

# CRESCIMENTO E FOTOSSÍNTESE EM PLANTAS DE RABANETE SOB ESTRESSE SALINO

## PLANT GROWTH AND LEAF PHOTOSYNTHESIS IN RADISH PLANTS UNDER NaCl STRESS

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### RESUMO

Os efeitos do estresse salino (0-200 mM NaCl) sobre o crescimento, a composição de pigmentos fotossintéticos e fotossíntese foram investigados em plantas de rabanete (*Raphanus sativus* L. cv. Cometo) irrigadas com solução nutritiva de Hoagland 1/2 força. A presença de 50 mM de NaCl resultou na redução da área foliar, da matéria seca da parte aérea e do conteúdo de clorofila e carotenóides. Porém, a matéria seca da raiz reduziu-se somente em concentrações iguais ou maiores que 100 mM de NaCl. O estresse salino não teve efeito sobre a eficiência máxima do fotossistema II ( $F_0$ ,  $F_m$ ,  $F_v/F_m$  and  $F_v/F_0$ ). Entretanto, o quenching fotoquímico ( $q_P$ ) diminuiu significativamente com o aumento da concentração salina em 50 mM, sem variação no quenching não-fotoquímico ( $q_N$ ). A taxa fotossintética líquida ( $A$ ) medida em folhas jovens completamente expandidas reduziu-se significativamente em concentrações salinas maiores que 100 mM. Nossos resultados mostram que altas concentrações salinas afetam o crescimento do rabanete através da redução da área foliar total e da produção de matéria seca, bem como através do decréscimo no conteúdo de clorofila. Embora não tenham ocorrido mudanças nos parâmetros da fluorescência da clorofila, elevadas concentrações de NaCl contribuíram para a redução da taxa fotossintética líquida.

Palavras-chave: *Raphanus sativus*, Concentração de clorofila, Fluorescência da clorofila, Salinidade.

### ABSTRACT

The effects of salt stress (0-200 mM NaCl) on growth, photosynthetic pigment composition and photosynthesis were investigated in radish (*Raphanus sativus* L. cv. Cometo) plants watered with half-strength Hoagland nutrient solution. The presence of 50 mM NaCl resulted in reductions in leaf area, shoot dry weight and total chlorophyll and carotenoids content. However, the root dry matter decreased only above 100 mM NaCl concentrations. Salt stress induced no effect on the maximal efficiency of PSII photochemistry ( $F_0$ ,  $F_m$ ,  $F_v/F_m$  and  $F_v/F_0$ ). However, the photochemical quenching ( $q_P$ ) decreased significantly in salt concentrations higher than 50 mM NaCl, with no variation in non-photochemical quenching ( $q_N$ ). The net photosynthetic rate ( $A$ ) measured in fully expanded young leaves decreased significantly in salt concentrations above 100 mM NaCl. Our results show that high salinity affected the radish growth as result of the total leaf area and dry weight reduction was parallel to decreased chlorophyll content. Although no detected changes occurred in the chlorophyll fluorescence parameters, high salt concentrations could have contributed to reduced leaf photosynthetic rate.

Keywords: *Raphanus sativus*, Chlorophyll concentration, Chlorophyll fluorescence, Salinity.

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(Recebido para Publicação em 10/04/2007, Aprovado em 21/12/2007)

## INTRODUCTION

Soil salinity is one of the most important negative factors in agriculture (VAIDYANATHAN et al., 2003). High salt content can influence physiological processes of plants (SUDHIR & MURTHY, 2004). In saline areas or areas irrigated with saline waters most crop plant species exhibit marked decrease in growth, photosynthesis and yield (AGASTIAN et al., 2000; LACERDA et al., 2001; LU et al., 2002).

