ISSN 1807-1929
Revista Brasileira de Engenharia Agrícola e Ambiental
v.21, n.4, p.232-237, 2017
Campina Grande, PB, UAEA/UFCG – http://www.agriambi.com.br

DOI: http://dx.doi.org/10.1590/1807-1929/agriambi.v21n4p232-237

Fluorescence of chlorophyll a and photosynthetic pigments in *Atriplex nummularia* under abiotic stresses

Hidelblandi F. de Melo[1], Edivan R. de Souza[1] & Jailson C. Cunha[2]

[1] Universidade Federal Rural de Pernambuco/Departamento de Agronomia/Programa de Pós-graduação em Ciência do Solo. Recife, PE. E-mail: hidelfarias@gmail.com; edivan.rodrigues@ufrpe.br (Corresponding author)
[2] Universidade Federal do Vale do São Francisco/Campus Ciências Agrárias. Petrolina, PE. E-mail: jailson.c.c@gmail.com

Key words:
photosynthesis
water and salt stress
halophyte

A B S T R A C T

Chlorophyll a fluorescence is a very useful tool in ecophysiological studies to analyze the photosynthetic performance of plants under biotic and abiotic stresses. This research aimed to evaluate the parameters of the fluorescence of chlorophyll a, contents of chlorophyll a, b and total, and carotenoids in *Atriplex nummularia* cultivated under water stress (37 and 70% of field capacity) and salt stress (irrigation water with electrical conductivity of 0, 5, 10, 20, 30 and 40 dS m^{-1}), besides two sources of salts: NaCl and a mixture of salts of Ca^{2+}, Mg^{+2}, K^+, Na^+ and Cl^-, in a 6 x 2 x 2 factorial, with 4 replicates, totaling 96 experimental plots. At 91 days after transplanting, the initial fluorescence (F_0), maximum fluorescence (F_m), potential quantum efficiency of photosystem II (F_v/F_m), F_v/F_0 ratio and the contents of chlorophyll a, b, and total and carotenoids were determined. All assessed parameters decreased as a standard response to salt stress, except F_0, which had, as a characteristic, the increase in its values under stress conditions. The emission parameters for *Atriplex nummularia* varied with the type of salt present in the irrigation water.

Palavras-chave:
fotossíntese
estresse salino e hídrico
halófita

Fluorescência da clorofila a e pigmentos fotossintéticos em *Atriplex nummularia* sob estresses abióticos

R E S U M O

A fluorescência da clorofila a é uma ferramenta de grande utilidade em estudos ecofisiológicos para a análise da performance fotossintética de plantas sob estresses bióticos e abióticos. Objetivou-se avaliar os parâmetros da fluorescência da clorofila a, teores de clorofila a, b e total e carotenoides em *Atriplex nummularia* cultivada sob estresses hídrico (37 e 70% da capacidade de campo) e salino (água de irrigação com condutividade elétrica de 0, 5, 10, 20, 30 e 40 dS m^{-1}), além de duas fontes de sais: NaCl e uma mistura com sais de Ca^{2+}, Mg^{2+}, K^+, Na^+ e Cl^-, em fatorial 6 x 2 x 2 com 4 repetições totalizando 96 parcelas experimentais. Aos 91 dias após o transplantio foram determinadas a fluorescência inicial (F_0), a fluorescência máxima (F_m), a eficiência quântica potencial do fotossistema II (F_v/F_m) e a razão F_v/F_0 e os teores de clorofila a, b, total e carotenoides. Os parâmetros de emissão avaliados reduziram como resposta padrão ao estresse salino, com exceção do F_0 que tem, como característica, o aumento de seus valores quando em condições de estresse. Os parâmetros de emissão para a *Atriplex nummularia* variaram de acordo com o tipo de sal presente na água de irrigação.

