

Division - Soil Processes and Properties | Commission - Soil Physics

Comparison of water and osmotic potentials on *Vigna unguiculata* stress response

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ABSTRACT: Drought and soil salinity are the main abiotic stresses in semiarid regions of the world. This study aims to evaluate the effect of water tensions generated by the reduction of soil moisture and salt on the leaf water potential of cowpea (*Vigna unguiculata* L. Walp). The experiments were conducted in a randomized complete block design, with a 6 × 2 factorial arrangement consisted of six soil water tensions (0.025, 0.265, 0.485, 0.705, 0.925, and 1.145 MPa) and two tension sources (water deficit and salt), with four replications. Two experiments were performed with the same environmental conditions to evaluate the influence of the tensions on vegetative and reproductive stages. Water and osmotic potentials, relative water content, leaf succulence, and shoot biomass yield were evaluated. Soil water tension was not the main factor of changes on water and osmotic potentials of *V. unguiculata* plants; the water deficit treatments at soil water tensions of up to 1.145 MPa did not reduce the water and osmotic potentials either at the vegetative or flowering phenological stages; high correlations were found between shoot biomass yield and the leaf water potential at seven days after stress. The osmotic potential was the main indicator of stress in plants at the vegetative and flowering stages subjected to water deficit by the presence of salts in the soil solution.

Keywords: water deficit, water relationships, drought tolerance, cowpea.

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INTRODUCTION

Low soil water availability to plants is the main limiting environmental factor to the growth of plants and, consequently, to agricultural productivity (Campos et al., 2014; Kardile et al., 2018). Water deficit in cultivated areas occurs worldwide and it is the main limiting abiotic stress (Singh and Reddy, 2011; Singh et al., 2020). The available water to plants can be reduced due to the decrease in soil water content and increasing salt concentrations in the solution (Sheldon et al., 2017).

Presence of soluble salts in the plant root region causes stomatal responses induced by the osmotic effect of the salts in the soil solution. Changes in stomatal conductance cause disturbances in carbon fixation, gas exchange, and water dynamics in plants (Munns and Tester, 2008; Rivas et al., 2013; Farooq et al., 2017). Leaf water potential is a measure of water energy inside the plant (Negrão et al., 2017). It can be used to evaluate the water status of plants (Oliveira et al., 2016), as a parameter for selection of cultivars (Nichols et al., 2015; Zegaoui et al., 2017), and as a tool to monitor plants under abiotic stresses (water and salt) in areas with a potentially stressful environment for plants (Medrano et al., 2015).

Plant responses to salt and water stress present similarities (Munns and Gilliam, 2015). Plants use survival strategies such as stomatal closure and reduction of water potential (Melo et al., 2018) to tensions capable of overcoming the soil water retention energy and allowing the absorption of water and nutrients (Ramoelo et al., 2015). In addition, plant stress tolerance is strongly correlated to the potential transpiration rate (Perelman et al., 2020). Therefore, it is also important to understand the increased transpiration by responding to changes in plant stress tolerance (Groenvelde et al., 2013).

Agricultural environments with low soil water availability conditions require the use of crop species that are tolerant to water stress and can maintain high productivity in these conditions (Zandalinas et al., 2018; Priya et al., 2019). However, tolerance values depend on the spatial and temporal distribution of salinity along the soil profile, environmental conditions, bulk soil properties, and plant cultivar differences (Jorda et al., 2017). In tropical regions with predominance of arid or semiarid characteristics, legume species are the main protein source for animals and humans (Farooq et al., 2017).

The objective of this study was to evaluate the effects of soil water tensions, caused by salt increase and water deficit, on water and osmotic potentials of *Vigna unguiculata* (cv. IPA-206) plants at the vegetative and flowering stages.

MATERIALS AND METHODS

Experimental conditions

The experiment was conducted in a greenhouse at the Federal Rural University of Pernambuco (UFRPE), Recife, Pernambuco State (PE), Brazil (8° 04' 03" S, 34° 55' 00" W, and altitude of 4 m a.s.l.), from December 2016 to March 2017.

Air temperature and relative humidity during the experiment were monitored using a hygrometer and a datalogger (Instrutherm, HT - 70). The temperature and relative humidity were recorded at seven days after the beginning of soil water stress, when the effects of the treatments applied at the vegetative and flowering stages were evaluated. At the vegetative stage, the air temperature and relative humidities were 27.33 °C and 75 %, and at the flowering stage, they were 27.1 °C and 73.28 %, respectively.

Soil characterization

The soil used in the experiment was classified as *Neossolo flúvico* (Fluvisols) (Melo et al., 2018); it was collected from the 0.00-0.30 m layer in a rural area of Pesqueira, PE, Brazil.

The soil samples were air-dried, dewatered, and passed through a 4-mm mesh sieve to maintain soil microaggregates. The soil was placed in pots, and five subsamples were taken, air-dried, pounded to break up clods, and homogenized to obtain the air-dried fine earth (ADFE); then they were passed through a 2-mm mesh sieve for physical and chemical analyses (Tables 1 and 2).

The soil moisture in which the plants were grown in the control treatment was determined based on the soil water retention curve (SWRC); gravimetric moisture of 0.25 g g^{-1} (86 % of the field capacity) was established using the criteria of aeration conditions and soil water available. The matric potential (-0.025 MPa) in the moist soil of the control and other treatments was obtained through the SWRC.