The reduction of growth is often accompanied by reduction of photosynthetic pigments and lower photosynthetic rate and it is largely attributed to the reduction in light interception due the reduced leaf area, while the effects of salt stress on photosynthesis may be attributed both to stomatal and non stomatal responses (FUNG et al., 1998; MARCELIS & HOOIJDONK, 1999; AGASTIAN, et al., 2000; LACERDA et al., 2001; LU et al., 2002). Studies have been shown that there were decreases in photosynthesis and/or photosynthetic pigments in salt-stressed pepino (*Solanum muricatum* Ait.) (CHEN et al., 1999), melon (MAVROGIANOPOULOS et al. 1999), rice (SULTANA et al., 1999) spinach (DELFINI et al., 1999), sultana (FISARAKIS et al., 2001) and soybean (KAO et al., 2006). In addition, the photosynthesis in several olive (*Olea europaea* L.) cultivars was inhibited at high salinity, while low salinity seems to stimulate growth in some cultivars. Nevertheless, at moderate salinity levels the reduction in shoot dry weight was comparable to that of the root, indicating that shoot and root were equally sensitive to salinity (CHARTZOULAKIS et al., 2002). Indeed, some studies have shown that salinity treatment no effects on photosystem II (PS II) photochemistry inducing the growth and pigments synthesis in some plant species (LU et al., 2002, LU et al., 2003). Many authors suggested that the effects of salt stress on

physiological processes in plants depend on the plant species (SUDHIR & MURTHY, 2004).

In south Brazil, radish is widely cultivated and generally planted in raised beds in spring and autumn. Although radish is described as a crop moderately sensitive to salinity, it yields and quality dramatically fluctuates in the presence of salt in the soil (MARCELIS & HOOIJDONK, 1999). On the other hand, although salinity reducing the growth, raising the salinity in the root zone is also used as a method to enhance the quality of vegetables (KANG & WAN, 2005).

The objective of this study was to investigate the effects of the NaCl on growth, photosynthetic pigment content and photosynthesis of radish. For this purpose, we examined (1) how salt stress affects plant growth; (2) whether salt stress induces a change in photosynthetic pigment composition, (3) how salt stress affects PSII photochemistry and net CO<sub>2</sub> assimilation in radish (*Raphanus sativus* L.).

## MATERIAL AND METHODS

*Plant material.* Radish (*Raphanus sativus* L. cv. Cometo) plants were grown in greenhouse from June to August 2005 on sand in plastic pots (8 cm in diameter and 10 cm in height) and watered with half-strength Hoagland nutrient solution with pH adjusted to 6.0-6.5. The average temperature was 15 °C (20/10.1 °C day/night), average relative air humidity was 80-100%, the photoperiod for the day/night cycle was 12 h, and the maximum photosynthetically active radiation was about 230  $\mu\text{mol m}^{-2} \text{s}^{-1}$ . After 4 weeks, the seedlings were subjected to salt treatment. Salt concentrations used were 0, 50, 100 and 200 mM. NaCl was dissolved Hoagland nutrient solution and plants were watered each two days to drip approximate 0.02 L of salt solution. All measurements on the youngest and

expanded leaves were made 3 weeks after the start to salt treatment.

*Photosynthetic pigments and analysis.* Chlorophyll concentration was analyzed from 0.15 g fresh weight (FW) of the leaf tissue, which was excised from the youngest and expanded leaves. Samples were extracted in ice-cold 80% (v/v) acetone/water (ARNON, 1949). Chlorophyll and carotenoids pigment concentrations were calculated according to HENDRY & GRIME (1993).

*Photosynthesis measurements.* Chlorophyll fluorescence parameters were measured using a PAM fluorometer (*FMS-2, Hansatech*, U. K.). The radish leaves were dark-adapted for 15 min and irradiated by weak modulated measuring beam ( $0.12 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) to determine the initial fluorescence yield ( $F_0$ ). Maximum fluorescence yield ( $F_m$ ) was determined during a saturating photon pulse ( $4\ 000 \mu\text{mol m}^{-2} \text{s}^{-1}$ ). Variable chlorophyll fluorescence ( $F_v$ ) was calculated as  $F_v = F_m - F_0$ . Primary photosystem II photochemical efficiency and maximum quantum yield of PSII were expressed as  $F_v/F_m$  and  $F_v/F_0$ . The photochemical and non-photochemical quenching coefficient were calculated as  $q_p = (F_m' - F_s)/(F_m' - F_0')$  and  $q_n = (F_m - F_m')/(F_m - F_0)$ . The net photosynthetic rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) was measured outdoors at a  $\text{CO}_2$  concentration of about  $360 \mu\text{mol mol}^{-1}$ , with a portable open-system infrared gas analytical tool (LCi - ACD, U. K.).

