of stomatal conductance; one of the factors limiting net photosynthesis (Chaves *et al.*, 2009). Salt stress reduced the relative water content (RWC) in two wheat cultivars, 'Giza 168' and 'Gimeza 9', the reduction was more pronounced in 'Giza 168' (El-Bassiouny and Bekheta, 2005). RWC was also reduced and water deficit increased in roots and leaves of the seedlings of *Iris lactea* under NaCl stress (Wang *et al.*, 2012). In the salt-tolerant 'Astro', the osmotic potential decreased with increasing NaCl concentrations, while RWC decrease did not take place until 200 mM NaCl (Hniličková *et al.*, 2017).

The significance of photosynthetic characteristics in salt tolerance species is still discussed and varies according to species (Xu *et al.*, 2011; Saravanavel *et al.*, 2012; Hniličková *et al.*, 2017). Some investigators have shown that P_n was hardly reduced by salinity and sometimes even enhanced under low salt concentrations (Downton *et al.*, 1985; Dionisio-Sese and Tobita, 2000). Others have shown a distinctive decrease in P_n in plants exposed to salinity (Yeo *et al.*, 1985; Plaut, 1987). According to Yeo *et al.* (1985) and Galme's *et al.* (2007), salinity did not, initially, reduce P_n in the whole plant but only in the older leaves in which sodium accumulated. In salt-tolerant *Iris lactea*, under salt stress the P_n lowered, g_s decreased and CO_{2int} concentration increased, which belonged to the non-stomatal limitation (Wang *et al.*, 2012). The *Lens calinaris* plants close their stomata which lead to enhanced stomatal resistance (Turan *et al.*, 2007). Stomatal closure is known to be an effective mechanism for economical water utilisation under salt stress and limitation of the harmful salt ions uptake (Hasegawa *et al.*, 2000). The total chlorophyll concentration of *Lens calinaris* leaves were reduced by increasing the level of NaCl applied (Turan *et al.*, 2007). In natural conditions, *Avicennia germinans* tolerates

a range of salinity, from almost freshwater to up three times seawater. However, its P_n is lowered when salt concentration in the soil increases (Sobrado, 1999). In *Eruca sativa*, TR decreased at the concentration at low salinity level whereas g_s and P_n decreased at high salinity level (Hniličková *et al.*, 2017). Ashraf and Harris (2013) state that the mechanism of photosynthesis involves various components, including photosynthetic pigments and photosystems, the electron transport system, and CO_2 reduction pathways. Any damage at any level caused by a stress factor may reduce the overall photosynthetic capacity of a green plant.

Peanut (*Arachis hypogaea* L.) is one of the more important grain legumes in tropical cropping systems in Africa, being important to both small and large commercial producers (Nyabyenda, 2005). 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). Peanut is also useful sources of fat (34-54%) and very important in crop rotation systems as they help in biological nitrogen fixation (Taiz and Zeiger, 2002). It also contains polyphenols, polyunsaturated and monounsaturated fats, phytosterols and dietary fiber in amounts similar to several tree nuts (Musa, 2010). Efforts to enhance crop yield under salt stress have had a limited success because available knowledge of the mechanisms of salt tolerance has not been completed and also not turned into useful selection of species. Screening plant species for salinity tolerance or genetic potential to develop tolerance are promising approaches for developing salt-tolerant varieties for breeding program. Therefore, this study was undertaken to evaluate the effects of salt stress on plant growth, relative water content, chlorophyll content, stomatal conductance, transpiration rate, photosynthetic rate, yield of photosystem II and the intercellular CO_2 concentration of three peanut (*Arachis hypogaea* L.) varieties.

