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Chlorophyll fluorescence as a tool to select salinity-tolerant cowpea genotypes

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Abstract

The use of saline water reduces the growth and productivity of crops, so the need for techniques that make possible the use of this resource such as the use of salinity tolerant genotypes and efficient selection methods are of great importance. Thus, this study aimed to evaluate the tolerance of cowpea (*Vigna unguiculata* L. Walp.) genotypes to salt stress, through the chlorophyll fluorescence analysis. The experiment was conducted in a protected environment at the Federal University of Campina Grande, Paraíba, Brazil, using a completely randomized design in a 2 x 10 factorial arrangement, with three replications, consisting of two levels of irrigation water salinity (0.6 and 5.1 dS m^{-1}) and ten cowpea genotypes: (G1: MNCO1-649F-2-1, G2: MNCO3-736F-2, G3: PINGO DE OURO-1-2, G4: BRS GURGUÉIA, G5: BRS MARATAOÃ, G6: MNCO2-676F-3, G7: MNCO2-683F-1, G8: MNCO3-737F-5-4, G9: MNCO3-737F-5-9, and G10: BRS TUMUCUMAQUE). The stem length, stem diameter, SPAD index, and chlorophyll fluorescence transients were evaluated. The G2 and G4 genotypes had the lowest reductions in the growth, stem diameter, initial fluorescence, and primary and maximum photochemical efficiency of PSII, proving to be tolerant to salinity. Chlorophyll fluorescence is a tool that can be used in the selection of salinity-tolerant cowpea genotypes.

Keywords: photochemical efficiency, water salinity, *Vigna unguiculata*

Introduction

The cowpea (*Vigna unguiculata* (L.) Walp.) is a leguminous plant of great socioeconomic importance in the North and Northeast regions of Brazil, not only for its popular acceptance but also for being a source of protein, energy, fibers, and minerals (Pereira et al., 2016). It is cultivated mainly in the northeastern arid and semi-arid areas of Brazil, consisting of basic food for the population (Aquino et al., 2017; Oliveira et al., 2018). Due to its characteristics of rusticity and precocity, it is considered a plant adapted to semiarid conditions, and is widely cultivated in rainfed agriculture conditions (in which agriculture production occurs only during the rainy season) and in the irrigated perimeters of Northeast Brazil.

Despite its importance and wide distribution in these regions, some factors have limited their production, such as the water salinity in irrigated areas (Sá et al., 2016; Oliveira et al., 2018). Regarding this aspect, cowpea is considered a moderately salinity tolerant species, tolerating irrigation water salinity of up to 3.3 dS m^{-1}, without compromising productivity (Ayres & Westcot, 1999). However, the behavior regarding the water electrical conductivity may vary between genotypes, which may have different responses to salinity (Azevedo Neto et al., 2011; Brito et al., 2016; Sá et al., 2016).

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Salinity, in many arid and semi-arid regions, is a serious obstacle to the production system, due to changes in physical and chemical characteristics of soils and the action of specific ions on plant growth (Cavalcante et al., 2010; Melo et al. 2017a). In addition, excess salts in water may affect physiological and biochemical functions, such as the maximum efficiency of photosystem II, resulting in various disturbances which, consequently, compromises the development and production of the cultures (Brito et al., 2016; Oliveira et al. al., 2018). Among the physiological functions, the concentration of pigments (Qados, 2011) and chlorophyll fluorescence are effectively altered in plants under salinity conditions (Brito et al., 2016; Melo et al., 2017a), which allows us to distinguish tolerant from sensitive materials. Thus, this tool can be of great use in the selection of cowpea genotypes, making it possible to identify those most tolerant to salinity.

Some studies have reported the importance of the chlorophyll fluorescence evaluation technique as being efficient in the selection of genotypes more tolerant to salinity, such as in sunflower (Azevedo Neto et al., 2011), citrus (Brito et al., 2016), passion fruit (Freire et al., 2014), and barley (Kalaji et al., 2011). Thus, it was hypothesized in this study that bean genotypes more tolerant to salinity undergo less photosynthetic damage when evaluated by chlorophyll fluorescence.