*Data analysis.* The experimental design was complete randomized with five replications. The growth and photosynthetic characteristics were compared across salinity levels by entry to determine significant differences among salinity levels. Data were subjected to an analysis of variance and the means compared by Tukey's test at 5% of probability.

## RESULTS AND DISCUSSION

The effect of stress salinity on non-halophytes is a reduction in growth and yield (CHARTZOULAKIS et al., 2002). They affect plants via different mechanisms, causing osmotic stress and ionic imbalance (VAIDYANATHAN et al., 2003). The results of this work demonstrated a significant effect of NaCl salinity on *Raphanus sativus* plants.

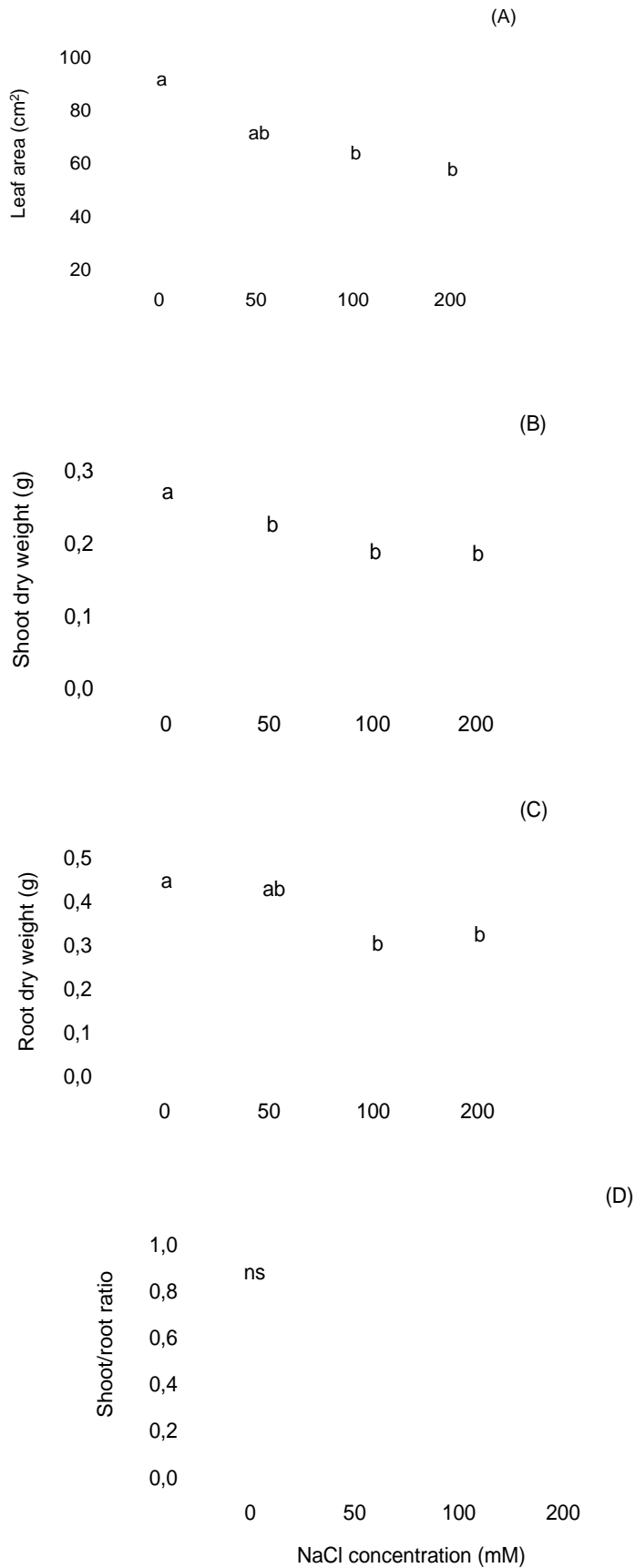
The total leaf area decreased significantly with the increase in salt concentration up to 50 mM NaCl (Figure 1A). In addition, decreases in leaf area were seen earlier than other evaluated parameters. This performance agrees with the observation that, a common salt stress symptom in plants is leaf expansion restriction (CHARTZOULAKIS et al., 2002; RODRÍGUEZ et al., 2005). Also, at high salinity levels (100 and 200 mM NaCl) leaf abscission occurred in *R. sativus* and contributed to the reduced leaf area. These symptoms could be a result of excess  $\text{Na}^+$  and  $\text{Cl}^-$  ions, which induce chlorosis and leaf senescence (VIÉGAS & SILVEIRA, 1999). While, in one hand, these senescence symptoms clearly showed a salt ionic toxicity, they suggest, in other hand, that this negative effect could result in substantial reduction in total plant photosynthetic  $\text{CO}_2$  assimilation, indicated by a reduction in the photosynthetic area available to support further growth.