Materials and Methods

Plant materials

Peanut (*Arachis hypogaea* L.) is a legume crop grown mainly for its edible seeds. As a legume, the peanut belongs to the botanical family of Fabaceae. It is widely grown in the tropics and subtropics, being important to both small and large commercial producers. Peanut plants grow best in light, sandy loam soil with a pH of 5.9-7. Their capacity to fix nitrogen means that, providing they nodulate properly, peanuts benefit little or not at all from nitrogen-containing fertilizer and they improve soil fertility. Also, the yield of the peanut crop itself is increased in rotations, through reduced diseases, pests and weeds. To develop well, peanut plants need warm weather throughout the growing season. They can be grown with as little as 350 mm of water, but for best yields need at least 500 mm. Peanut is also useful sources of fat (34-54%) and very important in crop rotation systems as they help in biological nitrogen fixation (Taiz and Zeiger, 2002). It also contains polyphenols, polyunsaturated and monounsaturated fats, phytosterols and dietary fiber in amounts similar to several tree nuts (Musa, 2010). Peanut is also rich in essential nutrients such as vitamins B and E. They also contain about 25% protein, a higher proportion than in many tree nuts. Seeds of three peanut varieties ('P1184948', 'P244601' and 'Vanda'), provided by the breeding program of the Agronomic Institute for Research and Development of Ghana, Tanzania and Cameroon, respectively, were used in the study.

Plant growth conditions and salt treatments

The experiment was conducted in the greenhouse and growth chamber of Leibniz Universität Hannover, Germany, from October to November 2017. Seeds of peanut plants were surface sterilized with 70% (v/v) ethanol solution for 15 min, then rinsed four times with deionized water. Seeds of each variety were sown in rock-wool cubes (36 mm * 36 mm * 40 mm) in the greenhouse at 26 °C and 55% of the relative air humidity averaged with deionized water. Eight days after sowing (DAS), when primordial leaves were fully established, seedlings were transplanted into 30-L plastic pots filled with 26 L of the nutrient solution in the growth chamber. The pots were arranged in a complete randomized design upon styrofoam floating with six

plants per pot and four replicates per treatment. All plants were fertilized with a modified nutrient solution containing (in mg L⁻¹): 57.7 mg NO₃⁻, 0.8 mg NH₄⁺, 58.4 mg N, 115.6 mg K, 86.2 mg Ca, 12.7 mg Mg and 18.1 mg P (Chen *et al.*, 2015). The pH value of the nutrient solution was included 5.9-6.2. Throughout the growth period, average day/night temperatures in the growth chamber were 29 °C/23 °C, the relative air humidity averaged 55 %, photosynthetic active radiation 200 μmol m⁻²s⁻¹ and day/dark 12 h. Thirteen days after seedlings tranfert, the growth chamber experiments (for 3 weeks) were designed to assess the physiological responses of peanut varieties to salt stress, each variety was subjected to 0, 40, 80 and 120 mM NaCl in nutrient solution.

Plant growth parameters

Plants were harvested 42 DAS. NB, NL and TLA were recorded. Leaves, stems, and roots were separately dried at 85 °C for 48 h, and their dry weights were determined. The RWC ((leaf FW-leaf DW) *100/leaf FW) and TLA (2/3* length*width*0.80*total no. of leaves*0.662) were calculated using the methodology described by Kumar *et al.* (2002). Leaf CHL was determined by chlorophyll meter (Opti-Sciences) CCM-200 PLUS device.

Photosynthetic characteristics

TR, g_s, Pn, ΦP_sII and CO_{2int} concentrations were determined using portable photosynthesis system Li6800 (LiCor Inc, NE, USA) equipped with multiphase flash fluorometer. The light condition was kept to 200 μmol m⁻²s⁻¹ and before each measurement, the leaf was left around 5 to 15 min the leaf get acclimatized to the growth chamber conditions. The leaf temperature was maintained close to the ambient temperature ranking from 28 °C until the end of the experiment (for 3-week treatments).

Statistical analysis

The experiment was conducted as a factorial completely randomized design with four NaCl treatments and three varieties in four replications. All data were statistically analysed using the software XLSAT (STATCON, NY, United States) and first subjected to analyses of variance ANOVA. Statistical differences between treatment means were established using the Fisher LSD test at p < 0.05.