Given the above, this study aimed to evaluate the chlorophyll fluorescence in cowpea genotypes subjected to different salinity concentrations of irrigation water.

Material and Methods

The experiment was conducted in a protected environment, a greenhouse at the Academic Unit of Agricultural Engineering (UAEAg) of the Federal University of Campina Grande – UFCG (Campina Grande, Paraíba, Brazil), located at the geographical coordinates 7°15'18'' S latitude and 35°52'28'' W longitude, at an altitude of 550 m.

A completely randomized experimental design in a 2 x 10 factorial arrangement was used, with three replications referring to irrigation water salinity levels, which were expressed by the electrical conductivity (ECw) of 0.6 and 5.1 dS m^{-1}, being the latter one prepared by adding sodium chloride (NaCl) to the local supply system water (ECw of 0.6 dS m^{-1}), and ten cowpea genotypes (G1: MNCO1-649F-2-1, G2: MNCO3-736F-2, G3: PINGO DE OURO-1-2, G4: BRS GURGUÉIA, G5: BRS MARATAOÃ, G6: MNCO2-676F-3, G7: MNCO2-683F-1, G8: MNCO3-737F-5-4, G9: MNCO3-737F-5-9, and G10: BRS TUMUCUMAQUE). The cowpea genotypes were obtained from Embrapa Meio-Norte. These genotypes were developed with the intention of being cultivated under the prevailing climatic conditions of the semi-arid region of Northeast Brazil.

Each experimental unit consisted of a 20-L plastic pot, which was filled with soil of which physicochemical characteristics are shown in Table 1.

Table 1. Physicochemical characterization of the soil used in the experiment

Physical characteristics						
Density		Particle size				
Soil	Particles	Sand	Silt	Clay		
(g cm^{-3})		(g kg^{-1})				
1.40	2.67	777.0	112.9	110.1		
Natural Moisture						
FC	PWP			Total porosity		
(%)						
13.22	3.77			47.57		
Chemical Characteristics						
pH	P	Na	Ca	Mg	H	Al
H_2O(1:2.5)	mg/dm³	$cmol_c/dm^3$				
4.8	5.4	0.05	0.51	0.20	0.56	0.40

FC: field capacity; PWP: permanent wilting point; pH: hydrogenation potential; P: phosphorus; Na: sodium; K: potassium; Ca: calcium; Mg: magnesium; H: hydrogen; Al: aluminum

The soil was fertilized according to the recommendations of Novais et al. (1991), for pots in protected cultivation. Three seeds were sown in each pot, and five days after germination, thinning was performed, leaving only one plant per pot.

The plants were irrigated daily with low salinity water (0.6 dS m^{-1}) in order to maintain the soil characteristics close to that of field capacity until thinning; then the treatments with saline water (5.1 dS m^{-1}) were started.

To obtain water electrical conductivity of 5.1 dS m^{-1}, NaCl was added to the water of 0.6 dS m^{-1}. The amount of sodium chloride (A NaCl) used in the water preparation was determined considering the water initial electrical conductivity, according to the method proposed by Richards (1954):

A NaCl (mg L^{-1}) = 640 x (desired ECw - initial ECw) Equation 1

Where: A NaCl corresponds to the amount of NaCl added to water, and ECw is the water electrical conductivity, in dS m^{-1}.

The water volume to be used was calculated according to evapotranspiration demand. The water consumption was determined by the difference between the water volume used and the drainage volume, in order to make the moisture close to that of field capacity, obtaining a leaching fraction of 0.2, as follow:

IV = (WV-DV)/(1-LF) Equation 2

Where: IV = Water volume to be used in the irrigation (mL); WV = Water volume used in previous irrigation (mL); DV = Water volume drained in previous irrigation (mL); and LF = Leaching fraction (0.2).

