# SALT STRESS CHANGE CHLOROPHYLL FLUORESCENCE IN MANGO[1]

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**ABSTRACT -**This study evaluated the tolerance of mango cultivars 'Haden', 'Palmer', 'Tommy Atkins' and 'Uba' grafted on rootstock 'Imbú' to salt stress using chlorophyll fluorescence. Plants were grown in modified Hoagland solution containing 0, 15, 30, and 45 mmol $L^{-1}$ NaCl. At 97 days the parameters of the chlorophyll fluorescence ($F_0$, $F_m$, $F_v$, $F_0/F_m$, $F_v/F_m$, $F_v'/F_m'$, $\Phi PSII = [(F_m'-F_s)/(F_m')]$, $D = (1- F_v'/F_m')$ and $ETR = (\Phi PSII \times PPF \times 0{,}84 \times 0{,}5)$ were determined. At 100 days, the leaf emission and leaf area, toxicity and leaf abscission indexes were determined. In all cultivars evaluated, in different degree, there were decreases in photochemical efficiency of photosystem II, enhanced concentrations from 15 mmol $L^{-1}$ NaCl. The decreases in the potential quantum yield of photosystem II ($F_v/F_m$) were 27.9, 18.7, 20.5, and 27.4%, for cultivars 'Haden', 'Palmer', 'Tommy Atkins', and 'Uba', respectively, when grown in 45 mmol $L^{-1}$ NaCl. It was found decreases in leaf emission and mean leaf area in all cultivars from 15 mmol $L^{-1}$ NaCl. There were increases in leaf toxicity of 33.0, 67.5, 41.6 and 80.8% and in leaf abscission of 71.8, 29.2, 32.5, and 67.9% for the cultivars 'Haden', 'Palmer', 'Tommy Atkins', and 'Uba' respectively, when grown in 45 mmol $L^{-1}$ NaCl. Leaf toxicity and leaf abscission were not observed in 15 mmol $L^{-1}$ NaCl. The decrease in $F_v/F_m$ ratio were accompanied by decreasing in leaf emission and increased leaf toxicity index, showing, therefore, the potential of chlorophyll fluorescence in the early detection of salt stress in mango tree.
**Index terms:** *Mangifera indica* L., photosystem II, vegetative growth, salt stress.

## ESTRESSE SALINO ALTERA A FLUORESCÊNCIA DA CLOROFILA EM MANGUEIRA

**RESUMO -** Este trabalho teve como objetivo avaliar o efeito do estresse salino sobre a eficiência fotoquímica do fotossistema II (*PSII*) nas cultivares de manga 'Haden', 'Palmer', 'Tommy Atkins' e 'Ubá' enxertadas sobre o porta-enxerto 'Imbu'. Foi utilizada solução nutritiva de Hoagland modificada contendo 0; 15; 30e 45 mmol $L^{-1}$ NaCl. Aos 97 dias após a exposição ao estresse salino, foram avaliados os parâmetros da fluorescência da clorofila ($F_0$, $F_m$, $F_v$, $F_0/F_m$, $F_v/F_m$, $F_v'/F_m'$, $\Phi PSII = [(F_m'-F_s)/(F_m')]$, $D = (1- F_v'/F_m')$ e $ETR = (\Phi PSII \times PPF \times 0{,}84 \times 0{,}5)$. Aos 100 dias, foram avaliados a emissão foliar, a área média de folhas ($cm^2$), o índice de toxidez nas folhas e o índice de abscisão foliar. Em todas as cultivares, em graus diferenciados, ocorreram decréscimo na eficiência fotoquímica do fotossistema II, na emissão de folhas, e aumento nos índices de toxidez e abscisão foliar, intensificados nas concentrações a partir de 15 mmol $L^{-1}$ NaCl. As plantas cultivadas em 45 mmol $L^{-1}$ NaCl apresentaram decréscimos na razão $F_v/F_m$ de 27,9; 18,7; 20,5 e 27,4%, incremento no índice de toxidez foliar de 33,0; 67,5; 41,6 e 80,8% e no índice de abscisão foliar de 71,8; 29,2; 32,5 e 67,9% para as cultivares 'Haden', 'Palmer', 'Tommy Atkins' e 'Uba', respectivamente. Os decréscimos na razão $F_v/F_m$ foram acompanhados de redução na emissão de folhas e aumento no índice de toxidez foliar, mostrando, portanto, o potencial da fluorescência da clorofila na detecção precoce de estresse salino em mangueira.
**Termos para Indexação:** *Mangifera indica* L., fotossistema II, crescimento vegetativo, estresse salino.

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## INTRODUCTION

The mango (Mangifera indica L.) under irrigated conditions is widely cultivated in the semi-arid northeast of Brazil, where many have problems of soil salinity (AUDRY; SUASSUNA, 1995, HECK et al., 2003). Environmental factors such as salinity, which affect plant growth, have been investigated using measurements of quantum efficiency of photosystem II (PS II) (HAVAUX et al., 1988). This Fv/Fm ratio expresses the quantum yield of photochemical processes of photosystem, namely the relative efficiency of light energy capture by PS II (BAKER, 2008).