Results and Discussion

Plant growth

Peanut growth was estimated by measuring LDW, SL, NB, NL and TLA of three varieties plants under four NaCl concentrations at vegetative stage (42 DAS). There were statistically significant differences among the varieties for all salt concentrations and plant growth parameters. Application of NaCl treatment led to a significant (P < 0.05) decrease in LDW, SL, NL and TLA of 'Vanda' and 'P244601' compared to untreated plants (Table 1). The effect of salt on plant growth inhibition was notably noted at 120 mM NaCl in 'P1184948' for LDW, SL and NB (Table 1). The main effect of NaCl on plant growth parameters was that plants of 'Vanda' and 'P244601' under 40 mM NaCl remained almost unaffected for LDW, SL, NB and NL and presented significantly increased values for LDW as compared to those of untreated plants (Table 1). A significant two-way interaction between variety and salt treatment was significant (P < 0.05) for LDW and TLA (Table 1). The responses of plants to high soil salinity and the mechanisms of salt tolerance have been discussed in many works published (Munns, 2002; Meguekam *et al.*, 2014; Nouck *et al.*, 2016; Taffouo *et al.*, 2017; Hniličková *et al.*, 2017). In this study, the detrimental effects of salt on 'Vanda' and 'P244601' plant growth parameter above 40 mM NaCl are the consequence of both a water deficit that results from the relatively high solute concentrations in the soil as well as a stress specific to Cl and Na, resulting in a wide variety of physiological and biochemical changes that inhibit plant growth and development and disturb photosynthesis, respiration, protein synthesis and nucleic-acid metabolism (Sairam *et al.*, 2002; Mekhadi *et al.*,

2008; Negrão *et al.*, 2017). 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 this study, the growth inhibition effect of salt in growth parameters studied was significantly ($p < 0.05$) noted above 40 mM NaCl in 'Vanda' while the growth of 'P1184948' was significantly ($p < 0.05$) affected only at 120 mM NaCl for LDW, SL and NB (Table 1). These results demonstrate that 'Vanda', in common with certain other plant leguminous (e.g. beans), is highly sensitive to salt with severe effects at 50 mM NaCl (Levitt, 1980; Taffouo *et al.*, 2009). Under salt stress 'P1184948' was observed to have relatively higher tolerance on average of all growth parameters than others (Table 1). Similar observations for plant growth were reported in 'White seed coat' (Taffouo *et al.*, 2010), 'Fleur 11" (Meguekam *et al.*, 2014) and 'Granada' (Taffouo *et al.*, 2017) and described as salt-tolerant varieties. In case of high salinity, oxidative stress occurs due to closure of stomata, interruption of photosynthetic electron transport and disruption of cellular membrane integrity and antioxidative defence systems of plants start work against oxidative damage (Sairam *et al.*, 2002; Mehr *et al.*, 2012).

Table 1. Effect of salt stress on plant growth in peanut varieties at the vegetative stage (42 DAS)

Variety	Treatment (mM NaCl)	LDW (g plant ⁻¹)	SL (cm)	NL	NB	TLA (cm ² plant ⁻¹)
'Vanda'	0	3.20±0.00b	14.60±0.24a	5.67±0.10b	1.58±0.13b	310.90±0.23a
	40	3.53±1.02a	14.48±0.01a	5.67±0.10b	1.58±0.10b	260.38±0.03b
	80	1.83±0.55d	14.25±0.06b	5.44±0.00c	1.58±0.13b	226.88±0.10f
	120	1.20±0.21f	13.84±0.02b	5.00±0.19e	0.83±0.07f	212.03±0.16g
'P1 244601'	0	2.09±0.30c	14.37±0.33b	5.11±0.13d	1.25±0.11d	199.73±0.18i
	40	2.14±0.25c	14.03±0.21b	5.00±0.08e	1.33±0.16c	164.85±0.01j
	80	1.79±0.41d	13.83±0.13c	4.89±0.03f	1.33±0.16c	158.69±0.05j
	120	1.54±0.28e	11.70±0.67de	4.33±0.23g	0.33±0.04g	146.78±0.12k
'P1 184948'	0	3.14±0.20b	12.23±0.18d	6.11±0.19a	1.08±0.01e	202.76±0.13d
	40	3.17±0.66b	11.90±0.04d	6.11±0.12a	1.75±0.32a	236.04±0.04e
	80	2.27±0.63c	11.84±0.01d	6.11±0.19a	1.17±0.06d	243.93±0.08c
	120	1.61±0.76e	11.25±0.24e	6.11±0.11a	0.25±0.03h	227.34±0.05h
Two-way ANOVA results						
Variety (V)		*	*	*	*	*
Salt treatment (S)		**	*	*	*	**
Interaction V X S		*	NS	NS	NS	*

Values shown are means ($n = 4$) ± SD, within columns, means followed by different letter are significantly different ($p < 0.05$). **, * significant at 1 and 5% probability levels, respectively, NS not significant. Abbreviations: LDW, Leaf dry weight; SL, Shoot length; NB, number of branches; TLA, Total leaf area.