At 60 days after saline treatment, plant height or the main branch length, stem diameter, and chlorophyll fluorescence were evaluated. The main branch length was measured from the root collar to the apical meristem, using a tape measure graded in centimeters. The stem diameter was measured at 3 cm above the ground surface, using a digital caliper.

Chlorophyll fluorescence was measured using a Portable Fluorometer (PEA - Plant Efficiency Analyser, Hansatech Norfolk, UK) in fully expanded leaves. The leaves were subjected to a dark adaptation period, using specific clips for ≈30 minutes to induce complete oxidation of the reaction centers (Maxwell & Johnson, 2000). The following chlorophyll fluorescence transients were evaluated: initial fluorescence (Fo), maximum fluorescence (Fm), variable fluorescence (Fv), primary photochemical efficiency of photosystem II (Fv/Fo), and maximum photochemical efficiency of photosystem II (Fv/Fm).

The data were subjected to analysis of variance ('F' test). In the case of significant effects, the Scott-Knott test ($p<0.05$) and the Tukey test ($p<0.05$) were performed for the genotype and the salinity factors, respectively.

Results and Discussion

The increase in irrigation water salinity resulted in a reduction in plant growth in all bean genotypes. The G2 and G4 had the lowest reductions whereas the other genotypes had the highest ones (Table 2). Similarly, Sousa et al. (2014) found that increased irrigation water salinity affected the growth of cowpea plants. The salinity of the irrigation water causes a reduction in plant growth because, firstly, it reduces soil osmotic potential, which consequently increases the retention forces, decreasing the absorption of water by the plant and the cell turgescence, which affects the elongation rates and cell division (Santos et al., 2016; Araújo et al., 2017). Therefore, it can be suggested that this condition led to the lowest growth in the studied genotypes, specifically, in the most sensitive ones.

The stem diameter significantly varied between the genotypes subjected to the lowest salinity (0.6 dS m^{-1}), and the G5, G6, G8, and G10 had the largest diameters (Table 2). However, the increase in salinity did not affect the stem diameter in G1 and G2. Similar to the main branch growth, this characteristic is also associated with the plant turgescence. Nevertheless, there seems to be no high variation in bean genotypes as a result of the increase in salinity.

The chlorophyll index (SPAD) did not vary among the genotypes subjected to low salinity. However, increase in salinity did not only affect the G1, G2, G4, and G7 genotypes; the G9, G10, G6, and G3 were the most affected ones, with reductions of 44, 43, 40 and 29%, respectively, in comparison with those subjected to low salinity (Table 2). As the SPAD index is well correlated with the pigment contents, its reduction indicates a decrease in the pigments in response to salinity (Qados, 2011; Koyro et al., 2013). In pepper plants (*Capsicum annuum* L.), photosynthetic pigments were drastically affected by salinity, and chlorophyll *a* was the most sensitive, with a reduction of 50% when subjected to water EC of 9 dS m^{-1} (Melo et al., 2017a). Pigment reduction also occurred in fava (*Vicia faba* L.) due to the increased salinity (Qados, 2011). These reductions probably affected the absorption and use of light energy, as it can be observed in the chlorophyll fluorescence measurements in this study.

The increase in water salinity affected the initial fluorescence (Fo) of the studied genotypes, except for G2, G4, and G8 (Table 2). The G9, G6, G5, and G3 were the genotypes most affected by the water salinity of 9 dS m^{-1}, with reductions of 19, 18, 16 and 10%, respectively, in comparison with those subjected to low salinity, indicating sensitivity to salt stress. The increase in Fo may be interpreted as a damage to the PSII light-collecting complex or as a decrease in the capacity of transferring the excitation energy from the antenna to the PSII (Baker, 2008; Azevedo Neto et al., 2011; Kalaji et al., 2011), as it is observed in the present study. These results involve a higher energy loss per chlorophyll complex, which may be related to the higher difficulty of plants to absorb water and to optimize the process of light energy consumption (Brito et al., 2016).