Environmental conditions that provide the concentration of intracellular $Na^+$ ions, $K^+$ and $Cl^-$, leads to irreversible inactivation of photosystem I (*PS I*) and *PS II*. This inactivation may also occur in the electron transport respiratory chain (ALLAKHVERDIEV et al., 2000). The use of fluorescence parameters allow to assess the reduction in electron transport disorder diagnosed by the emission of heat in the form of infrared radiation or by fluorescence. This methodology is based on the kinetics of light absorbed by antenna pigments and the excitation energy transferred to the reaction centers of photosystem I and II (KRAUSE; WEIS, 1991).

According to Schreiber et al. (1998), the relationship between $F_m$ (all reduced plastoquinone) and $F_0$ (all oxidized plastoquinone) is approximately 5 to 6 in healthy leaves and adapted to shade. Under optimal conditions for the plant, the proportion of radiant energy emitted as fluorescence is reduced. However, under stressful conditions, the chlorophyll fluorescence changes, (ALLAKHVERDIEV et al., 2000). Thus, in vivo fluorescence of chlorophyll provides an early indication of photosynthetic dysfunction and can be used as a test to locate possible sites of lesions induced by salinity within the chloroplasts (SMILLIE; NOTT, 1982).

Thus, the analysis of the performance of photosystem II in physiological parameters that is important for the diagnosis of stress in plants, which show stress responses in a short time. This study aimed to evaluate the effect of salt stress on the photochemical efficiency of photosystem II (*PS II*) from mango cultivars 'Haden', 'Palmer', 'Tommy Atkins' and 'Uba'.

## MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the Plant Science Department, at the Federal University of Viçosa, in Viçosa-MG, from March 26$^{th}$ to August 8$^{th}$, 2008. Hydroponic system was used static, aerated with Hoagland solution modified with the following concentrations: N (13.0 mmol $L^{-1}$), P (1.0 mmol $L^{-1}$), K (4.0 mmol $L^{-1}$), S (2.0 mmol $L^{-1}$), Ca (5.0 mmol $L^{-1}$), Mg (2.0 mmol $L^{-1}$) e B (25.0 µmol $L^{-1}$), Mn (2.0 µmol $L^{-1}$), Zn (2.0 µmol $L^{-1}$), Cu (0.5 µmol $L^{-1}$), Mo (0.5 µmol $L^{-1}$) and Fe (80 µmol $L^{-1}$).

Seedlings were grafted in mango trees on the rootstock 'Imbu' with approximately 18 months from the nursery. The experiment was conducted in a factorial (4 x 4) in randomized blocks, with five replicates and one plant each. The factorial design consisted of four concentrations of NaCl (0, 15, 30, and 45 mmol $L^{-1}$) and four mango cultivars ('Haden', 'Palmer', 'Tommy Atkins', and 'Uba'). The EC ($dS.m^{-1}$) for treatments 0, 15, 30 and 45 mmol $L^{-1}$ NaCl were 1.26, 2.46, 4.04 and 5.68 $dS.m^{-1}$, respectively.

Plants were grown in a volume of seven $dm^3$ of nutrient solution, the volume being restored to its initial value with deionized water on alternate days. The pH was adjusted to 5.5 ± 0.2 with an acid solution (0.1 mol $L^{-1}$ $HNO_3$) and/or basic solution (0.1 mol $L^{-1}$ KOH), also on alternate days. The electrical conductivity (EC) of the solution was monitored weekly, and performed the renewal of the solution when it was observed a depletion of 20% of the initial value of the electrical conductivity of the control (0 mmol $L^{-1}$ NaCl). Analyses of chlorophyll fluorescence were performed at 97 days after exposure to salt stress with the aid of model modulated fluorometer PEA (Plant Efficiency Analyzer, Hansatech Instruments Limited, UK). The induction kinetics of chlorophyll fluorescence followed the pattern described by Rohácek (2002). At the end of the experiment 100 days after exposure to salt stress, plants were collected by evaluating the leaf numbers, the average leaf area ($cm^2$) model LICOR LI-3100 (LI-COR, Inc., Lincoln, Nebraska, EUA1987), the rate of leaf abscission (%) obtained by the ratio ($LN_b/LN_e \times 100$), where $LN_b$: the leaf numbers at the beginning of the experiment, and $LN_e$: is the leaf numbers at the end of the experiment, the index of toxicity in leaves (%) obtained by ratio ($LA_d/LA_t \times 100$), where $LA_d$: the damaged leaf area and $LA_t$: is the total leaf area, using all the leaves of each plant, obtained with the aid of image analysis system (Win Days Delta-Device, UK). The results were subjected to analysis of variance and regression at 5% probability using the "Systems for Genetic Analysis and Statistics – SAEG" (UFV, 2000).