The control treatment tension (0.025 MPa) was used as standard soil moisture for seed germination in the treatments with salt and water stresses. The soil water tensions for the water stress treatments were chosen with intervals to allow the last level to be an intermediate value of the maximum tension tolerated by the plant, which is 2.5 MPa (Boyer, 1978). Once the tensions' values were determined, they were converted to gravimetric moisture through the SWRC to allow the control of the soil water tensions in the pots by weighing them.

For the calculation of salt to be applied to the soil solution in the salt stress treatments, the gravimetric moisture was used in the control treatment to calculate the amount of water in the soil volume used during the experiment. Based on this water volume, six tensions were used in the Van't Hoff equation to estimate the salt concentration required to generate the desired osmotic potential in each treatment. The solutions were prepared in a laboratory, using NaCl. Thus, plants in the salt stress treatments were maintained

Table 1. Chemical properties of the soil (*Neossolo flúvico*) used in the experiment

Saturation Extract		Exchange Complex		Relation (soluble)	
Properties	Values	Properties	Values	Properties	Values
pH(H ₂ O)	7.3	pH(H ₂ O) (1:2.5)	6.07	Na/Ca	4.49
EC (dS m ⁻¹)	1.12	Ca ²⁺ (cmol _c kg ⁻¹)	3.4	Na/Mg	4.20
Ψ _o (MPa)	- 0.06	Mg ²⁺ (cmol _c kg ⁻¹)	4.3	Na/K	3.94
Ca ²⁺ (mmol _c dm ⁻³)	1.12	Na ⁺ (cmol _c kg ⁻¹)	0.78	Na/Cl	0.92
Mg ²⁺ (mmol _c dm ⁻³)	1.15	K ⁺ (cmol _c kg ⁻¹)	0.45	Cl/Ca	4.64
Na ⁺ (mmol _c dm ⁻³)	4.8	BC (cmol _c kg ⁻¹)	8.94	Cl/Mg	4.52
K ⁺ (mmol _c dm ⁻³)	1.22	CEC (pH 7.0)	9.92	Cl/Na	1.08
Cl ⁻	5.2	ESP (%)	7.86	Cl/K	4.26

EC: electrical conductivity; Ψ_o: osmotic potential; BC: sum of base cations; ESP: exchangeable sodium percentage; CEC: cation exchange capacity, pH in water (1:2.5) and exchangeable Ca, Mg, Na, and K contents, which were extracted with ammonium acetate 1 mol L^{-1} (Thomas, 1982). A saturation extract was obtained from the soil paste (Richards, 1954) to determine the ions in the soil solution, electrical conductivity (EC), and soluble bases; the chloride ion was determined by titration with AgNO₃ (Teixeira et al., 2017). CEC was determined using the index cation method (Richards, 1954). BC and ESP were determined based on the exchange complex.

Table 2. Physical properties of the *Neossolo flúvico* used in the experiment

Sand			Silt	Clay	WDC	DD	DF	BD	PD	TP	FC	PWP	TC
Fine	Coarse	Total											
g kg ⁻¹						g cm ⁻³			%		g g ⁻¹		
303	107	410	435	155	120	0.77	0.23	1.26	2.50	50.4	0.29	0.14	Loam

WDC: water-dispersible clay; PD: particle density; BD: soil bulk density; DD: degree of dispersion (WDC/Clay); DF: degree of flocculation (1 - DD); TP: total porosity; TC: textural classification; FC: field capacity; PWP: permanent wilting point. Soil granulometry analysis according to Donagemma et al. (2017); WDC, DF, and DD according to Donagemma and Viana (2017); BD (Almeida et al., 2017a); PD (Viana et al., 2017). TP was estimated based on the values of the BD and PD (Almeida et al., 2017b). The FC and PWP were determined based on the soil water retention curve (SWRC) (Teixeira and Bhering, 2017).

in the same soil moisture of the control treatment, and only the amounts of salt applied through the salt solution were changed to establish the stress.

Ten-liter pots were filled with 8 kg of soil to ensure sufficient soil for the whole crop cycle (Rivas et al., 2013; Oliveira et al., 2016). These pots were moistened until saturation and subjected to daily weighing to monitor the soil gravimetric moisture until it reaches the moisture of the control treatment (86 % of the field capacity) to uniform water distribution throughout the soil before sowing.

The *V. unguiculata* (cv. IPA-206) seeds used in the experiment were sterilized by placing them in an ethanol solution (1 %) for 3 min and, subsequently, in a sodium hypochlorite solution (0.1 %) for 1 min (Cavalcanti et al., 2004); they were carefully washed in distilled water and, then, six seeds were sown per pot. A thinning was performed three days after sowing (DAS), leaving two plants per pot, when the plants had a pair of leaves, to ensure the supply of plant material to all analyses, considering that most plant material would be lost in the analysis process.

The maintenance of soil moisture was performed by daily weighing and replacing the water lost by evapotranspiration. This process was carried out by the end of the afternoons to avoid losses by evaporation and provide a more uniform water redistribution inside the pots, allowing the rehydration of the plants through the soil moisture during the night.