Leaf relative water content

In this study, the depressive effect of salt on RWC was recorded at 120 mM NaCl in peanut leaves of all varieties (Figure 1). Similar results were reported by El-Bassiouny and Bekheta (2005), Wang *et al.* (2012) and Taffouo *et al.* (2017) with wheat, pepper and *Iris lacteal* plants, respectively. Water deficit causes a leaf turgor decrease, further causing stomata closure and decreases of stomatal conductance; one of the factors limiting photosynthesis rate (Chaves *et al.*, 2009). RWC was also reduced and water deficit increased in roots and leaves of the seedlings of *Iris lacteal* under NaCl stress (Wang *et al.*, 2012). In the salt-tolerant 'Astro', the osmotic potential decreased with increasing NaCl concentrations, while RWC decrease did not take place until 200 mM NaCl (Hniličková *et al.*, 2017). According to Munns (2002), salinity reduces the ability of plants to take up water and this quickly causes reduction in growth rate, along with a suite of metabolic changes. There are two phases growth response to salinity (Munns, 1993). The first phase of growth reduction is quickly apparent, and is due to the salt outside the roots. It is essentially a water stress or osmotic phase for which there is

surprisingly little genotypic variation. The growth reduction is presumably regulated by hormonal signals coming from the roots. Then there is a second phase of growth reduction which take time to develop, and results from internal injury. It is due to salts accumulating in transpiring leaves to excessive levels, exceeding the ability of the cells to compartmentalize salts in the vacuole. This will inhibit growth of the younger leaves by reducing the supply of carbohydrates to the growing cells.

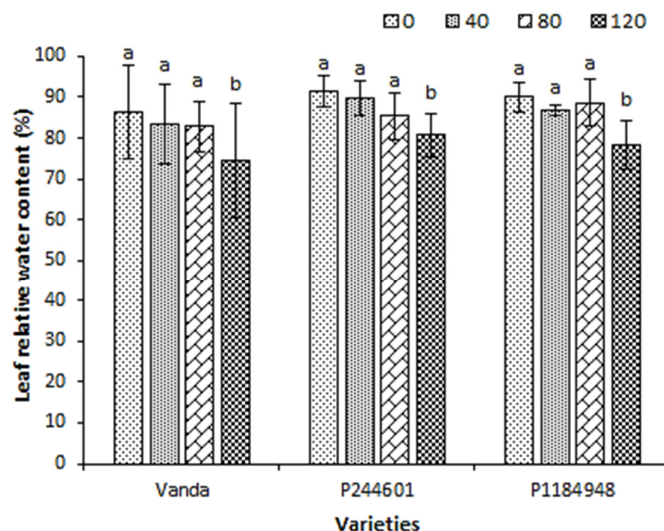


Figure 1. Effect of salt stress on leaf relative water content in peanut varieties at vegetative stage (42 DAS). Bars are means ($n=4$) \pm SD. Means followed by different letter are significantly different ($p < 0.05$)

Chlorophyll concentrations

In this study, salinity increased the leaf CHL content in moderately-tolerant 'P244601' and salt-tolerant 'P1184948' at 80 mM NaCl compared to salt sensitive 'Vanda' and untreated plants (Figure 2). The depressive effect of salt on leaf CHL content in peanut plants was recorded at 120 mM NaCl (Figure 2). This effect of salt was attributed to a salt-induced weakening of protein-pigment-lipid complex (Strogonov *et al.*, 1970) or increased chlorophyllase enzyme activities (Stivsev *et al.*, 1973). The decreased in chlorophyll content under salt stress is a commonly reported phenomenon and in various studies, because of its adverse effects on membrane stability (Hajer *et al.*, 2006; Meguekam *et al.*, 2014). Similar results were reported by El-Iklil *et al.* (2002), Turan *et al.* (2007) and Taffouo *et al.* (2017) with tomato, lentil and pepper plants, respectively.