## RESULTS AND DISCUSSION

***Leaf emission, abscission and toxicity indexes***

There was a decrease in the leaf numbers, in all cultivars with increasing concentrations of NaCl (*p≤0.05*). In plants grown at lower concentration (15 mmol $L^{-1}$ NaCl) the decreased estimate average of the leaf numbers, was 25.0 ± 2.0% for all cultivars. However, at higher concentration (45 mmol $L^{-1}$ NaCl) the decrease in the leaf numbers was around 75.0% in the all cultivars (Figure 1A).

The low emission of shoots results in a decrease in leaf area, causing an excessive increase in the $Na^+$ and $Cl^-$ levels in developed leaves (data not shown) and trigger the process of senescence (Fig. 1C) and necrotic leaves (Fig. 1D), which alter the photosynthetic process (data not shown), reducing the amount of photoassimilates important for plant growth (MUNNS, 2002).

Besides reducing the emission of leaves, the average leaf area, was lower with increasing salinity in all cultivars. The decrease in average leaf area (Figure 1B) probably occurred due to the reduction of meristematic activity, and mainly due to lower potential wall, initially caused by water and osmotic stress, leading to lower occurrence of cell turgor and consequently a lower cell expansion (RADIĆ et al., 2005).

With the increase of the period of plant exposure to stress, there was likely an ionic imbalance due to the excessive content of $Na^+$ and $Cl^+$ in tissues (Figures 1E and 1F). These results suggest that there may have been limited ability to compartmentalization of ions in the vacuole with consequent loss of membrane integrity, followed by the appearance of foliar symptoms of phytotoxicity (Figure 1D).

The leaf abscission was increased from a concentration of 15 mmol $L^{-1}$ NaCl (*p≤0.05*). In 15 mmol $L^{-1}$ NaCl the rate of leaf abscission was similar to control plants (Figure 1C). The cultivar 'Tommy Atkins' showed lower rates of leaf abscission, whereas the cultivars 'Haden' and 'Uba' have higher rates of leaf abscission at concentrations of 15 mmol $L^{-1}$ NaCl. These results show that the salinity tolerance depends on the combination scion/rootstock, recorded by the index of abscission around 70% in the cultivars 'Haden' and 'Uba', while cultivars 'Palmer' and 'Tommy Atkins' index showed around 30.0%, both at the highest concentration of salts. Bañuls e Primo-Millo (1995) also observed different rates of leaf abscission depending on the rootstock in citrus cultivars subjected to salt stress.

Although it was observed reduction of leaf emission (Figure 1A) and average leaf area (Figure 1B), only after the concentration of 30 mmol $L^{-1}$ NaCl phytotoxicity symptoms in the leaves became apparent and were observed in all cultivars analyzed (Figure 1D).

***Initial fluorescence ($F_0$)***

The cultivars showed increases in initial fluorescence ($F_0$) with increasing salinity (*p≤0.05*). However, in 15 mmol $L^{-1}$ NaCl with all cultivars the photosynthetic apparatus appears to be little affected by salinity (Figure 2A). Hipkins and Baker (1986) define $F_0$ as a benchmark for determining the fluorescence of the other variables. However, the $F_0$ is not always a constant; its value may increase if the *PSII* reaction center is compromised or if the transfer of excitation energy from the antenna to the reaction centers is impaired (SCHREIBER et al., 1998). Thus, the increase in $F_0$ observed in mango cultivars studied may be associated with damage to the photosynthetic apparatus, such as inactivation partially reversible or even irreversible reaction centers of *PSII* (YAMANE et al., 1997), when these plants were subjected to higher concentrations of NaCl.

***Maximal fluorescence ($F_m$)***

The cultivars 'Palmer' and 'Tommy Atkins' had higher maximum fluorescence ($F_m$) at all NaCl concentrations, while 'Haden' and 'Uba' had lower mean values of $F_m$ (Figure 2B). However, the greatest mean maximum fluorescence ($F_m$) observed in 'Palmer' cannot be related to the greater efficiency of energy capture and converted into chemical energy in step biochemistry of photosynthesis, as seen in the actual efficiency of photosystem II (Figure 3B) and the rate of electron transport (Figure 3D) was observed in this cultivar when submitted to salt stress. According to Silva et al. (2008) the highest values of $F_m$ and $F_v/F_m$ in 'Palmer' can be associated with a higher number of active reaction centers of PSII ($RC/CS_0$) as well as to higher levels of chlorophyll observed in this cultivar, since the increase of these relations did not reflect a significant increase in energy absorption, indicating a low capacity to transfer energy from the light-collecting systems (antenna).

Thus, it appears in Figure 2B that the reduction of $F_m$ can be associated with increased non-photochemical dissipation as heat (Figure 3C), associated with the xanthophyll cycle (MÜLLER et al., 2001). The reduction of electron acceptors such as $NADP^+$, and energy requirements as ATP, depending on enzyme-sensitive $Na^+$ and $Cl^-$ are undermining the activity cycle of carbon fixation (ABDEL-LATIF, 2008) can also be a reason for the rate of reoxidation of the plastoquinone (Qa) being less than the rate of reduction, thus leading to a reduction of $F_m$ with increasing salinity.