Ref. 060-2015 – Received 22 Apr, 2016 • Accepted 30 Dec, 2016 • Published 3 Mar, 2017

Introduction

The use of chlorophyll a and photosynthetic pigments is an important tool to evaluate the performance in the light energy harvest by plants under abiotic stress (Shu et al., 2013). The high concentration of salts causes damages to the photosynthetic apparatus and to the metabolic processes in most plants (Glenn et al., 2012). Under these conditions, the contents of pigments constituting the antenna complex may be reduced, leading to a deficit in the harvest of light energy (Bouchenak et al., 2012).

The integrity of the photosynthetic apparatus and the photosynthetic rates are among the main metabolic phenomena affected by salt and water stresses (Glenn et al., 2012). Thus, the evaluation and monitoring of these metabolic phenomena can be used as quantitative and qualitative tools of the stress level and damages associated with it (Ahmed et al., 2012; Shu et al., 2013).

The use of fluorescence parameters allows to analyze, qualitatively and quantitatively, the absorption and use of light energy, besides detecting possible damages in the photosynthetic apparatus of the plant (Vieira et al., 2010; Azevedo Neto et al., 2011). Non-invasive and non-destructive techniques allow the researcher to evaluate the plant during a longer period along the crop cycle and make more-consistent inferences about any hypotheses tested (Baker, 2008). *Atriplex nummularia* is a halophyte widely used in soils degraded by salinity and has been the target of studies in the Northeastern semi-arid region (Souza et al., 2011; 2012; 2014; Melo et al., 2016); however, investigations on its photosynthetic apparatus are still necessary.

This research aimed to evaluate alterations in the parameters of fluorescence emission, as well as in the contents of chlorophyll a, b and total and carotenoids (carotenes + xanthophylls) in *Atriplex nummularia* in response to water and salt stresses.

Material and Methods

The soil used in the experiment was collected in the municipality of Pesqueira-PE, Brazil, in the layer of 0-30 cm, classified as Fluvic Neosol (EMBRAPA, 2013), free from problems of salinity and sodicity. Subsequently, the soil was air-dried, pounded to break up clods, homogenized and sieved through a 4-mm mesh, thus preserving its microaggregation.

The soil was chemically characterized (Table 1) using air dried fine earth (ADFE), through the determination of pH_{H2O} at the ratio of 1:2.5. The exchangeable cations Ca^{2+}, Mg^{2+}, Na^+ and K^+ were extracted using 1 mol L^{-1} ammonium acetate (Thomas, 1982). The saturation extract was obtained by preparing the saturation paste (USSLS, 1954), which was analyzed for electrical conductivity (EC) and pH, determining the soluble bases and the chloride ion, through the method of titration with $AgNO_3$ (EMBRAPA, 1997). The cation exchange capacity (T) was determined through the index cation method using ammonium acetate as extractor (USSLS, 1954). The results for the exchange complex were used to calculate the values of sum of bases (SB) and exchangeable sodium percentage (ESP).

Table 1. Chemical characterization of the Fluvic Neosol

Variables	Values
Saturation extract	
pH_{se} 1/	8.17
EC (dS m^{-1})	1.17
Ca^{2+} ($mmol_c$ L^{-1})	1.00
Mg^{2+} ($mmol_c$ L^{-1})	1.04
Na^+ ($mmol_c$ L^{-1})	5.34
K^+ ($mmol_c$ L^{-1})	1.12
Cl^- ($mmol_c$ L^{-1})	6.804
Exchange complex	
pH $_{(1:2.5)}$	7.70
Ca^{2+} ($cmol_c kg^{-1}$)	5.53
Mg^{2+} ($cmol_c$ kg^{-1})	2.22
Na^+ ($cmol_c$ kg^{-1})	0.26
K^+ ($cmol_c$ kg^{-1})	0.50
SB 2/ ($cmol_c$ kg^{-1})	8.51
Ratio (soluble)	
Na/Ca	5.34
Na/Mg	5.13
Na/K	4.77
Na/Cl	0.79
Cl/Ca	6.80
Cl/Mg	6.54
Cl/Na	1.27
Cl/K	6.07

[1] pH determined in the saturation extract; [2] Sum of Bases; [3] Exchangeable sodium percentage

Physical characterization (Table 2) consisted of granulometric analysis and clay dispersed in water in the ADFE through the hydrometer method, calculating the indices of clay dispersion and flocculation, soil density through the cylinder method and particle density through the volumetric flask method (EMBRAPA, 1997). Field capacity and permanent wilting point were determined based on the soil-water retention characteristic curve (SWRCC). Total porosity was estimated using the values of particle and bulk density.