Treatments and analyses

Pe maintained at the same soil water tension of the control treatment (0.025 MPa) until 17 days after germination (DAG). Then, the treatments were applied gradually for three days, and the days of stresses began to be counted. The plants were subjected to six soil water tensions (0.025, 0.265, 0.485, 0.705, 0.925, and 1.145 MPa) equivalent to gravimetric moistures of 0.25, 0.184, 0.17, 0.162, 0.157, and 0.152 g g⁻¹, respectively. These soil water tensions were generated by two tension sources: water deficit (reduction of soil moisture) and salt stress (addition of salt to the soil solution).

The amount of salt needed to obtain the soil water tensions for the salt treatments was diluted into 100 mL of distilled water. The application of the salt solution was divided into three times, together with the water irrigation, applying approximately 33 mL of the salt solution at the time of irrigation. This fractionation was necessary to prevent osmotic shock to the plants and was concluded after three days, along which the established soil moistures for the plants of the water deficit treatments were applied.

Irrigation was suspended until the soil reaches the established moistures for each treatment, with a daily weighing of the pots to monitor their weights; then, irrigation was resumed. The established moisture for each treatment was achieved within three days of the suspension of irrigations when the days of water stress began to be counted.

The stress treatments were applied to plants at the first development stage (vegetative) when these plants presented two pairs of completely expanded leaves (20 DAG) to ensure their resistance to the stressful conditions. The stress treatments were applied to plants at the second development stage (flowering) when 50 % of the plants were at the flowering stage (35 DAG). The evaluations were performed seven days after the beginning of stress.

Leaf water potential of plants (Ψ_w)

Leaf water potential (Ψ_w), osmotic potential (Ψ_o), turgor pressure potential (Ψ_p), relative water content (RWC), leaf succulence, and the yield shoot biomass were evaluated to determine the water status of the evaluated plants. All physiological evaluations were performed on healthy, fully expanded leaves from the middle third of the plants,

at predawn after seven days of stress, corresponding to 27 and 42 DAG, respectively, for plants that received the stress treatments at the vegetative and flowering stages.

The Ψ_w was determined immediately after the sectioning of the leaves, which were directly analyzed in the Scholander pressure chamber (Model 1515D, Pressure Chamber Instrument - PMS Instrument Company).

Osmotic potential (Ψ_o)

The same leaves were used to evaluate Ψ_w and Ψ_o . The Ψ_o was analyzed by macerating the leaves in liquid nitrogen until obtaining their saps, which were filtered and placed into 2-mL microtubes. The samples were centrifuged (10,000 *g* for 10 min at 4 °C) until the decantation of the plant residues and obtaining a translucent supernatant. The supernatant was collected to determine the osmolality of the sap using an osmometer (Melo et al., 2018).

Based on the osmolality values, Ψ_o of the plants was estimated by applying the Van't Hoff equation, according to Equation 1:

$$\Psi_o = -R T C \quad \text{Eq. 1}$$

in which *R* is the general gas constant (0.00831 kg MPa mol⁻¹ K⁻¹), *T* is the temperature (K), and *C* is the osmolality of the sap, expressed in mol kg⁻¹ (Gimenez et al., 2005; Ben-Gal et al., 2009).

Turgor pressure potential (Ψ_p)

Water potential is the sum of the pressure potential and osmotic potential (Equation 2); thus, the turgor pressure potential was estimated by subtracting the osmotic potential from the water potential previously obtained, as shown in equation 3:

$$\Psi_w = \Psi_o + \Psi_p \quad \text{Eq. 2}$$

$$\Psi_p = \Psi_w - \Psi_o \quad \text{Eq. 3}$$

Relative water content (RWC) and leaf succulence (LS)

The relative water (RWC) content was determined during the Ψ_w measurements. Seven leaf discs of known area were weighed in a precision analytical balance to obtain their fresh weights (FW). Subsequently, these discs were placed on Petri dishes coated with a filter paper and 5 mL of distilled water, and covered with a second filter paper; these Petri dishes were placed in a refrigerator at 4 °C under the absence of light over 48 h to obtain the mass of the leaf discs at complete turgidity; then, they were weighed to quantify the turgid disc weights (TW). Finally, the discs were dried at 65 °C until constant weight and weighed to obtain the disc dry weights (DW). The RWC was calculated according to equation 4:

$$RWC(\%) = [(FW - DW) / (TW - DW)] \times 100 \quad \text{Eq. 4}$$

Leaf succulence was determined based on the difference between the fresh and dry weights of the leaf discs used in the determination of RWC; the leaf succulence is the result of the difference between the weights and disc areas (*A*), as proposed by Delf (1912), according to equation 5:

$$LS = (FW - DW) \times A \quad \text{Eq. 5}$$

Statistical design and data analysis

The experiment was conducted in a randomized complete block design with a 6 × 2 factorial arrangement consisted of six soil water tensions and two stress sources, with four

replications, totaling 48 plots in each phenological crop stage (vegetative and flowering). The data were analyzed through analysis of variance, comparison test of means, and adjustment of regression for the interactions with the salinity levels.

RESULTS

The evaluated soil water tensions and stress sources presented a significant interaction for leaf water potential (Ψ_w) and osmotic potential (Ψ_o) of the *V. unguiculata* plants (cv. IPA-206) subjected to stress treatments at the vegetative and flowering stages. In the evaluations at seven days after stress; no significant difference was found for turgor pressure potential, whose mean values are presented with the standard deviation bars (Figure 1).