***Quantum yield baseline ($F_0/F_m$)***

The quantum yield baseline ($F_0/F_m$) was increased due to the high concentrations of NaCl in the nutrient solution (Figure 2D). The higher $F_0/F_m$ indicates that the initial rate of reduction of the plastoquinone (Qa) was higher than the rate of plastoquinone reoxidation by b (Qb) and the activity of photosystem I (PSI) when plants were exposed to higher concentrations of NaCl. Rohácek (2002) points to the increase relation $F_0/F_m$ as stress indicative suggesting normal values as standard, observed between 0.14 and 0.20.

***Potential quantum yield ($F_v/F_m$)***

The increase in salt concentration provided the decline in maximum quantum yield ($F_v/F_m$). Decrease of efficiency of the capture of light energy in all cultivars (Fig. 2C). All cultivars in the absence of stress (control) had $F_v/F_m$ ratio within the range established by Bolhàr-Nordenkampf and Orquist (1993). These authors have established the value of $F_v/F_m$ ratio between 0.750 and 0.850 in healthy plants in the absence of stress (biotic or abiotic).

***Effective quantum yield ($F_v'/F_m'$)***

The increase in salt concentration brought the decline of effective quantum yield ($F_v'/F_m'$) ($p \leq 0.05$). At higher concentrations of NaCl (45 mmol $L^{-1}$) $F_v'/F_m'$ showed significant decline, and we observed 36.0% reduction in 'Haden', 25.3% in 'Palmer', and 34.8% in 'Uba', especially the cultivar 'Tommy Atkins' which showed a smaller reduction (18.7%) (Figure 3A). The term $F_v'/F_m'$ (Genty parameter) represents the conversion efficiency of the energy of electrons by open reaction centers of photosystem II (*PSII*) into chemical energy (SCHREIBER et al., 1994). The effective quantum yield best represents the variations in quantum yield of photosynthesis than the ratio $F_v/F_m$ (potential quantum yield) and can be used together with photochemical quenching (qP) to estimate the rate of electron transport is the photosynthetic photon flux (PPF) on incident photosynthetic tissue is known (GENTY et al., 1989).

***Photosystem II Efficiency (ΦPSII)***

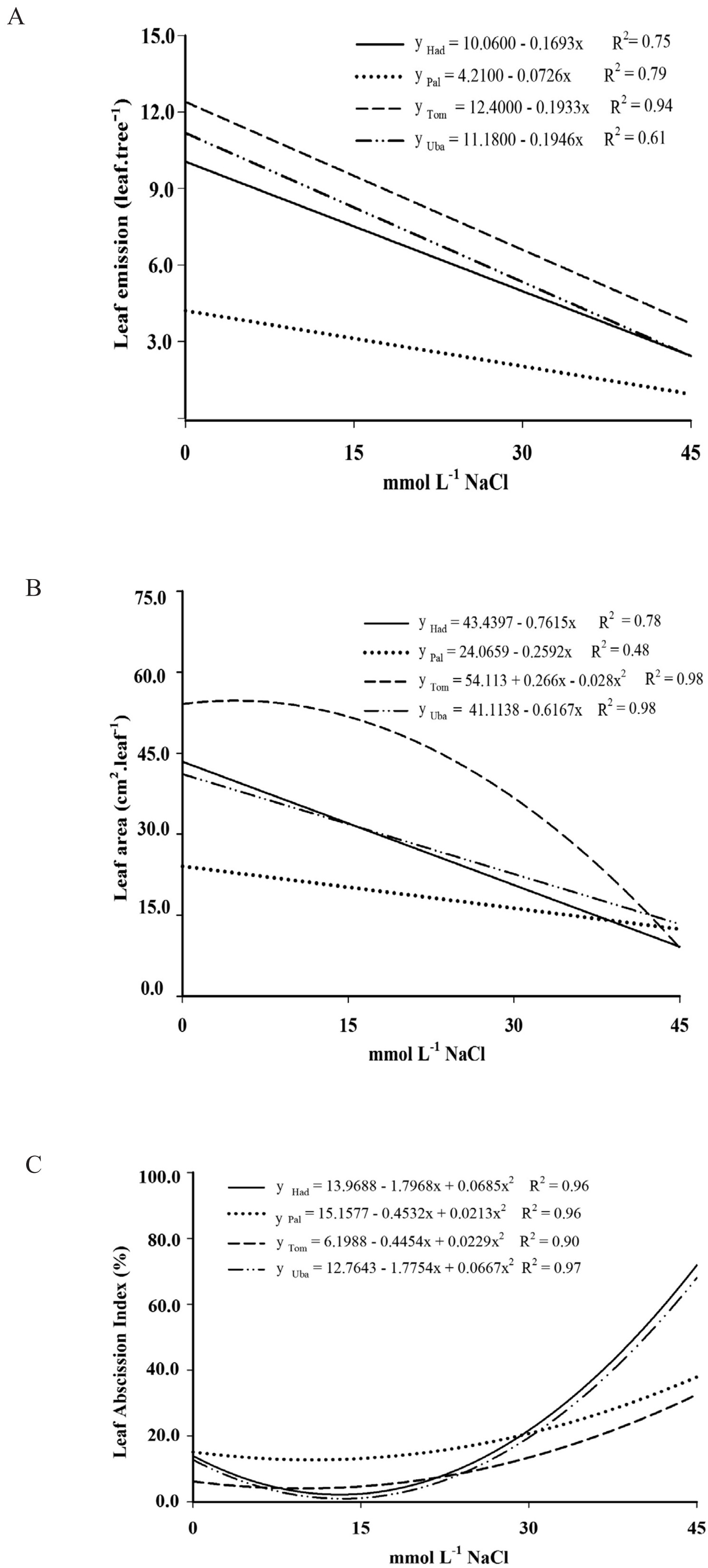
The efficiency of photosystem II (*ΦPSII*) showed decreases with increasing the salt concentration in the nutrient solution ($p \leq 0.05$). According to Schreiber et al. (1998), 1 mol of photons causes excitation 1 µmol electrons of the chlorophyll in this condition, it appears that the efficiency of photosystem II (*ΦPSII*) represents the proportion of these electrons that are used during photochemical reduction of $NADP^+$. The cultivars 'Palmer' and 'Uba' showed the greatest reduction in *ΦPSII*, while 'Haden' and 'Tommy Atkins' showed tolerance up to a concentration of 15 mmol $L^{-1}$ NaCl (Figure 3B).