The reduction of leaf canopy surface has been considered an avoidance mechanism which minimize water losses when the stomata are closed, minimizing osmotic effects generated by salt treatments (RUIZ-SÁNCHEZ et al., 2000). Besides reduction of canopy surface, plant species have a number of tolerance and avoidance mechanisms such as osmotic adjustment, decrease in leaf osmotic potential and decrease of stomatic conductance. All these mechanisms maintain the plant water status under water deficit conditions. The osmotic adjustment can be achieved by the accumulation

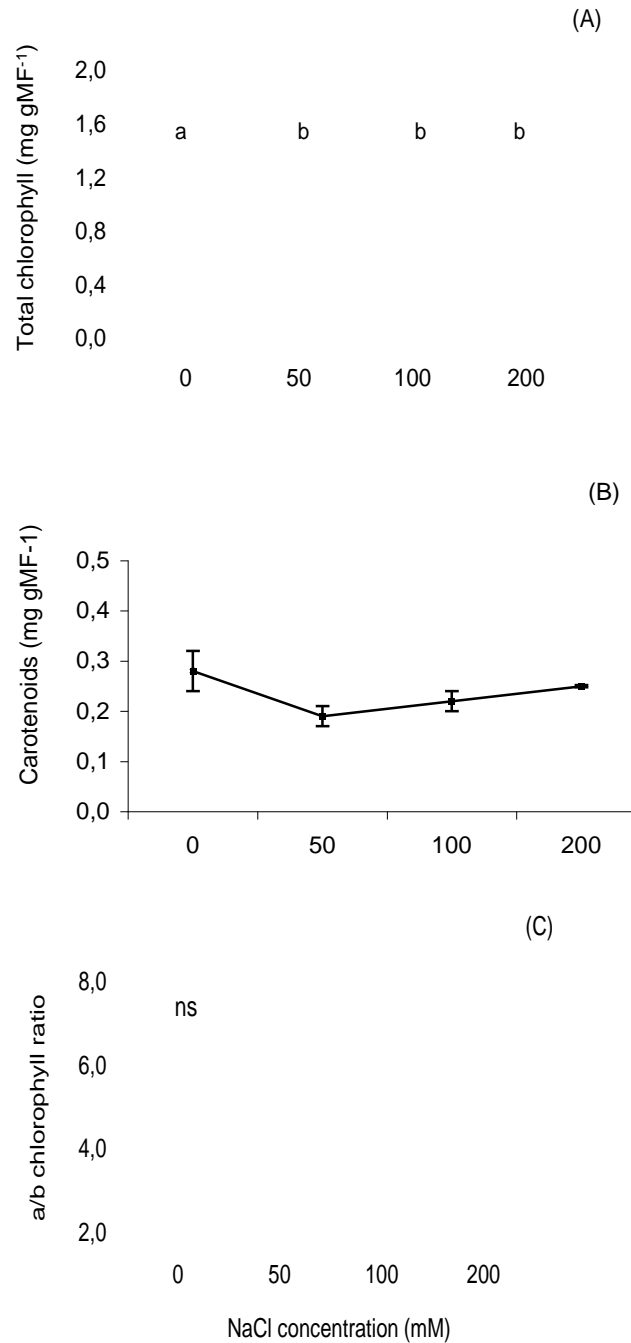
of inorganic ions and/or organic substances and permitted the maintenance of turgor (MOGHAIEB et al., 2004).