The experiment was carried out in a greenhouse at the Federal Rural University of Pernambuco, during the months from March to June 2013, totaling 100 days of monitoring. *A. nummularia* plants were cultivated in pots with capacity for 10 L per pot. After transplanting, the plants were subjected to two soil gravimetric moisture contents: 0.17 g g^{-1} (-0.06 MPa), referring to 70% of field capacity and 0.09 g g^{-1} (-0.52 MPa), referring to 37% of field capacity. The moisture contents were selected based on the SWRCC. The waters for irrigation were prepared in the laboratory using two sources of salts, one composed of NaCl and the other composed of a mixture of salts in proportions similar to those found in a well located in the experimental area, which were produced maintaining

Table 2. Physical characterization of the Fluvic Neosol

Sand			Silt	Clay	CDW 1/	Ds 2/	Dp 3/	ID 4/	IF 5/	TP 6/	FC 7/	PWP 8/
Fine	Coarse	Total								%		
g kg^{-1}						kg dm^{-3}					g g^{-1}	
435	17	452	386	162	117	1.36	2.66	0.72	0.28	48.87	0.24	0.05

[1] Clay dispersed in water; [2] Soil bulk density; [3] Soil particle density; [4] Index of dispersion (CDW/Clay); [5] Index of flocculation [IF: (1 – ID)]; [6] Total porosity; [7] Field capacity; [8] Permanent wilting point

six values of electrical conductivity: 0, 5, 10, 20, 30 and 40 dS m^{-1} (Araújo et al., 2006; Silveira et al., 2009; Belkheiri & Mulas, 2013).

In order to justify the composition of the mixture of salts necessary for the treatments, water samples were collected in an Artesian well located close to the area of soil collection. The samples were analysed for the determination of pH, electrical conductivity, Ca^{2+}, Mg^{2+}, through atomic absorption spectrophotometry, Na^+ and K^+, through flame photometry, and Cl^- through the Mohr method (EMBRAPA, 1997).

After determination of the chemical composition and the proportion of each element in the samples, salts of NaCl, KCl, $MgCl_2$ and $CaCl_2$ were weighed to maintain the same proportion of the samples (Table 3) and the electrical conductivity of the solution was determined.

To guarantee genetic uniformity, clones of a single plant were used, produced through cuttings and cultivated in unfertilized substrate (material composed of clay, sand and humus), obtained in seedling trays. The cuttings were cultivated in greenhouse and transplanted 90 days after propagation.

The seedlings were transplanted one day after establishment and equilibrium of the moisture content required in each treatment. Plants were initially irrigated with distilled water for a period of 20 days, with gradual increase (EC increased every two days to achieve the highest value) to avoid osmotic shock on the transplanted plants. Along the entire experiment, the moisture content in the pots was maintained by weighing and replenishment of the volume evapotranspired every day, always in the late afternoon, to allow the soil to come into equilibrium with the desired moisture content during the night.

The variables of chlorophyll a fluorescence [initial fluorescence (F_0), variable fluorescence (F_v), maximum fluorescence (F_m) and quantum yield (F_v/F_m) and (F_v/F_0)] were measured using a fluorometer, FluorPen, model F100 (Photon Systems Instruments), at 91 days after transplanting. The measurements were taken in healthy, fully expanded leaves from the middle third section, exposed to the sun. After the measurements, the leaves were collected for the determination of the contents of chlorophyll a, b and carotenoids.

The contents of chlorophylls a, b and carotenoids were determined according to Lichtenthaler & Buschmann (2001).