Table 2. Main branch length (MBL), stem diameter (SD), SPAD index, and initial fluorescence (Fo) in different cowpea genotypes under salt stress

Genotypes	MBL (cm)		SD (mm)		SPAD Index		Fo	
	Salinity (dS m^{-1})							
	0.6	5.1	0.6	5.1	0.6	5.1	0.6	5.1
G1	53.5Ad	24.0Bb	7.01Ac	6.48Aa	46.7Aa	41.2Ab	384.6Ba	487.3Aa
G2	186.1Aa	64.5Ba	7.49Ac	6.47Aa	55.7Aa	50.0Aa	389.5Aa	363.6Ab
G3	45.0Ad	30.5Bb	8.95Ab	6.43Ba	58.2Aa	40.9Bb	421.3Ba	465.0Aa
G4	198.5Aa	48.5Ba	8.63Ab	6.23Ba	56.4Aa	49.4Aa	417.3Aa	402.6Ab
G5	53.3Ad	32.0Bb	9.39Aa	7.63Ba	55.3Aa	44.6Ba	393.0Ba	469.0Aa
G6	47.0Ad	30.0Bb	9.36Aa	6.22Ba	58.9Aa	35.0Bb	380.6Ba	468.0Aa
G7	52.6Ad	27.5Bb	8.45Ab	5.55Ba	58.5Aa	54.7Aa	393.3Ba	474.3Aa
G8	34.0Ad	19.5Bb	10.19Aa	6.27Ba	63.2Aa	46.7Ba	382.6Aa	393.6Ab
G9	78.5Ac	24.0Bb	8.12Ac	6.35Ba	65.2Aa	36.2Bb	375.6Ba	468.0Aa
G10	131.0Ab	35.0Bb	8.77Ab	6.23Ba	63.4Aa	35.6Bb	400.3Ba	454.0Aa
MSD	5.20		0.33		2.98		11.06	
CV%	16.42		8.64		11.27		5.06	

Different uppercase letters indicate a significant difference between water salinity levels by the Tukey test ($p<0.05$); different lowercase letters indicate a significant difference between genotypes at each salinity level by the Scott-Knott test ($p<0.05$); MSD: Minimum significant difference, CV: coefficient of variation.

The maximum fluorescence (Fm) and variable fluorescence (Fv) were only influenced by the increase in water salinity, regardless of the bean genotype (Table 3). The reduction in Fv and Fm is due to the decrease in chlorophyll efficiency in response to the increased salinity, indicating ionic toxicity (Melo et al., 2017b), although there has been no salinity effect regarding the genotypes, as it was observed in Fv/Fo and Fv/Fm.

The primary photochemical efficiency of PSII (Fv/Fo) was not affected by water salinity only in the G2 and G4 genotypes (Table 3). With regards, the other genotypes, the Fv/Fo was reduced in response to the increased salinity, and the G5, G10, and G6 were the most affected genotypes, with decreases of 32, 28 and 26%, respectively, in comparison with those subjected to low salinity.

The Fv/Fo ratio has also been recommended to detect changes induced by salt stress (Kalaji et al., 2011) because, although it provides the same basic information like the Fv/Fm, it is able to identify small variations (Azevedo Neto et al., 2011). This was confirmed in our study, as it can be observed in the comparison between both variables, corroborating the results found by Azevedo Neto et al. (2011) in sunflower genotypes under salt stress. The Fv/Fo ratio has also been used to select salinity tolerant barley genotypes (Kalaji et al., 2011). According to these authors, the Fv/Fo is the most sensitive component of the electron transport chain and, therefore, a decrease in this ratio results from the damage in the photosynthetic electron transport. Thus, these results confirm the importance of evaluating the Fv/Fo ratio in the selection of bean genotypes considering their tolerance to salinity.