***Thermal Dissipation (D)***

The dissipation (*quenching*) of light energy focused on the photosynthetic apparatus to heat increased with increasing NaCl in the nutrient solution ($p \leq 0.05$). The increase in the dissipation of light energy in the form of heat (*D*) reached average values of 75% in the cultivar 'Haden' and the other 30% when cultured in 45 mmol $L^{-1}$ NaCl (Figure 3C). According to Schreiber et al. (1998), during application of saturating light pulse and after the tissue has been kept in the dark, the quantum yield of photochemical process (*Ph*) reduces to zero and the emission of fluorescence and heat dissipation become maximum ($F_m$ and $D_m$). In plants under stress, the increase in non-photochemical in the form of thermal energy (*D*) is correlated with increasing proportion xanthophyll/chlorophyll and the rapid conversion of violaxanthin to zeaxanthin in the presence of light, featuring photoinhibition (Demmig-Adams; Adams, 1992).

***Electron Transport Rate (ETR)***

The mango cultivars analyzed showed a reduction in transport rate (*ETR*) with increasing NaCl concentration ($p \leq 0.05$). However, the reduction in *ETR* occurred differentially among cultivars (Figure 3D). In 45 mmol $L^{-1}$ NaCl, only the cultivar 'Tommy Atkins' showed less reduction (29.8%) of *ETR*, with the other cultivars showing an average reduction of 50% in the *ETR*. The electron transport reduction mediated by *PSII* and *PSI* in isolated thylakoid membrane of *Synechococcus sp*. subjected to salt stress was reported by Allakhverdiev et al. (2000). The effect of ionic caused by the influx of sodium ions through the channels $K^+/Na^+$ increases the concentration of salt in the cytosol and causes the dissociation of plastocyanin or cytochrome c553 complex PSI causing decrease in the rate of electron transport mediated by *PSI* and *PSII* (ALLAKHVERDIEV; MURATA, 2008).


A
15.0
12.0
9.0
6.0
3.0
Leaf emission (leaf.tree$^{-1}$)
$y_{Had} = 10.0600 - 0.1693x$ $R^2 = 0.75$
$y_{Pal} = 4.2100 - 0.0726x$ $R^2 = 0.79$
$y_{Tom} = 12.4000 - 0.1933x$ $R^2 = 0.94$
$y_{Uba} = 11.1800 - 0.1946x$ $R^2 = 0.61$
0
15
30
45
mmol L$^{-1}$ NaCl
B
75.0
60.0
45.0
30.0
15.0
0.0
Leaf area (cm$^2$.leaf$^{-1}$)
$y_{Had} = 43.4397 - 0.7615x$ $R^2 = 0.78$
$y_{Pal} = 24.0659 - 0.2592x$ $R^2 = 0.48$
$y_{Tom} = 54.113 + 0.266x - 0.028x^2$ $R^2 = 0.98$
$y_{Uba} = 41.1138 - 0.6167x$ $R^2 = 0.98$
0
15
30
45
mmol L$^{-1}$ NaCl
C
100.0
80.0
60.0
40.0
20.0
0.0
Leaf Abscission Index (%)
$y_{Had} = 13.9688 - 1.7968x + 0.0685x^2$ $R^2 = 0.96$
$y_{Pal} = 15.1577 - 0.4532x + 0.0213x^2$ $R^2 = 0.96$
$y_{Tom} = 6.1988 - 0.4454x + 0.0229x^2$ $R^2 = 0.90$
$y_{Uba} = 12.7643 - 1.7754x + 0.0667x^2$ $R^2 = 0.97$
0
15
30
45
mmol L$^{-1}$ NaCl