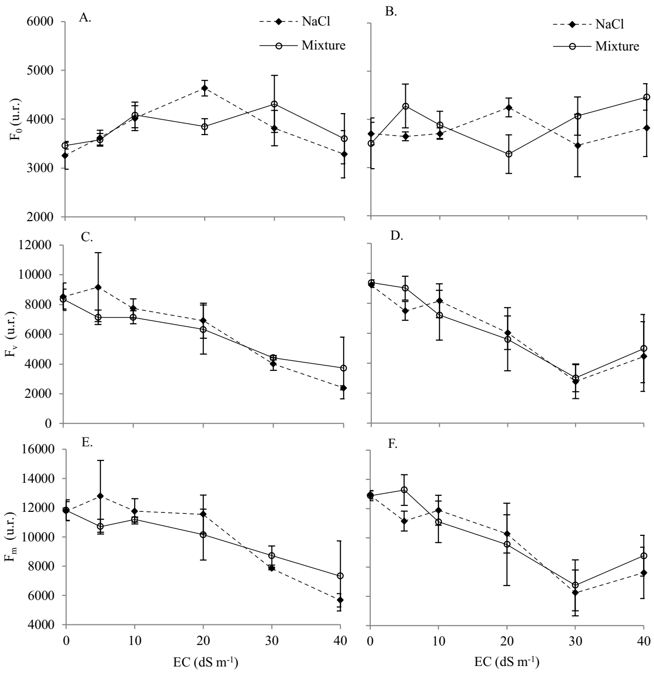
The treatments were arranged in a randomized block design, with four replicates in a 6 x 2 x 2 triple factorial scheme, corresponding to six levels of electrical conductivity (0, 5, 10, 20, 30 and 40 dS m^{-1}); two matric tensions (0.06 and 0.52 MPa) and two sources of salts, NaCl and a mixture of salts of $CaCl_2$, $MgCl_2$, NaCl and KCl, with four replicates, totaling 96 experimental units. After the tests of assumptions (homogeneity of variances and normality), the data were subjected to analysis of variance, test of comparison of means and fits of regressions for the interactions with the salinity levels.

Results and Discussion

Due to the increase in salinity, plants subjected to 37 and 70% of field capacity and different sources of salts showed trend of reduction in chlorophyll a fluorescence for all evaluated parameters (Figures 1 and 2), except F_0, which increased with the increment of salts up to the EC of 20 dS m^{-1} at the soil moisture corresponding to 70% field capacity (Figure 1A).

Under salt stress, the increase in F_0 values eventually indicates damages to the PSII reaction center (Baker, 2008), a behavior observed in plants subjected to 70% FC, in which the higher evaporation demand resulted in greater entry of salts through irrigation water, leading to increments in F_0 values.

Increments in F_0 values associated with reductions in F_m values can be interpreted as an indication of the damage in the



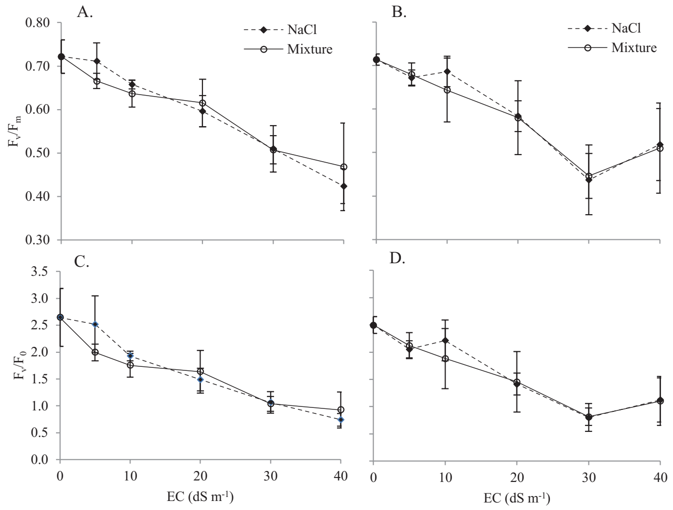
Bars indicate standard deviation around the mean