Salt stress resulted in a significant reduction of 32% in shoot dry matter at 50 mM NaCl. However, the root dry matter decreased only up to 100 mM NaCl (Figure 1B-C). The reduction in shoot dry weight was due to a more substantial effect of the salinity in the stem than in root dry weight values. Therefore, moderate salinity in *R. sativus* may alter the pattern of dry matter distribution favoring the root, although no

significant change in shoot/roots ratio values has been observed after saline stress in radish plants (Figure 1D). In contrast, high NaCl salinity alters of the shoot/root ratio values in kiwifruit (CHARTZOULAKIS et al., 1995), olive (CHARTZOULAKIS et al., 2002) and soybean (KAO et al., 2005), suggesting that this plant characteristic could be an important tolerance indicator of salt stress, at least in this species.



**Figure 1-** Leaf area (A), shoot dry weight (B), root dry weight (C) and shoot/roots ratio of radish (*Raphanus sativus* L. cv. Cometo) plants treated with NaCl for 3 weeks. Values represent mean  $\pm$ S.E. of five replicates. Different letters indicate significant difference according Tukey's test ( $P \leq 0.05$ ). Vertical bars represent standard deviations. ns = not significant



**Figure 2-** Total chlorophyll (A), carotenoids contents (B) and *a/b* chlorophyll ratio (C) expressed on a leaf fresh weight basis in radish (*R. sativus* L. cv. Cometo) plants treated with NaCl for 3 weeks. Values represent mean  $\pm$ S.E. of five replicates. Different letters indicate significant difference according Tukey's test ( $P \leq 0.05$ ). Vertical bars represent standard deviations. ns = not significant.

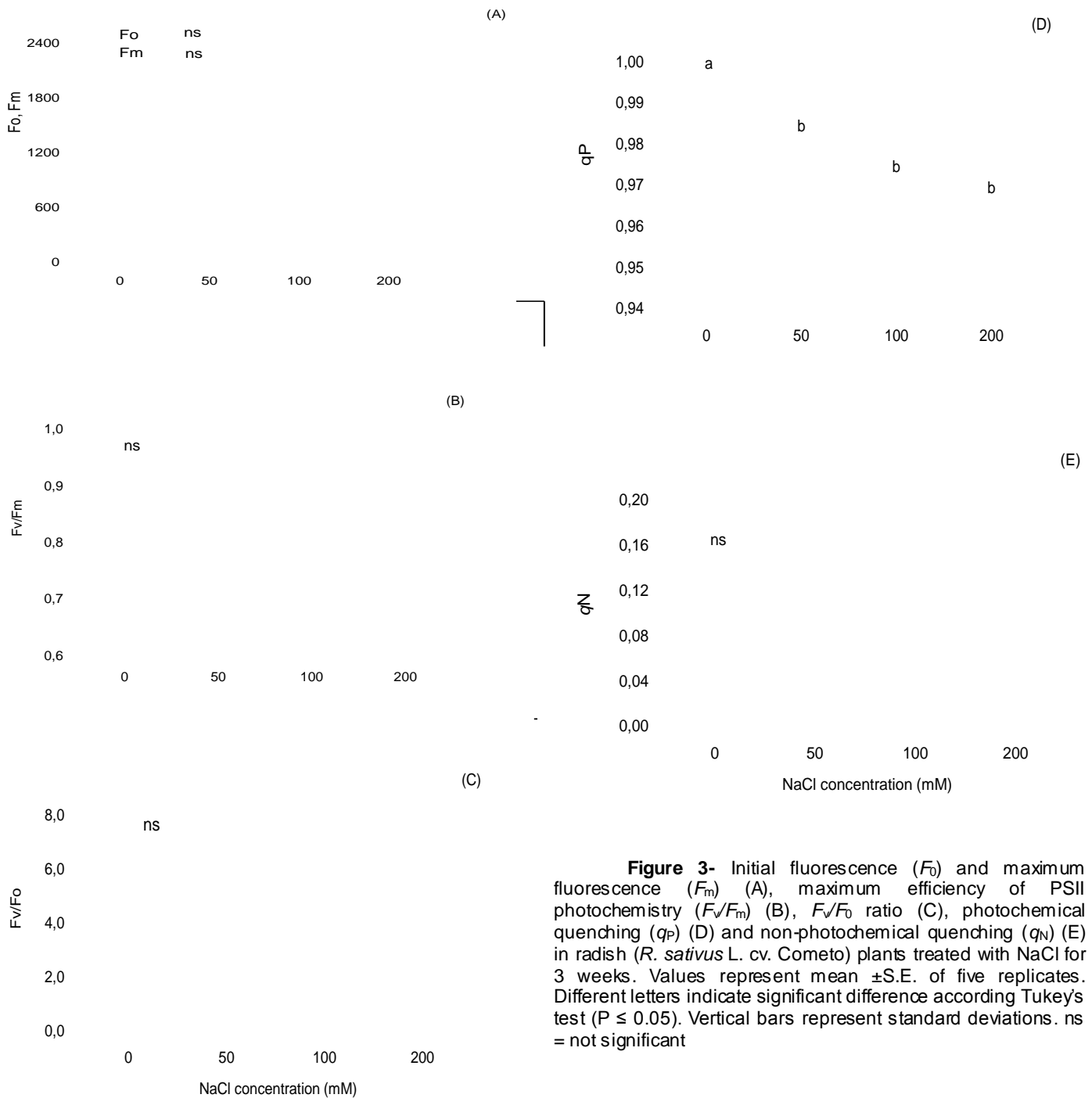
Total contents of Chl and carotenoids decreased significantly at 50 mM NaCl in *R. sativus* (Figure 2A-B). The