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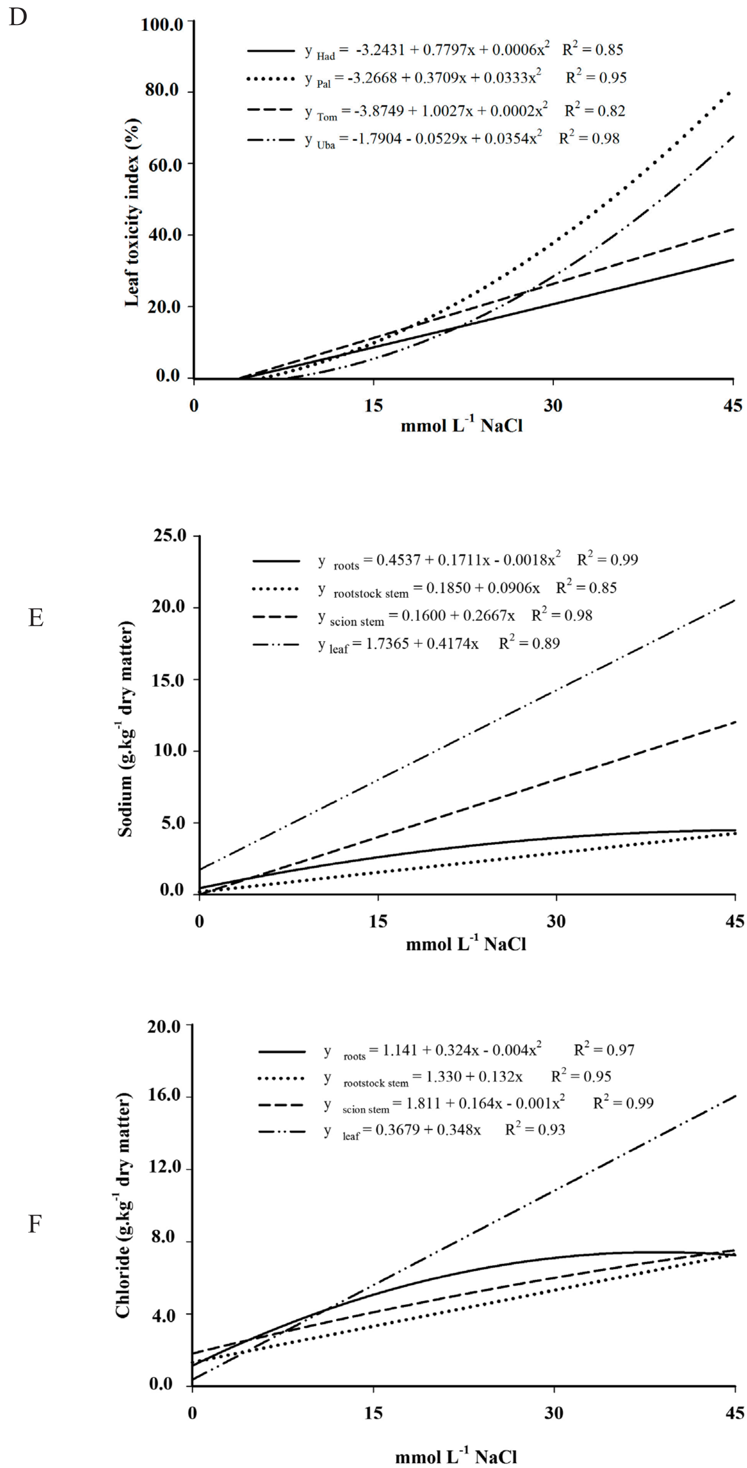
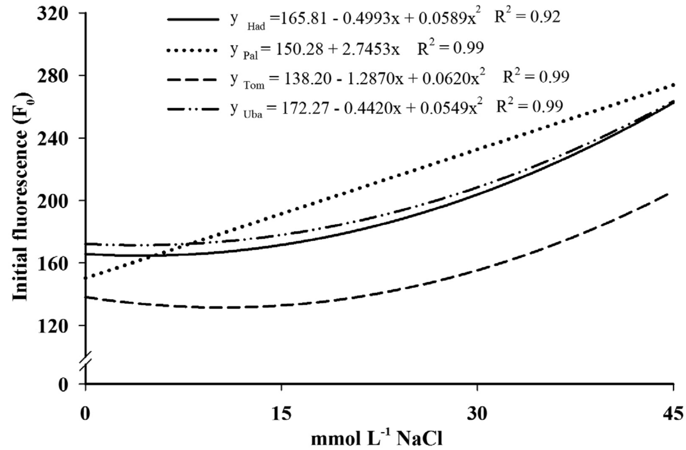
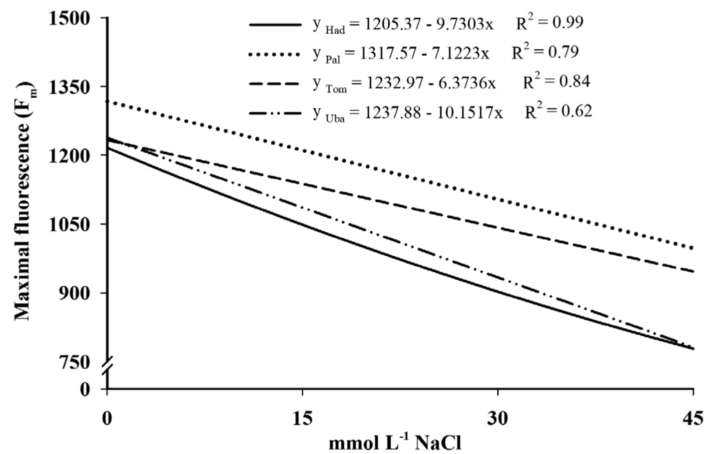