Figure 1. Effect of the increase in salinity on the parameters of chlorophyll a fluorescence in leaves of *Atriplex nummularia* cultivated at levels of electrical conductivity (EC), soil moisture (70%: A, C and E; 37%: B, D and F) and different sources of salts

Table 3. Amounts of salts (g L^{-1}) necessary to obtained the values of electrical conductivity (EC) used for the irrigation waters of both sources of salts and the osmotic potential of the solutions (Ψ_o)

EC dS m^{-1}	Sources of water							
	NaCl		Mixture of salts					
	NaCl	Ψ_o [1]	NaCl	KCl	$MgCl_2$	$CaCl_2$	Total	Ψ_o [1]
0	-	-0.02	-	-	-	-	-	-0.02
5	2.2202	-0.21	1.6688	0.0100	0.4705	0.3769	2.5263	-0.19
10	5.2591	-0.30	4.3744	0.0262	1.2333	0.9881	6.6222	-0.58
20	13.279	-1.24	10.619	0.0637	2.9941	2.3988	16.0760	-1.17
30	23.128	-2.21	20.454	0.1228	5.7672	4.6206	30.9655	-2.31
40	37.672	-2.99	28.914	0.1736	8.1524	6.5315	43.7721	-3.07

[1] Osmotic potential in MPa



Bars indicate standard deviation around the mean

Figure 2. Maximum quantum yield of PSII (F_v/F_m) and F_v/F_0 ratio of leaves of *Atriplex nummularia* cultivated at levels of electrical conductivity (EC), soil moisture (70%: A and C; 37%: B and D) and different sources of salts

light-harvesting complex of PSII (Fernandez et al., 1997; Li et al., 2010), as observed in the present study. On the other hand, the reduction of F_v and F_m was due to the decrease in the levels of chlorophyll with the increment in EC. The reduction in the parameters of chlorophyll a fluorescence with the increment in salinity was also reported by Correia et al. (2009) in peanut irrigated with saline water (EC 6.0 dS m^{-1}) and is directly related to the degradation of chlorophyll due to the ionic toxicity.

Hence, the differences of F_v and F_m between the moisture contents represent the higher damages caused by the water deficit to the photosynthetic apparatus between the same levels of salinity, at which F_v and F_m were more sensitive than F_0, especially at the highest EC values (Figure 1).

According to Azevedo Neto et al. (2011), the F_v/F_m ratio has been used to detect disturbances in the photosynthetic system favored by salt stress, since its decrease indicates a reduction in the photochemical efficiency of PSII and damages to the photosynthetic apparatus. According to Bjorkman & Demming (1987), F_v/F_m is virtually constant for different species when measured under no-stress conditions and the photosynthetic apparatus of the plant is intact, being equal to $0.75 \leq F_v/F_m \leq 0.86$.

Increments in the values of F_v/F_m indicate increase in the photosynthetic conversion efficiency of PSII (Shu et al., 2013; Mehta et al., 2011). The values of F_v/F_m presented in the literature (Li et al., 2010; Shu et al., 2013) for plants under no-stress conditions (0.75) are above those observed in the present study (0.72).

Bjorkman & Demmin (1987), working with the fluorescence parameters in *Atriplex triangularis*, observed F_v/F_m values of 0.805 for plants with intact photosynthetic apparatus. Such result contrasts with that found in this research, not corresponding to the values of plants under conditions of no stress. It should be noticed that the difference between plants under high moisture and water deficit was not more than 0.01 (Figure 2). Thus, only the water stress applied in the present study was not sufficient to reduce the values of F_v/F_m.

The F_v/F_0 ratio is a very sensitive indicator of the potential photosynthetic activity, in both stressed and healthy plants (Ozfidan et al., 2013). The evaluation of F_v/F_0 has also been recommended to detect changes induced by salt stress, since it amplifies the small variations detected by the F_v/F_m ratio (Azevedo Neto et al., 2011).

Reductions in these values in plants under severe salt stress indicate that the efficiency of the photosynthetic process and electron transport chain were affected (Li et al., 2010; Shu et al., 2013). Thus, analyzing these variables in *A. nummularia* plants, it is possible to observe the reduction in the photochemical efficiency of PSII (Figures 2A and 2C), when there is an increment in irrigation water EC.

