Journal of Animal & Plant Sciences, 2011. Vol. 10, Issue 1: 1219- 1231.
Publication date: 28/3/2011, http://www.biosciences.elewa.org/JAPS; **ISSN 2071 - 7024**

Identifying multiple physiological responses associated with salinity-tolerance for evaluating three tomato cultivars selected from Moroccan territory.

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Key words: Salinity, tomato; Gas exchange; Chlorophyll fluorescence, stress tolerance,

1 SUMMARY

Crops differ in their ability to grow under saline conditions; their responses are quite different and not fully understood. To study the response of tomato (*Solanum lycopersicon cv.*) to salinity, three tomatos (A5, T8 and T4) (*Lycopersicon esculentum.*) genotypes were subjected to salt treatment and their responses were monitored in a set of experiments . The objective was to advance them as potential salt tolerant tomato scions and/or rootstocks. The strong correlations between the foliar content of sodium (Na+) and Chloride (Chl), the photochemical quenching (qP), the non photochemical quenching (NPQ), the linear electron transport rate (ETR), and plant growth suggest that these parameters can be used to screen genotypes for salt tolerance.

2 INTRODUCTION

Crops are subjected to a number of abiotic and biotic stresses due to their sedentary nature. Among the most important crop stresses is salinity that is considered as the major land degradation problem worldwide (Pichu et al 2006). Currently, more than 800 million hectares of land throughout the world are salt affected accounting for more than 6% of the world's total land area (Munns and Tester, 2008). The problem of salinity tolerance in agriculture is probably best tackled by either altering farming practices to prevent soil salinization occurring in the first place, or by implementing schemes to remediate salinized soils (Mark and Romola. 2003). Salinity is considered as one of the most lethal abiotic stresses for plants since it induces a number of secondary stresses including water deficit stress, ionic stress, and nutritional imbalance. The interest in salt tolerance of plants, especially those of economic importance like tomato, is increasing as more saline lands are brought under cultivation. Salt tolerance can be defined as the ability of plants to survive and maintain growth

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under saline conditions. Consequently, searching for strategies that will generate improved tolerance in plants is a priority (Estañ et al, 2005; Martinez-Rodriguez et al, 2008). In this way yields can be increased on affected soils and plants can also maintain increased growth when they encounter saline subsoil thus enabling them to form part of the remediation process itself (Mark et al. 2003). Salt stress affects many physiological aspects of plant growth at different level of organization from cell to whole plant. The great variability shown by the species and varieties under this stress can be used to consider the selection of cultivar especially being well adapted to salt stress. Salinity has deleterious effects on different stages of plant growth due to reduced water availability (osmotic effect) and accumulation of ions, particularly Na+ and Cl-, to toxic concentrations (Ehsan et al 2010). Decreased RLWC (Real Leaf Water Content) under salt stress induces stomatal closure, resulting in reduced rate of photosynthesis. However, this stomatal closure has no effect on photosynthetic events of PSII like maximal quantum yield of the photochemical reaction in PSII (Fv/Fm) (Lawlor and Cornic 2002) Moreover, lower NPQ values was indicative of increased heating of the photosynthetic apparatus and therefore there may be degradation of NADPH and ATP (Baker 2008), representing reduced photosynthetic rates. The less accumulation of Na+ and more of K+, seed germination and plant total weight were used as indicator for salt stress tolerance. All these parameters are considered as a good index to evaluate the photosynthetic performance of plants under salt stress (Baker 2008).

Tomato (*Lycopersicon esculentum*) is a major vegetable crop that has achieved tremendous popularity over the last century. It is grown practically in almost every country, and it is moderately sensitive to salinity (Pertala et al, 2005). In this research three cultivars were selected from different Moroccan regions, and subjected them to salt stress. An Extensive research was done to confirm this selection and also to develop growing conditions in moderate salinity to produce good vegetative growth and find cultivars available for breeding or using as root-stock.

3 MATERIALS AND METHODS

3.1 Plant material, growth, and treatment conditions: Tomato (*Solanum. Lycopersicon escenlum CV.*) was used as plant material. The experiment was carried out in a controlled culture chamber. Seeds were germinated in petri dishes with sterile Whatman filter paper. From germination to transplantation into the pots, the plants were grown in the same growth chamber, where the environmental conditions were optimized for the growth of tomato seedlings. The environmental conditions were 24/18 °C (day/night) temperature, 16 h light (450 µmol m−2 s−1) and 60–70% RH (relative humidity). Seedlings were individually transplanted into plastic pots filled with 250 cm3 sterile sand and irrigated with a nutrient solution. The composition of trace elements was 24 µM H3BO3, 10 µM MnSO4.4 H2O, 3 µM ZnSO4, 0.9 µM CuSO47H2O, 0.04 µM (NH4) 6Mo7O27 H2O. The plants were grown under greenhouse and natural light conditions, temperature day / night of 22 ± 5 ° C / 15 ± 2 ° C and 70 ± 10% relative humidity during the day. For salinity treatment, four salt levels were applied by daily irrigation with 0 (water), 100, 150, and 200 and 250mM NaCl solutions for one month after the true leaves start growing. The salt treatment was gradually increased

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in order to avoid osmotic shock. For each cultivar and for each batch, there were 12 plants including one per pot, or 3 repetitions per treatment. Irrigation with nutrient solution was conducted in alternating with an injection of distilled water to avoid significant variations in osmotic potential during treatment. All the parameters were checked for the plants under 100mM NaCl which considered as selective concentration. The plants were harvested after one month of treatment.