**FIGURE 1** – (A) Number of emitted leaves ($leaf.tree^{-1}$), (B) mean leaf area ($cm^2.leaf^{-1}$), (C) leaf abscission index (%), (D) leaf toxicity index (leaf necrosis area or chlorosis/leaf total area), (E) sodium content ($g.kg^{-1}$ dry matter) and (F) chloride content ($g.kg^{-1}$ dry matter) in mango tree cultivars ‘Haden’ (Had), ‘Palmer’ (Pal), ‘Tommy Atkins’ (Tom) and ‘Ubá’ (Uba) grafted on rootstock ‘Imbú’, grown in nutrient solution and submitted to salt stress.

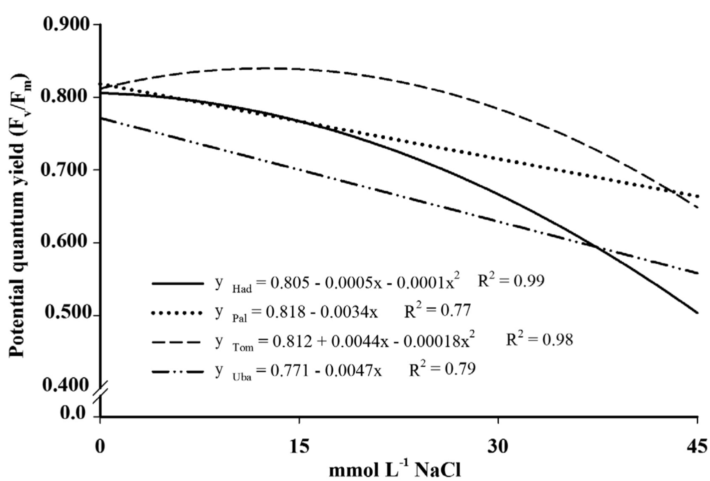
A




B




C

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D

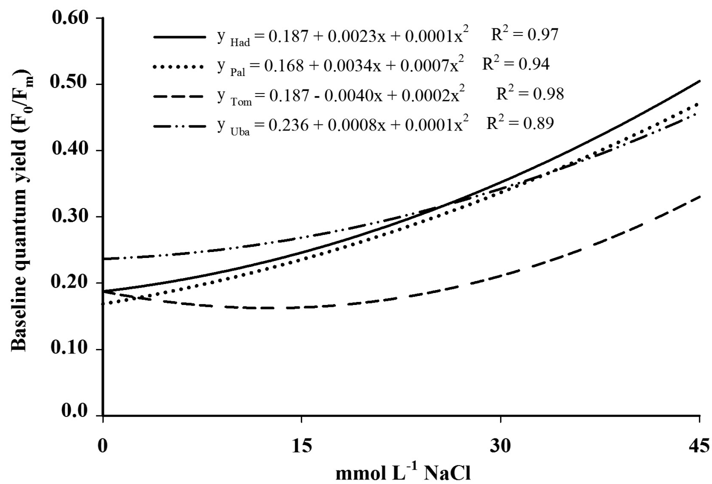
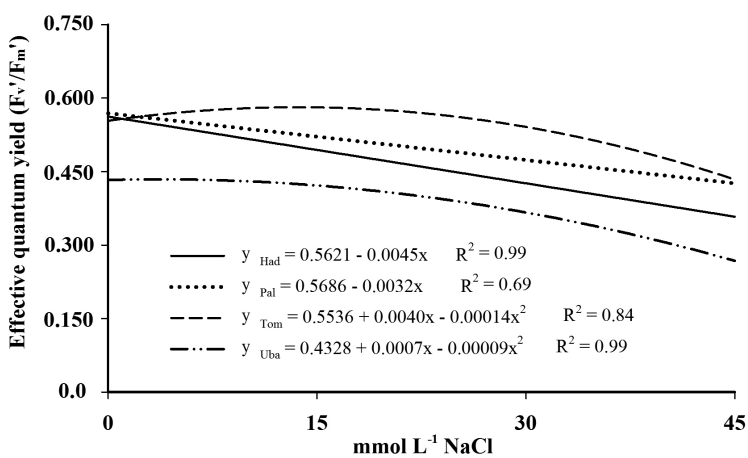
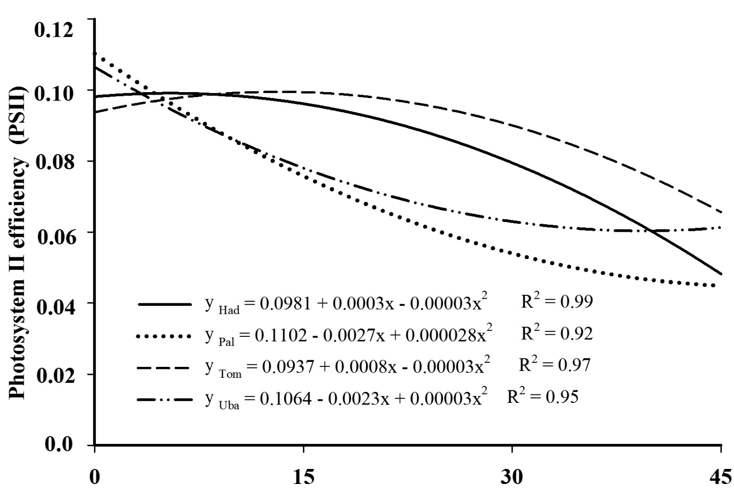