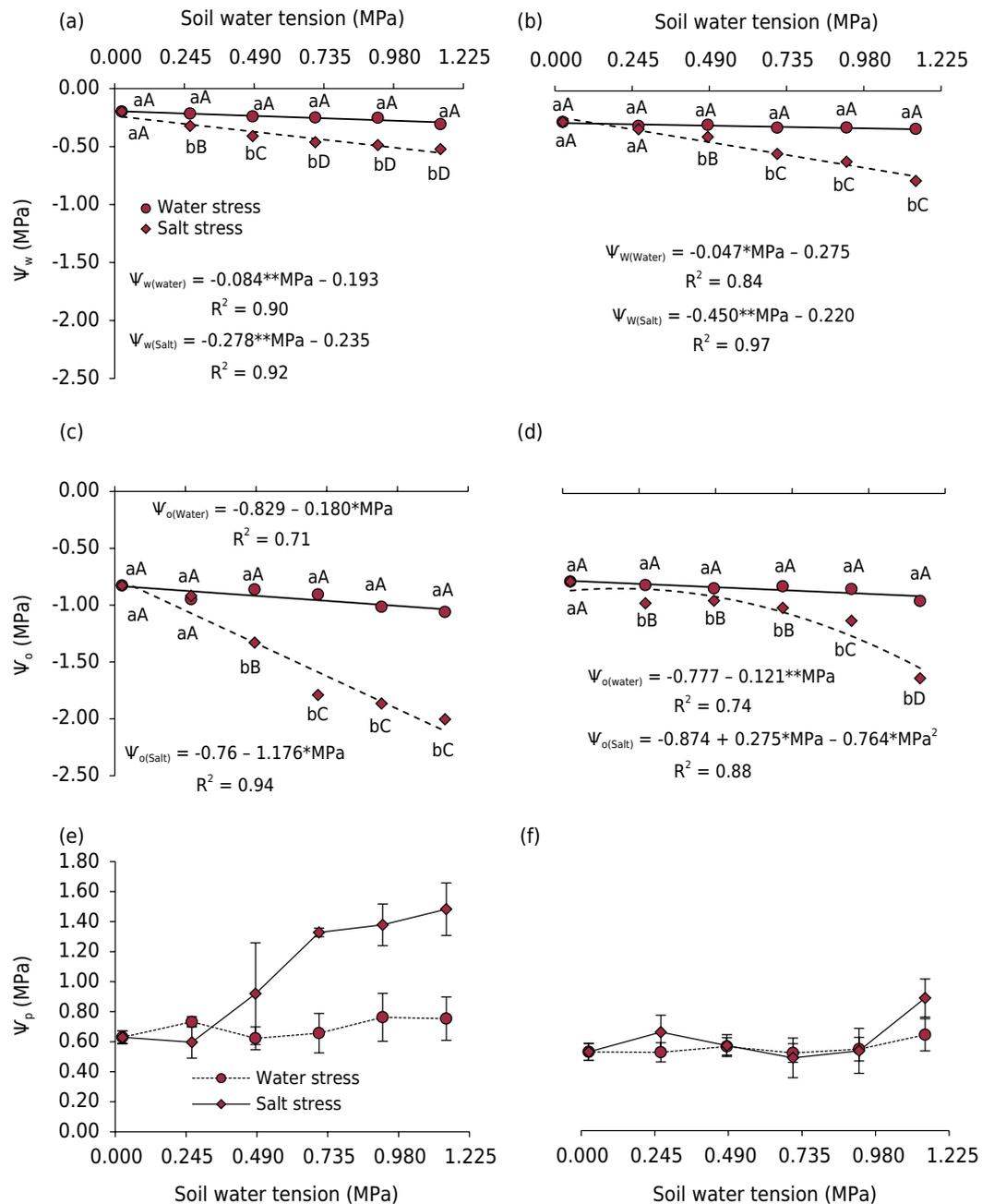


Figure 1. Leaf water potential (Ψ_w) (a and b), osmotic potential (Ψ_o) (c and d); turgor pressure potential (Ψ_p) (e and f) of *Vigna unguiculata* plants (IPA-206 cultivar) evaluated seven days after stress by treatments applied at the vegetative and flowering stages, respectively. * Means followed by the same uppercase letter for the soil water tensions or same lowercase letter for stress sources were statistically similar by the Scott-Knott test at 5 % probability.

The Ψ_w of plants in the treatments with different soil water tensions generated by reduction of soil moisture presented no significant differences. None of the tensions differed from the control, regardless of the crop phenological stage (vegetative and flowering) in which they were applied. Linear regressions were adjusted for the vegetative and flowering stage data, with a coefficient of determination of $R^2 = 0.90$ and 0.84 , respectively (Figures 1a and 1b).

Linear equations with coefficients of determination of $R^2 = 0.92$ and $R^2 = 0.97$ were adjusted for the Ψ_w data of plants subjected to salt stress at the vegetative and flowering stages, respectively (Figures 1a and 1b). The angular coefficients (vegetative stage = 0.278 ; flowering stage = 0.450) found denoted that salts affect growing environments, with a decrease of the Ψ_w of plants as the salt levels increased.

Despite the decrease in Ψ_w as the salt concentration was increased, the RWC of the evaluated plants were similar, regardless of the tension sources (water and salt), soil water tensions, and phenological stages; no significant differences were found either for relative water content or leaf succulence.