Photosynthetic characteristics

The significance of photosynthetic characteristics (P_n , g , TR, ΦP_s II and CO_{2int} concentration) in salt tolerance species is still discussed and varies according to species. In the present study, the presence of NaCl resulted in a significant decrease in P_n in 'Vanda' and 'P244601' compared to untreated plants (Figure 3A and B). On the contrary, a significant decrease in P_n was found only after one week in salt-tolerant 'P1184948' (Figure 3C). Salt induced reduction of P_n has been previously reported in a number of species (Sobrado, 1999; Lawlor and Cornic, 2002; Hniličková, 2017). According to Munns *et al.* (2006) and Chaves *et al.* (2009), photosynthesis is the primary processes to be affected by salinity and it also varies according duration of the stress as well as with the leaf age (Galme's *et al.*, 2007). This decrease was related to decline in leaf CHL content in all varieties at 120 mM NaCl (Figure 2). CHL, one of the most important pigments in photosynthesis, is not only the main pigments of energy absorption and transfer, but also essential for electron transfer (Hajer *et al.*, 2006). In this experiment, leaves from untreated plants grown without NaCl addition did not show any

symptoms of necrosis, chlorosis or leaf tissue damage. This is contrary to results of salt-sensitive ‘Vanda’ that show anomalous leaf development and poor growth.

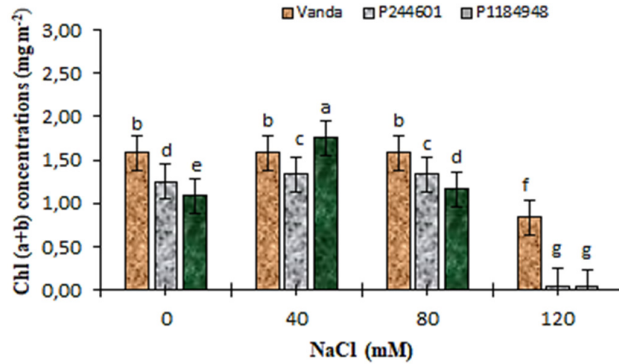


Figure 2. Effect of salt stress on chlorophyll concentrations in peanut varieties at vegetative stage (42 DAS). Bars are means (n=4) ± SD. Means followed by different letter are significantly different (p < 0.05)

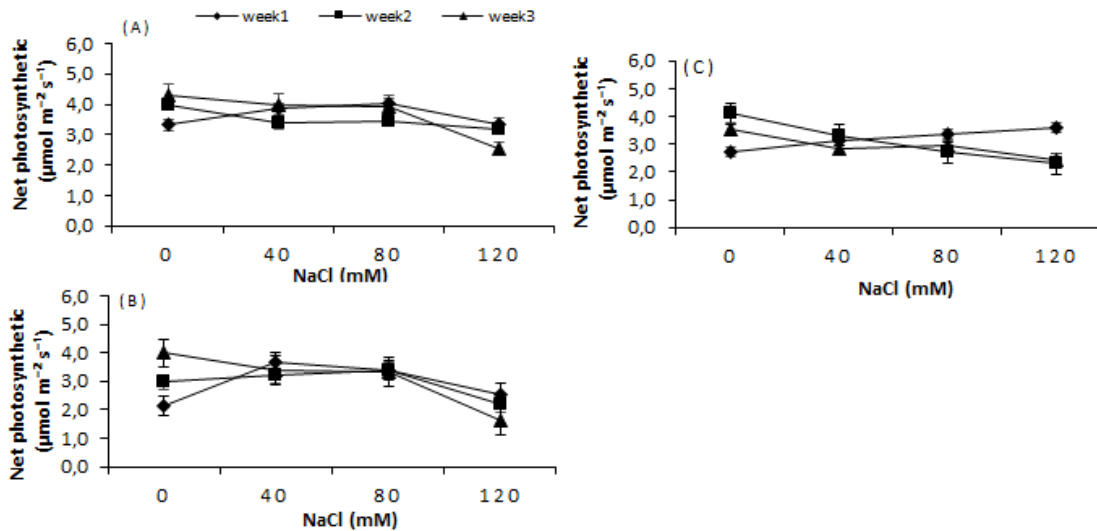


Figure 3. Effect of salt stress on net photosynthetic of leaves in peanut varieties at the vegetative stage (42 DAS)