**FIGURE 2** – (A) Initial fluorescence ($F_0$), (B) maximal fluorescence ($F_m$), (C) potential quantum yield ($F_v/F_m$), (D) baseline quantum yield ($F_0/F_m$) in mango tree cultivars 'Haden' (Had), 'Palmer' (Pal), 'Tommy Atkins' (Tom) and 'Ubá' (Uba) grafted on rootstock 'Imbú', grown in nutrient solution and submitted to salt stress.

A




B

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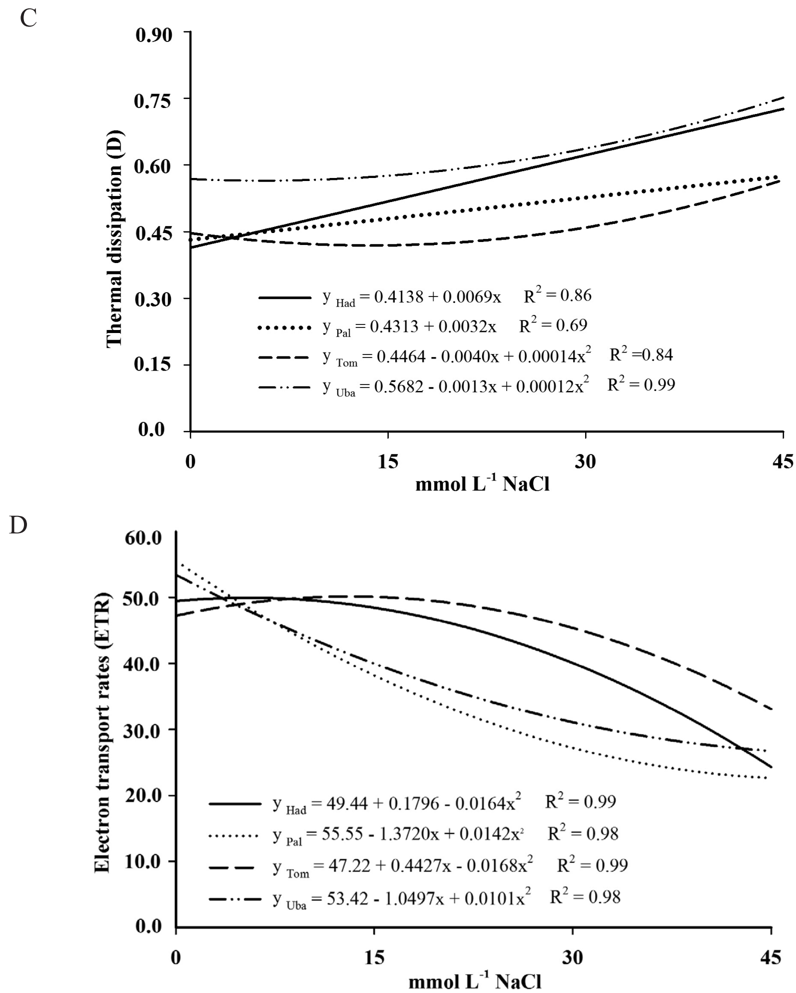


**FIGURE 3** – (A) Effective quantum yield ($F_v'/F_m'$), (B) photosystem II efficiency (*ΦPSII*), (C) thermal dissipation (*D*), (D) electron transport rate (*ETR*) in mango tree cultivars 'Haden' (Had), 'Palmer' (Pal), 'Tommy Atkins' (Tom) and 'Ubá' (Uba) grafted on rootstock 'Imbú', grown in nutrient solution and submitted to salt stress.

## CONCLUSIONS

The mango cultivars analyzed showed reduction in leaf and the average area of leaves when exposed to concentrations greater than 15 mmol $L^{-1}$ NaCl. There were no visible symptoms of foliar phytotoxicity at a concentration of 15 mmol $L^{-1}$ NaCl. All chlorophyll fluorescence parameters evaluated were altered in the presence of NaCl in the nutrient solution. The decrease in photochemical efficiency of photosystem II, in differing degrees, occurred in all cultivars and was enhanced at concentrations above 15 mmol $L^{-1}$ NaCl.

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