At both vegetative and flowering stages, plants were able to maintain hydration of their tissues, regardless of the soil water tension applied. Souza et al. (2014) confirmed the capacity of cowpea plants to keep high RWC on water deficit conditions. In this study, the RWC was of approximately 90 % even when the IPA-206 cultivar cowpea plants were subjected to the highest soil water tension (1.145 MPa), regardless of tension source. Even with no significant variations in the response of RWC to the different soil water tensions and tension sources, the water potential of plants was more affected by the salt stress than by the water deficit (Figures 1a and 1b).

Linear regressions for both sources of stress were adjusted for the Ψ_o of plants evaluated at the vegetative stage, although with a lower determination coefficient for the water stress ($R^2 = 0.71$). The coefficient of determination of the adjusted equation for Ψ_o of plants in the salt stress treatments was $R^2 = 0.94$ (Figure 1c). The linear equation adjusted with the Ψ_o means showed a high angular coefficient ($b = 1.176$), with a significant response to the increase in soil water tensions.

The plants at the vegetative stage subjected to water deficit treatments presented similar Ψ_o , regardless of the soil water tension. No significant decreases were observed as the tensions were increased, with no differences from the control treatment up to the tension of 1.145 MPa. The plants grown under salt stress conditions presented decreases in Ψ_o as the soil water tension was increased above 0.485 MPa, with similar results when using the three highest tensions (0.705 , 0.925 , and 1.145 MPa).

The plants at the flowering stage subjected to salt stress treatments presented greater Ψ_o decreases than those under water-deficit treatments. The results show that the water tension in the soil not alter the water and osmotic potentials and the rehydration capacity of cowpea plants of the IPA-206 cultivar subjected to water deficit, regardless of the phenological stage, as shown by assessment of the RWC. The turgor pressure potential of the plants presented no significant differences, or interaction with soil water tensions nor with tension sources, regardless of the physiological stage (Figures 1e and 1f).

Shoot biomass yield was reduced with the increase of the tension of water in the soil for both sources of tension, salt, and water deficit, showing higher reductions when stresses were applied at the flowering stage (Figures 2a and 2b). When the stresses (water deficit) were applied at the vegetative stage, biomass values reduced little in relation to the control ($\cong 3.17$ g per MPa), showing that up to 1.145 MPa, the phenological stage of stress exposure was more decisive in reducing biomass than the values of the tensions applied in the treatments. Differently from the values observed for the salt source, which gradually reduced with increasing tension, with reductions of $\cong 11.38$ g per MPa.

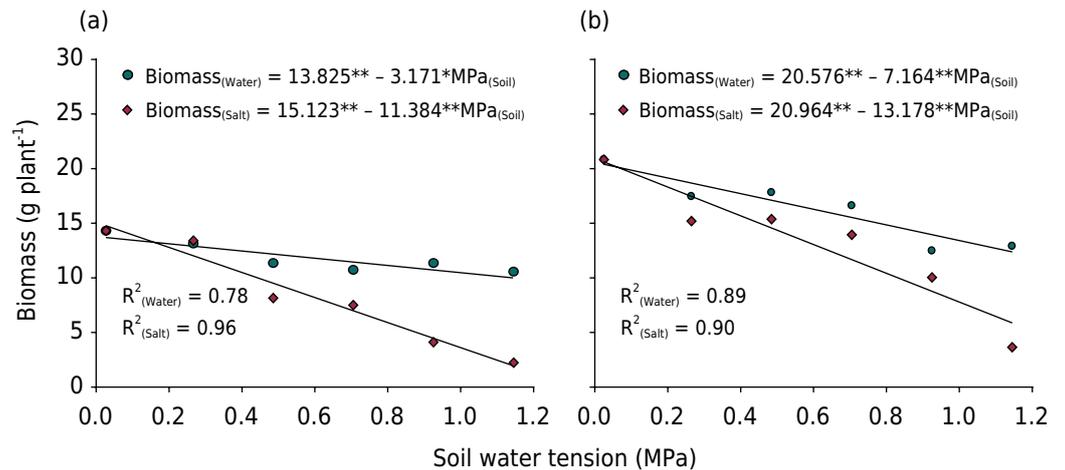


Figure 2. Shoot biomass yield (dry matter) of *Vigna unguiculata* plants (IPA-206 cultivar) evaluated 90 days after stress by treatments applied at the vegetative (a) and flowering (b) stages, respectively.