decrease in chlorophyll concentration might possibly due to changes in the lipid protein ratio of pigment-protein complexes

or increased activity of the chlorophyll-degrading enzyme chlorophyllase (SULTANA et al., 1999; PARIDA et al., 2004). The chlorophyll *ab* ratio remained unaffected differences by NaCl treatment (Figura 2-C). The decrease in carotenoids under salt stress in *R. sativus* (Figure 2B) could be an indicative of the protection of the photosynthetic machinery against damage caused by salt stress and would allows the excessive energy to be dissipated harmlessly in the pigment bed (BEHERA & CHOUDHURY, 2002).

Initial ( $F_o$ ) and maximum ( $F_m$ ) chlorophyll fluorescence remains constant with the NaCl concentration range applied

(Figure 3A). It has been reported that stability of LHC from the PS II complex, the activation of PS II reaction center and the normal electron flow within the PQ pool are dependent of these invariability of the  $F_o$  and  $F_m$  (YAMANE et al., 1997). No change in maximal efficiency of photosystem II ( $F_v/F_m$ ) (Figure 3B) in control and salt-stressed plants measured suggest that salt stress had no effects on PSII photochemistry in *R. sativus*. This can be better envisaged by taking into account the  $F_v/F_o$  ratio (Figure 3C), which exhibits much larger amplitude than  $F_v/F_m$  and thus, more sensitively reflecting changes in photosynthetic activity.

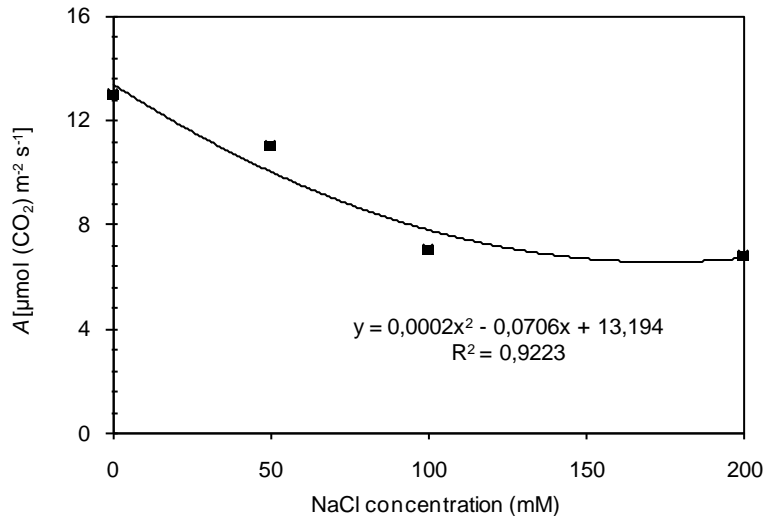


**Figure 3-** Initial fluorescence ( $F_0$ ) and maximum fluorescence ( $F_m$ ) (A), maximum efficiency of PSII photochemistry ( $F_v/F_m$ ) (B),  $F_v/F_0$  ratio (C), photochemical quenching ( $q_p$ ) (D) and non-photochemical quenching ( $q_N$ ) (E) in radish (*R. sativus* L. cv. Cometo) plants treated with NaCl for 3 weeks. Values represent mean  $\pm$  S.E. of five replicates. Different letters indicate significant difference according Tukey's test ( $P \leq 0.05$ ). Vertical bars represent standard deviations. ns = not significant