In general, the reductions in the quantum yield of PSII can be associated with the degradation of chlorophyll (Figure 2A and 2B), since there was a decrease in the contents of chlorophyll, especially chlorophyll a, in the leaves of *A. nummularia* with the increment in salinity (Figure 3A).

The chlorophyll content is an important physiological index directly related to photosynthesis in plants (Ma et al., 2011). The reduction in the chlorophyll contents in Atriplex is associated with the damages caused by the increase in salinity (Nedjimi, 2014). The degradation of chlorophyll in plants under abiotic stress can be partially attributed to the sensitivity of the membranes to the oxidative stress (Hasegawa et al., 2000).

These environmental conditions lead to the increment in the level of reactive oxygen species, culminating in the

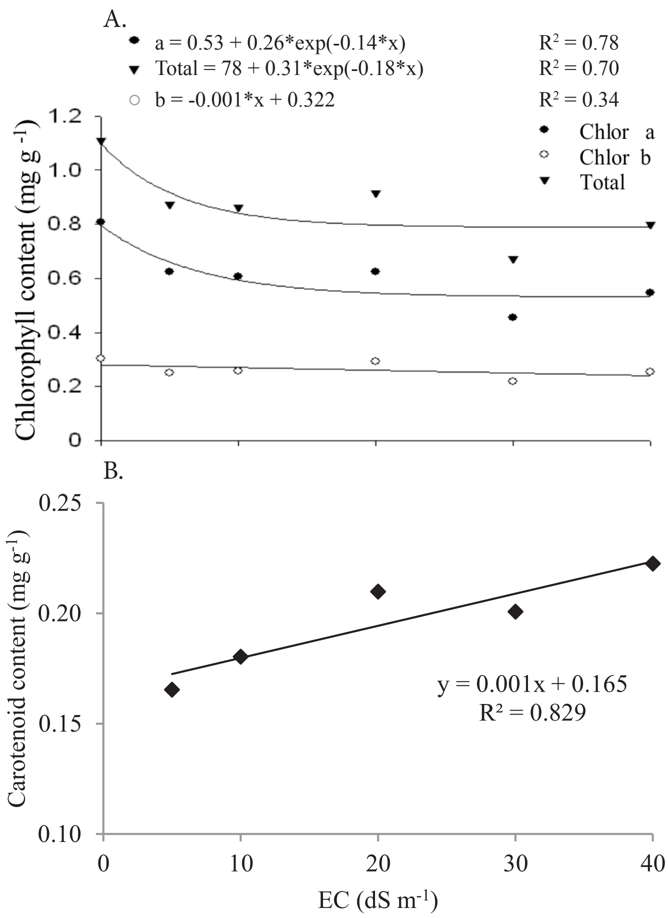


Figure 3. Contents of chlorophyll (A) and carotenoids (B) in leaves of *Atriplex nummularia* Lindl, 91 days after transplanting as a function of the electrical conductivity (EC) levels

peroxidation of the lipid of the membranes, disintegration of thylakoids and reduction in chlorophyll concentration (Paridas & Das, 2005; Ma et al., 2011).

Therefore, Atriplex plants were more efficient in the accumulation of carotenoids with the increment of salts in the irrigation water (Figure 3B). The increase of carotenoids can be associated with mechanisms of protection to the antenna complex, since they act as reducing agents and protecting pigments of oxidative reactions.

Conclusions

1. The reductions in the contents of chlorophyll a, b and total indicate possible oxidative stress and degradation of membranes in plants subjected to high salt concentrations.

2. The increment in salinity up to the maximum studied value of electrical conductivity increases the contents of carotenoids in Atriplex leaves, avoiding greater damages to the photosynthetic apparatus.

3. Atriplex plants were more sensitive to salt stress than to water stress.

4. Plants were more affected by the mixture of salts when subjected to the first four values of electrical conductivity and, from these levels on, they were more sensitive to NaCl.

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