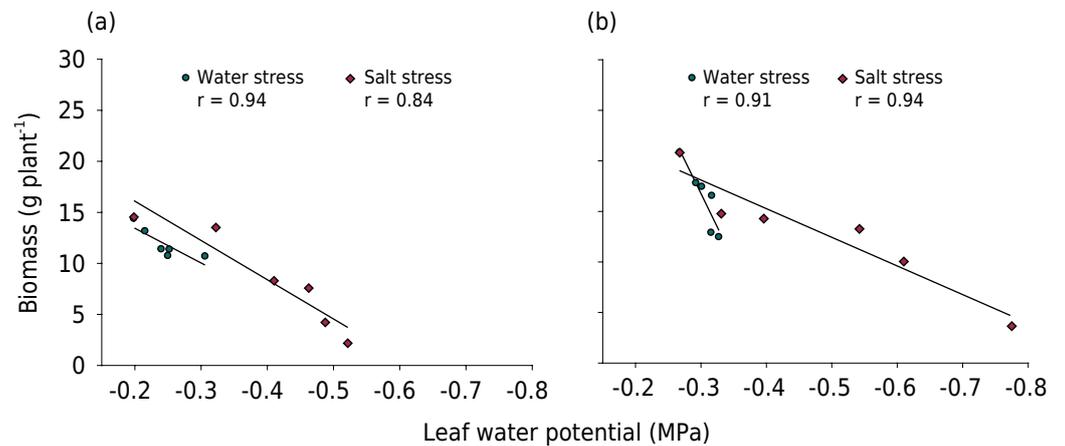


Figure 3. Simple linear correlation between leaf water potential in *Vigna unguiculata* (cultivar IPA-206) as measured 7 days after stress and shoot biomass yield (dry matter) for treatments applied at the vegetative (a) and flowering (b) stages for each soil water tension.

In the flowering stage, the reductions were greater for both sources of tension, salt, and water deficit, being more intense on salt source than in the water deficit source, where the reductions were $\cong 13.18$ and 7.16 g per MPa, respectively. Two facts can be highlighted, the first one is: the reductions found for the water deficit in the flowering stage were practically double the ones registered at the vegetative stage for the same tensions; and, secondly is: even the application of the treatments being in a phenological stage in which the plants had greater accumulation of dry matter, the observed reductions reinforce the plants' greater sensitivity in flowering phenological stage. In both phenological stages, high correlations were observed between shoot biomass yield and leaf water potential estimated seven days after stress (Figure 3).

At the vegetative stage, the highest correlation coefficient was found in the water deficit source of tension ($r = 0.94$) followed by the salt tension source (0.84) (Figure 3a). It is worth mentioning that for water deficit source the range of leaf water potential and biomass yield in relation to soil tension varied $\cong 0.1$ MPa and 4 g plant⁻¹, respectively, between the control treatment and the maximum water tension in the soil. To salt source, the values varied between 0.3 MPa and 12 g plant⁻¹ for leaf water potential and shoot biomass yield, respectively, between maximum tension and control treatment.

In the flowering stage, the correlation coefficients were 0.91 and 0.94 for the water deficit and salt as tension sources, respectively (Figure 3b). For the water deficit as tension source, the values of leaf water potential and biomass production varied $\cong 0.1$ MPa and 9 g plant^{-1} , respectively. Meanwhile, for salt as a source of tension, the values varied $\cong 0.5$ MPa and 17 g plant^{-1} for the leaf water potential and shoot biomass production, respectively, between the maximum tension and control treatment.

DISCUSSION

In the hottest hours of the day, the plants were exposed to soil water tensions above 1.145 MPa due to the increase of evapotranspiration. However, none of the plants under water deficit treatments had their rehydration compromised. Considering the response to the soil water tensions generated by the water deficit, plants of the IPA-206 cowpea cultivar can be grown under soil water tensions of up to 1.145 MPa without a reduction in water potential when measured at predawn. This denotes that this cultivar can maintain tissue hydration even when grown under water deficit when the soil does not present water tensions higher than 1.145 MPa. Similarly, Souza et al. (2014) evaluated *V. unguiculata* plants under suspensions of irrigation of up to a zero CO_2 assimilation rate and found reductions of -1.27 MPa in the water potential of plants subjected to the most stressful conditions, and reductions of -0.71 MPa in control plants, with a similarity of this variable among treatments after rehydration.

The identification of the role of the osmotic component as the main source of stress probably resulted from the higher concentration of salts at the soil-root interface. Although this was not our object of study, the verification of the osmotic potential in the rhizosphere could support us in understanding the phenomenon as a whole, since osmotic differences between the rhizosphere and the soil is a fact observed in studies of the same nature (Jorda et al., 2017; Perelman et al., 2020). In addition, the adoption of tensions lower than half that supported by the crop may also have had an effect on the observed responses, since the plant may not have experienced stress due to the matric tension of the soil. Jorda et al. (2017) evaluated the effect of osmotic and matric components on the development of stress in plants and were able to verify that frequent irrigation was fundamental for the stress generated to be exclusively due to osmotic stress. We believe that this occurs because under irrigation conditions with saline water, the total potential of the soil was composed mainly of osmotic potential, which affects both water potential and hydraulic conductivity, two of the plant-water relations. This is likely the explanation for the differences found between matric and osmotic potentials in this study.

Plants subjected to high evapotranspiration conditions restore their water status at night, when temperatures are mild and evaporation is practically zero (Fini et al., 2013; Ramalho et al., 2014). Thus, plants can establish a balance between water status and soil moisture during predawn, when they are rehydrated by absorption of water, without great water losses due to transpiration (Martínez-Vilalta et al., 2014).

For a halophyte plant species (*Atriplex nummularia*), Melo et al. (2018) found a variation of -0.80 MPa in Ψ_w between evaluations at predawn and noon in plants grown under the moisture of 70 % of field capacity. This value is within the limits reported by Martínez-Vilalta et al. (2014) when evaluating tree species of different environments (tropical and arid regions) and comparing variations in Ψ_w between measurements at predawn and noon (-0.86 MPa). The main factors that affect the rebalancing of the plant water potential are transpiration rate, hydraulic conductance, and soil water availability (Martínez-Vilalta et al., 2014). Martínez-Vilalta et al. (2014) found a variation of -0.19 MPa in Ψ_w in a tropical-region tree (*Acacia etbaica*) with measurements at night and noon; this variation was -1.36 MPa for an arid-region tree (*Poposis glandulosa*).