‘Vanda’ (A), ‘P244601’ (B) and ‘P1184948’ (C). Bars are means (n=4) ± SD

The changes in P_n of ‘Vanda’ and ‘P244601’ may result from the closure of stomata induced by osmotic stress, or from salt damaging of photosynthetic apparatus or other metabolic processes (Sobrado, 1999). In the present study, different NaCl levels supply had significant effects on g_s in all peanut varieties (Figure 4A, C and D). Application of NaCl treatment led to a significant ($p < 0.05$) decrease in g_s of all varieties at 40 mM NaCl compared to untreated plants. The highest depressive effect was detected in g_s of ‘Vanda’ while the lowest were recorded in g_s of salt tolerant ‘P1184948’ at high salinity level (Figure 4A, B and C). Similar observations were reported by Sobrado (1999) and Acosta-Motos *et al.* (2015) with *Avicennia germinans* and *Myrtus communis*. It has been reported that salinity affects plant physiology through changes of water and ionic status in the cells because of ionic imbalance due to excessive accumulation of Na and Cl and reduced uptake of other mineral nutrients, such as K, Ca and Mg (Hasegawa *et al.*, 2000). Water deficit causes a leaf turgor decrease, further causing stomata closure and decreases of g_s ; one of the factors limiting P_n (Chaves *et al.*, 2009). According to Sairam *et al.* (2002) and Mehr *et al.* (2012), in case of high salinity, oxidative stress occurs due to

closure of stomata, interruption of photosynthetic electron transport and disruption of cellular membrane integrity and antioxidative defense systems of plants start work against oxidative damage (Sairam *et al.*, 2002; Mehr *et al.*, 2012).

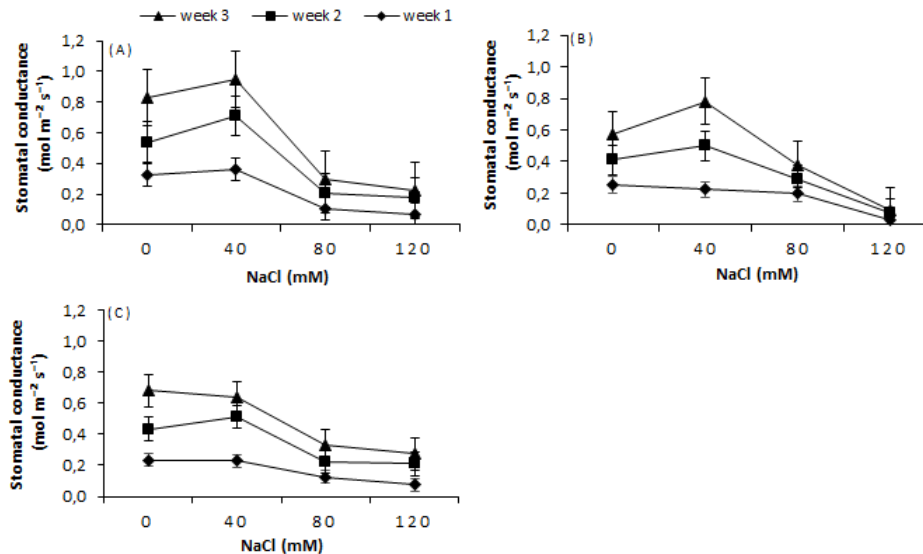


Figure 4. Effect of salt stress on stomatal conductance in peanut varieties at the vegetative stage (42 DAS) 'Vanda' (A), 'P244601' (B) and 'P1184948' (C). Bars are means (n=4) \pm SD

Means of the TR in leaves of the peanut varieties (42 DAS) are depicted in Figure 5A, B and C. TR in leaves of all varieties of peanut was affected by different NaCl levels supply (Figure 5A, B and C). According to Asch (1996), environmental factors had some effects on transpiration among rice varieties subjected to salt stress. The main effect of NaCl on TR under salt stress showed significant ($P < 0.05$) decreases as compared to control plants (Figure 5A, B and C.) This is consistent with the reports that NaCl reduces the ability of the plant to take up water, and this leads to slow growth; then when excessive amounts of salt entering the transpiration stream will eventually injure cells in the transpiring leaf and this may further reduce growth (Munns, 2002). Water deficit causes a leaf turgor decrease, further causing stomata closure and decreases g_s ; one of the factors limiting P_n (Chaves *et al.*, 2009). Water transpiration drives the water uptake by the roots and transport until the leaves. The amount of water lost is regulate by opening and closing stomata plants, with sacrificing CO_2 uptake, when the environmental conditions are unfavourable (Hniličková *et al.*, 2017).