Like the primary photochemical efficiency, the maximum photochemical efficiency of PSII (Fv/Fm) was not affected by water salinity only in the G2 and G4 genotypes (Table 3). On the other hand, the G5, G1, and G10 were the most affected genotypes, with reductions of 9, 6 and 5.9%, respectively, in comparison to those subjected to low salinity. The genotypes studied here had the same behavior both regarding the Fv/Fm and Fv/Fo; however, there were more pronounced reductions in the Fv/Fo, which evidences the importance of evaluating this ratio to detect and/or confirm the Fv/Fm variations. Nevertheless, the Fv/Fm is widely reported as efficient in selecting salinity tolerant genotypes, such as in citrus (Brito et al., 2016) and passion fruit (Freire et al., 2014). Thus, it can be suggested that Fv/Fo, Fv/Fm, and Fo, together, were efficient in the selection of salinity tolerant bean genotypes, as it was observed in the distinction between G2 and G4.

Table 3. Maximum fluorescence (Fm), variable fluorescence (Fv), primary photochemical efficiency (Fv/Fo), and maximum photochemical efficiency of PSII (Fv/Fm) of different cowpea genotypes under salt stress

Genotypes	Fm		Fv		Fv/Fo		Fv/Fm	
	Salinity (dS m^{-1})							
	0.6	5.1	0.6	5.1	0.6	5.1	0.6	5.1
G1	2282.0Aa	2307.3Aa	1897.6Aa	1820.0Aa	4.93Aa	3.73Bb	0.83Aa	0.78Bb
G2	2340.6Aa	2018.0Ba	1951.1Aa	1654.3Ba	5.01Aa	4.55Aa	0.83Aa	0.81Aa
G3	2251.0Aa	2074.6Aa	1829.6Aa	1609.6Aa	4.35Ab	3.46Bb	0.81Ab	0.77Bb
G4	2199.6Aa	2089.0Aa	1782.3Aa	1686.3Aa	4.26Ab	4.18Aa	0.80Ab	0.80Aa
G5	2306.6Aa	2020.3Ba	1913.6Aa	1551.3Aa	4.90Aa	3.30Bb	0.83Aa	0.76Bb
G6	2210.0Aa	2130.3Aa	1829.3Aa	1662.3Aa	4.80Aa	3.55Bb	0.82Aa	0.78Bb
G7	2228.3Aa	2192.0Aa	1835.0Aa	1717.6Aa	4.66Aa	3.62Bb	0.82Aa	0.78Bb
G8	2262.3Aa	1927.0Ba	1879.6Aa	1533.3Ba	4.93Aa	3.91Bb	0.83Aa	0.79Bb
G9	2154.0Aa	2197.0Aa	1778.3Aa	1729.0Aa	4.73Aa	3.69Bb	0.82Aa	0.78Bb
G10	2254.0Aa	2195.0Ba	2153.6Aa	1741.0Ba	5.38Aa	3.83Bb	0.84Aa	0.79Bb
MSD	74.45		69.59		0.16		0.005	
CV%	6.49		7.50		7.52		1.38	

Different uppercase letters indicate a significant difference between water salinity levels by the Tukey test ($p<0.05$); different lowercase letters indicate a significant difference between genotypes at each salinity level by the Scott-Knott test ($p<0.05$). MSD: Minimum significant difference, CV: coefficient of variation.

Conclusions

Chlorophyll fluorescence is a tool that can be used to select salinity tolerant bean genotypes, specifically, through initial fluorescence (Fo), primary photochemical efficiency of PSII (Fv/Fo), and maximum photochemical efficiency of PSII (Fv/Fm), considering their combined behavior and relationship to plant growth.

Based on the chlorophyll fluorescence analysis, the G2: MNCO3-736F-2 and G4: BRS GURGUÉIA were the most salinity tolerant genotypes; the others proved to be sensitive to the conditions studied.

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