Depending on the environmental conditions, the plant rehydration capacity can be compromised (Melo et al., 2018). These authors evaluated *Atriplex nummularia* plants subjected to irrigation with six electrical conductivity (EC) in the irrigation water and two water regimes (70 and 37 % of the field capacity) and found that plants treated with the highest EC (30 and 40 dS m⁻¹) presented no differences in relative water content (RWC) measured at noon, time of maximum evaporation; but, the RWC differed between evaluations at predawn, when plants subjected to 30 dS m⁻¹ presented higher RWC compared to those treated with 40 dS m⁻¹, therefore, this level of stress (40 dS m⁻¹) affected the rehydration capacity of plants, even with high water content in the soil. Thus, this ability to maintain water content in tissues at optimal levels in the face of high salinity (30 dS m⁻¹) is an important trait to this plant species function, cause the loose water from tissues plants under saline stress can have effects on important plant physiological processes (Negrão et al., 2017). According to Cavalcanti et al. (2004), the tolerance of cowpea to salt stress is mainly due to its ability to exclude Na⁺ from leaves and concentrate it in roots, maintaining high leaf Ψ_w . According to Cavalcanti et al. (2004), the tolerance of cowpea to salt stress is mainly due to its ability to exclude Na⁺ from leaves and concentrate it in roots, maintaining high leaf Ψ_w .

The effects observed on the plant-water relations under salt stress are the results of the osmotic pressure exerted by the salts in solution. In addition, we can argue that the stress experienced by the plant was not essentially osmotic. The exposure time to salinity stress could cause an increasing plant ionic response, a recognizable indication of salinity stress and which induce physiological disorders in plants (Munns and Tester, 2008). These responses indicate that there are significant energy costs and damage to root cells leading to mortality and high root turnover. Therefore ionic responses will occur long before ions accumulate to toxic levels in leaves (Negrão et al., 2017; Perelman et al., 2020).

Plants evaluated after treatments applied at the vegetative stage (salt source) presented different water potential from the control for all evaluated soil water tensions; and plants under the treatments with the three higher tensions (0.705, 0.925, and 1.145 MPa) had similar results. The plants treated with the highest tension had a decrease of 130 % compared to the control. Oliveira et al. (2016) found a similar decrease (116 %) in plants of the same cultivar subjected to tension of 1.0 MPa when compared to the control. Comparing the evaluated tensions sources, all tensions higher than 0.265 MPa presented differences (Figure 1a).

The presence of salt in the soil solution affected more the leaf water potential than the tension in which water was retained in the soil, since the plants under salt stress were grown under the same soil moisture of the control treatment (86 % of pot capacity). The presence of Na⁺ and K⁺ ions in the soil solution activates specific transporters located in the plant cell membrane, which overlaps the effect of the osmotic component; when these transporters are of high affinity, the activation occurs under low concentrations (Munns and Tester, (2008). Ion transporters and their localization in key cell types underpin plant salinity tolerance (Negrão et al., 2017). In addition, as a previous study suggested that more sensitive species tend to have low Na⁺ concentrations in leaves, lower than in the external solution, there is a correlation between salt tolerance and Na⁺ exclusion as an important adaptive trait (Munns and Gilliam, 2015).

In general, cowpea plants at the vegetative stage are more tolerant of reductions of water in the soil than to the increase of salt contents in the soil solution (Ahmed and Suliman, 2010). The relationship between soil water tension and leaf water potential found in this study (Rivas et al., 2013; Ramoelo et al., 2015) confirms the higher tolerance of the plants when compared to the lower Ψ_w found using the highest three soil water tensions (0.46, 0.49, and 0.52 MPa) in salt stress treatments.

This study showed clearly that *V. unguiculata* plants could efficiently reduce their leaf water potential below the substratum water potential as a consequence of decreased tissue osmotic potential. This implies that cowpea plants were able to effectively adjust osmotically, maintaining a positive water balance in response to water salinity. This can be inferred from the improvement of plant water status (RWC).

It should also be noted that as a response to signals from roots to balance the decrease in water potential (Negrão et al., 2017). Most plants in saline environment could accumulate a large amount of osmoregulators in the protoplasm to maintain osmotic balance, due to ion accumulation in vacuole (Wang et al., 2019).

In response to osmotic stress, plant hormones such as ABA can act as adaptive signals to induce the synthesis and concentration of osmoregulators, especially in leaves, so that the observation of lower leaf osmotic potentials may be due to these secondary metabolites and not just to the decrease in the total potential of water in the soil (Zhou and Memelink, 2016).

Cowpea plants at the flowering stage have greater sensitivity to salt stress (Ahmed and Suliman, 2010). The Ψ_w of the IPA-206 cultivar presented this same pattern. The adjusted linear regressions showed approximately 10-fold increase in the angular coefficient of the equation for the salt stress treatments when compared do those of the water deficit source treatments, and practically double angular coefficient of the equation adjusted for the data of the salt stress treatments applied at the vegetative stage.