Enhanced NaCl concentration led to a significant ($P < 0.05$) increase in $\Phi PSII$ of salt-tolerant 'P1184948' compared to 'Vanda', 'P244601' and untreated plants (Figure 6A, B and D). These results are consistent with the results obtained by Lu and Vonshak (2002) in halophyte *Suaeda salsa*. According to Ashraf and Harris (2013); Chen *et al.* (2015), under the salt stress the P_n , g_s and $\Phi PSII$ of tolerant species were significantly increased compared with the untreated plants. Increased of NaCl concentration could contribute to create a water potential in the cells which would rather guarantee water availability for physiological function for the plant (Taffouo *et al.*, 2017). In this study, the slightly decline in $\Phi PSII$ in salt-sensitive 'Vanda' and moderately-tolerant 'P244601' during their exposure to salt stress (Figure 6A, B and D) could be mainly be due to the synthesis of chlorophyllase which destroys the produced chlorophyll (Meguekam *et al.*, 2014) or to the inhibition of the nitrogen absorption which is an essential in the chlorophyll atom (Paul and Lade, 2014) and to orientation of the photosynthetic activity towards the production of resistance proteins; knowing that, in both crop plants, chemical energy expended in a number of metabolic processes derived from the photosynthetic process (Pan *et al.*, 2012; Ashraf *et al.*, 2013); lipid metabolism (Paul and Lade, 2014) or maintain a relatively high Na concentration in their shoots to developed mechanism for salinity tolerance

(Munns *et al.*, 2016; Zaki and Yokoi, 2016). Enhanced salinity provokes changes in the photosynthetic apparatus, by affecting both its structure and function (Lu and Vonshak, 2002).

In the present study, CO_{2int} concentrations of leaves were significantly ($P < 0.05$) reduced with increasing salinity in all varieties (Figure 7A, B and C). These results corroborate the findings of Sobrado (1999) with *Avicennia germinans*, Turan *et al.* (2007) with *Lens calinaris* but they contradict those of Asch *et al.* (2000) with *Oryza sativa* plants who reported that salinity increases CO_2 assimilation but reduces growth in field-grown. The highest decrease of CO_{2int} concentrations was detected in salt-sensitive ‘Vanda’ while the lowest was recorded in salt-tolerant ‘P1184948’ at high salinity level (120 mM NaCl) compared to moderately-tolerant ‘P244601’ and untreated plants (Figure 7A, B and C). The decreased CO_{2int} concentration availability observed in leaves of these varieties, mainly in ‘Vanda’ can be caused by the diffusion limitations through the stomata and the mesophyll (Flexas *et al.*, 2007; Bacha *et al.*, 2017) or the alterations of photosynthetic metabolism (Law and Cornic, 2002). It is stated that high level of Na inhibits Ca and K absorption, which results in a N/K antagonism (Taffouo *et al.*, 2009) and P_n is affected strongly by NaCl saline conditions, which is related directly to the closure of stomata as well as to low CO_{2int} levels (Turan *et al.*, 2007).

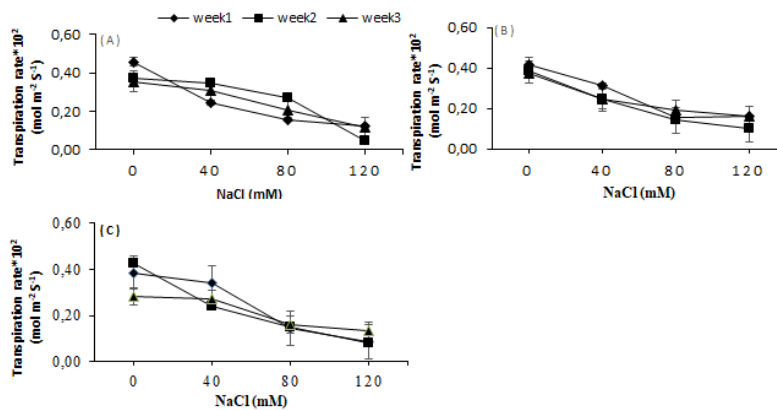
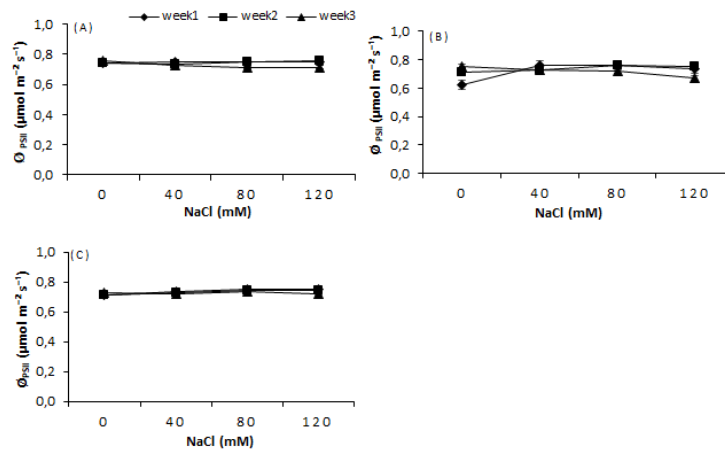