Since the same leaves had marked reductions in their net photosynthesis (A) (Figure 4), there would be a surplus of chemical energy and reducing power, obtained from photosynthetic electron transport, that must be dissipated in processes other than carbon fixation. Our results showed

that radish affected by salinity could dissipate efficiently the excess of energy not used in photosynthesis. The increase in  $q_N$  indicated that the excess of excitation energy had been dissipating by heat, minimizing the effects of salinity on the photosynthetic apparatus (Figure 3E).





**Figure 4-** Net photosynthetic rate,  $A$  [ $\mu\text{mol (CO}_2\text{) m}^{-2} \text{s}^{-1}$ ] measured in radish (*R. sativus* L. cv. Cometo) plants treated with NaCl for 3 weeks. Means of at least 5 replicates for each treatment.

The photochemical quenching ( $q_p$ ) in dark-adapted leaves decreased in the presence of salinity (Figure 3D).  $q_p$  quantifies the photochemical capacity of PSII in light-adapted leaves. Decreases in  $q_p$  indicate weak photochemical conversion efficiency of PSII, which can be implied in reduction in the photosynthetic activity and carbon fixation (MACFARLANE, 2003).

The rate of leaf  $\text{CO}_2$  assimilation ( $A$ ) is sensitive to a wide range of environmental perturbations, although the sites of limitation of  $\text{CO}_2$  assimilation during these various stresses can be quite different. LORETO et al. (2002) studied the photosynthetic limitations in olive cultivars with different sensitivity to salt stress concluded that the main limitation of photosynthesis in salt-stressed olive, as well as in cultivars with inherently low photosynthesis is the low chloroplastic  $\text{CO}_2$  concentration, caused by both stomatal and mesophyll conductance reduction. This result was confirmed by CHARTZOULAKIS et al. (2002). In rice (*Oryza sativa* L.) cv. Koshihikari, moderately salt resistant variety, the decline in

net photosynthesis under salinity is attributed partly to reduced stomatal conductance partly be due to a reduction in protein concentration and partly to a decline in photosynthetic pigment concentrations or ionic concentrations. The inhibition of net photosynthesis in rice by salinity is also mediated by water deficit in the leaf cells due to accumulation of salt in the apoplast (SULTANA et al., 1999). Thus seems that salinity affected the photosynthetic capacity of plants by several ways.

Some studies have classified radish as a crop moderately sensitive to salinity, while others reported a low sensitivity (MARCELLIS & HOOIJDONK, 1999). However, differences between different genotypes does not have been explored. Salt tolerance in glycophytes is associated with the ability to limit uptake and/or transport of saline ions (mainly  $\text{Na}^+$  and  $\text{Cl}^-$ ) from the root zone to aerial parts. Ion exclusion and compartmentation at the root level regulates ion concentration in the xylem sap preventing accumulation of potentially toxic ions in the aerial parts. This mechanism

seems to work effectively at low and moderate levels of salinity, but it considerably slows plant growth (CHARTZOULAKIS et al., 2002). Nevertheless, other salt tolerance mechanisms are observed in photosynthesizing organisms as the production of the  $\omega$ -solutes (sucrose, trehalose, proline, glucosyl-glycerol, and glycine-/ glutamato-betaine) (SUDHIR & MURTHY, 2004).

In conclusion, the present results show that high salinity affected the growth through the leaf area and dry weight reduction and decreasing of chlorophyll content. Thus, the decreasing of leaf area and chlorophyll content in presence of NaCl, although it has not caused changes in the chlorophyll fluorescence parameters ( $F_0$ ,  $F_m$ ,  $F_v/F_m$  and  $F_v/F_0$ ), could have contributed to reduced leaf photosynthetic rate (A).

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