However, at the flowering stage, differences were found only for tensions above 0.485 MPa. The Ψ_w showed no differences with tensions above 0.705 MPa, despite the significant decrease in Ψ_o as the soil water tension was increased (Figure 1d). This result denotes the importance of the turgor pressure for the plant water status. Oliveira et al. (2016) evaluated this same cultivar under irrigation with salt waters ($\Psi_w \cong -1.0$ MPa) throughout the growing cycle and found that the turgor pressure potential contributed with approximately 0.60 MPa.

Plants at the flowering stage are more susceptible to stresses (Hall, 2012; Kazan and Lyons, 2016). Thus, the plants at the flowering stage subjected to salt stress conditions presented the greatest Ψ_o decreases. This is a response to the presence of salts in the soil solution and not to the tension in which the water was retained in the soil. This was observed in plants in the water deficit treatments, which presented similar Ψ_o for all evaluated tensions, whereas those under salt stress conditions had significant differences in tensions (0.265 to 1.145 MPa).

Similarly, Chaves et al. (2009) found a negative correlation between RWC below 50 % and photosynthetic activity of plants; however, the RWC found ranged from 85 to 91 %. Despite a reduction in the osmotic potential of the IPA-206 cultivar, Oliveira et al. (2017) found no significant difference in RWC in plants under salt stress, with electrical conductivity of the irrigation water varying from 0 to 12.5 dS m⁻¹ (osmotic potential of irrigation water ranging from -0.7 to 0 MPa).

The maintenance of RWC under stress conditions at the both evaluated crop stages was due to an efficient mechanism of stomatal closure (Ramalho et al., 2014) since even differing in osmotic and water potentials, the plants maintained RWC close to 90 %, regardless the tension sources, tensions, and phenological stages.

The reductions observed in shoot biomass at 90 DAS are the result of the sum of the osmotic and ionic effects in salt source treatment (Munns and Tester, 2008). The osmotic potential was highly sensitive to the presence of salts in the medium even in initial conditions stress. These changes can be noticed by the changes in the values of leaf water potential of the plants, corroborating the fact that the tension did not act as the main factor of alteration of the potentials in the plants of cowpea cultivar IPA-206.

Studies have shown that the accumulation of salts around the roots depends on the plant transpiration rate and salinity level (Groenvelde et al., 2013). Changes in Na^+ concentrations due to the transpiration rate are probably caused by water uptake (Tack et al., 2015). Consequently, high soil hydraulic conductivity is most likely the most important soil property reducing water and salinity stresses (Hoffman and Rawlins, 1971; Perelman et al., 2020). It has been argued that the soil-root interface osmotic potential is potentially lower than the bulk soil osmotic potential, exhibiting an accumulation of salt around the root compared with the bulk soil (Jorda et al., 2017).

This is particularly important and implies that osmotic stress is highly dependent on root traits, its hydraulic conductivity, and plant's transpiration, which have a consistent influence on salinity tolerance, and cause tolerant plants to decrease their roots hydraulic conductance, thereby reducing the delivery of saline water to the shoot. Another important indicator of salt tolerance is the ability of plants to maintain normal rates of transpiration under saline conditions, particularly because transpiration is related to normal rates of CO_2 uptake for photosynthesis (Negrão et al., 2017).

Another approach that should be considered is that the hydraulic gradient between the soil and root is constantly dynamic, with a positive flow of water from the soil toward the root as a result of the transpiration flow (Jorda et al., 2017). Therefore, root water uptake distribution along the root system is influenced by the hydraulic conductivity of the soil and will influence the differences in solute concentration within the root zone, uptake, and plant response to salinity (Perelman et al., 2020).

The influence of evapotranspiratory demand in decreasing osmotic potential is recognized for many years and identified in several studies that have evaluated the effect of salinity on tolerance responses of some species, as previously demonstrated (Hoffman and Rowlings, 1971; Groenvelde et al., 2013; Tack et al., 2015). However, we cannot assume that in the present study, this variable had a significant influence, since although not presented, both temperature and relative humidity were monitored daily and provided an estimate of vapor pressure deficit estimate corresponding to 0.91 and 0.96 kPa, in the vegetative and reproductive stages, respectively.

Studying water and osmotic potentials are important for the evaluation of tolerant species to water and salt stresses (Negrão et al., 2017; Zegaoui et al., 2017). The results of these parameters are directly related to the ecophysiological mechanisms of tolerance to stressful conditions (Ramalho et al., 2014). The evaluation of these potentials can subsidize the evaluation of the tolerance level of plants based on the differentiation of the phenological stages and types of stresses (Fini et al., 2013; Zegaoui et al., 2017).

CONCLUSIONS

Soil water tension was not the main factor of changes in water and osmotic potentials of the evaluated *V. unguiculata* plants (cv. IPA-206); the water deficit treatments at soil water tensions of up to 1.145 MPa did not reduce the water and osmotic potentials of *V. unguiculata* plants at the vegetative and flowering phenological stages; the presence of ions in the soil solution was the main factor of changes in water and osmotic potentials of the *V. unguiculata* plants; water potential presented high correlation to shoot biomass yield; the flowering stage was the more sensitive one, showing high biomass reduction even for the plants under water source tension treatments; the osmotic potential was the main indicator of stress in plants at the vegetative and flowering stages subjected to water deficit by the presence of salts in the soil solution.

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