Figure 5. Effect of salt stress on transpiration rate of leaves in peanut varieties at the vegetative stage (42 DAS) ‘Vanda’ (A), ‘P244601’ (B) and ‘P1184948’ (C). Bars are means (n=4) ±SD



Figuri 6. Effect of salt stress on the yield of photosystem II in peanut varieties at the vegetative stage (42 DAS) ‘Vanda’ (A), ‘P244601’ (B) and ‘P1184948’ (C). Bars are means (n=4) ±SD

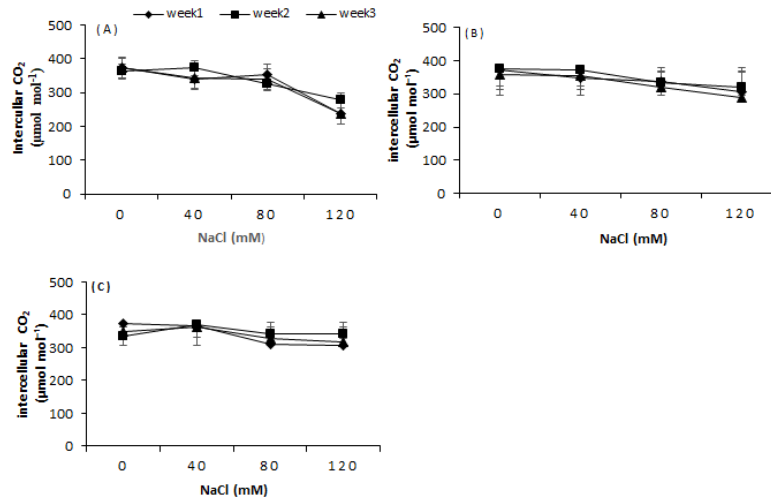


Figure 7. Effect of salt stress on the intercellular CO₂ concentration in peanut varieties at the vegetative stage (42 DAS) 'Vanda' (A), 'P244601' (B) and 'P1184948' (C). Bars are means (n=4) ±SD

Conclusions

Salt stress in peanut affected all monitored parameters. Application of NaCl treatment led to a significant decrease in LDW, SL, NL, TLA, P_n, g_s, TR and CO_{2int} concentrations of salt-sensitive 'Vanda' and moderately-tolerant 'P244601' compared to untreated plants while the plant growth inhibition was notably noted at 120 mM NaCl in salt-tolerant 'P1184948' for LDW, SL and NB. The highest depressive effect was detected in g_s of salt-sensitive 'Vanda' while the lowest were recorded in g_s of salt-tolerant 'P1184948' at high salinity level. Enhanced NaCl concentrations led to a significant increase in ΦPSII of 'P1184948' compared to 'Vanda', 'P244601' and untreated plants. Leaf CHL content was significantly increased in moderately-tolerant 'P244601' and salt-tolerant 'P1184948' at 80 mM NaCl compared to salt sensitive 'Vanda' and untreated plants. The depressive effect of salt on RWC was recorded at 120 mM NaCl in peanut leaves of all varieties. Under salt stress 'P1184948' was observed to have relatively higher tolerance on average of all growth and physiological traits than 'Vanda' and 'P244601' suggesting that it could be grown in salt-affected soils. This study conducted in peanut varieties provides additional information on the mechanisms of salt tolerance and may be helpful for breeding program.

Authors' Contributions

All authors read and approved the final manuscript.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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