3.2 Determination of plant growth: Shoot length, the number of leaves, and stem diameter were measured. At the end of the experimental period, plants were carefully removed from the soil, the roots were washed with distilled water, and plants were partitioned in different tissues (leaf, stem and root). After drying at 65 °C in an oven until constant dry weight, mass of leaves, stem, and root dry was determined. These data were used to calculate biomass allocation in leaves, stem, and roots as well as root-to-shoot ratio (R/Sh), and all the measurement was taken for the samples under 100mM NaCl stress.

3.3 Determination of relative water content (RWC): The first fully expanded leaves of three plants under 100mM NaCl stress were used for measuring leaf RWC as described by Weatherley (1950). Briefly, the samples were first weighed to determine fresh weight (FW), soaked in distilled water at 25 °C for 8 h and weighed again to record the turgid weight (TW), then oven dried at 80 °C for 48 h to determine the dry weight (DW). RWC was defined as follows: RWC (%) = [(FW−DW)/(TW−DW)] × 100.

3.4 Fluorescence measurements and chloroplasts content : Leaf chlorophyll fluorescence was measured with a FMS-2 pulse-modulated fluorometer (Hansatech, UK). The minimal fluorescence (Fo) was determined by a weak modulated light which was low enough not to induce any significant variable fluorescence. The maximal fluorescence (Fm) was determined by a 0.8 s saturating light of 8000 µmol m-2s-1 on dark-adapted leaf. When measuring the induction, the actinic light was offered by the light source of FMS-2. The steady-state fluorescence (Fs) was thereafter recorded and a second 0.8 s saturating light of 8000 µmol m-2s-1 was given to determine the maximal fluorescence in the light-adapted state (Fm'). The actinic light was then turned off; the minimal fluorescence in the light-adapted state (Fo') was determined by illumination of 3 s far red light. The following parameters were then calculated: (1) Fv/Fm, the maximal PSII efficiency, Fv/Fm = (Fm - Fo)/Fm; (2) Fv'/Fm', the efficiency of excitation energy capture by open PSII reaction centers, Fv'/Fm' = (Fm' - Fo')/Fm'; (3) qP, the photochemical quenching coefficient, qP = (Fm' - Fs)/(Fm'-Fo'); (4) ΦPSII, quantum yield of PSII electron transport, ΦPSII = (Fm' -Fs)/Fm. For all cases, the air temperature, air relative humidity, CO2 concentration, and PPFD were maintained at 25 °C, 70-85%, 360 µmol mol-1, and 800 µmol m-2s-1, respectively, and all the measurement was taken for the plants under different NaCl stress.

3.5 Determination of MDA, proline and soluble sugar contents: Malondialdehyde (MDA) was assayed for indirect evaluation of lipid peroxidation using TBA as described earlier by Hodges et al. (1999). The MDA contents of the samples were expressed as µmol·g-1 fresh weight. For extraction of proline and soluble sugars, samples were ground in liquid nitrogen and 0.5 g of each sample was incubated with 5 ml of 75% ethanol. After overnight shaking on shaker, samples were centrifuged at 5500 rpm for 20 minutes and stored at 4 °C for further use. For proline estimation, an aliquot of 2 ml was mixed with 2 ml of acid ninhydrin reagent (2.5% w/v ninhydrin, 60% glacial acetic acid and 40% of 6M H3PO4) and 2 ml of glacial acetic acid and incubated at 100 °C for 1 h. After cooling and centrifugation, 3 ml of toluene was added followed by vortexing. The chromophore was aspirated and absorbance was read at 520 nm (Bates et al. 1973). For measurement of soluble sugars, an aliquot of 80 µl of the ethanol

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extract was mixed with 4 ml of anthrone reagent (0.15% (w/v) anthrone, 72% (v/v) H2SO4, 28% (v/v) H2O). Samples were incubated in water-bath at 100 °C for 1 h. After cooling the samples to room temperature, absorbance was read at 625 nm and soluble sugars were expressed as glucose equivalents (Bravo et al. 1998). All the measurement was taken under 100mM of NaCl stress.

4 RESULTS

4.1 Plant growth: NaCl stress had significant effect on the seed germination, plant stem, leaf and root dry weight. The preliminary results showed an interesting germination for the cultivar A5 compared with the two others, while the T8 showed the lowest rate. For the mature plants under salt stress and compared with the unstressed plants, the salt stress decreased the plant shoot dry weight of A5, T8 and T4 by 20%, 69% and 36% at day 30. The root dry weight decreased gradually with NaCl concentrations increasing at day 30, the root of the A5 were less affected by NaCl stress. Salt stress induced significant differences on plant growth during the experimental period, at the end of the stress period, the mean values of plant height were 10.48, 10.8, and 13.4, respectively in 100mM and 9.81, 6.28, 10.22 in 200 mm. The number of leaves was not sensitive as plant height in all the treatments. Salt stress resulted in considerable decreases in leaves, stem, and total dry and fresh masses verified in NaCl levels of 150 and 200 mM. These reductions were approximately 22%, 24%.and 21% respectively in under the concentration of 150mM and 32%; 64% and 40%, respectively under 200mM of salt. For root dry masses A5 have less decrease than T8 and T4 (fig 1), the decreasing in under 150mM was 33%, 42% and 35%,, and 30%, 75% and 59% in 200mM respectively. Root-to-shoot ratio (R/Sh) increased with salt stress significantly for A5 and moderately for T8, for root length the T4 show a high rate than other cultivars (Figure 1).

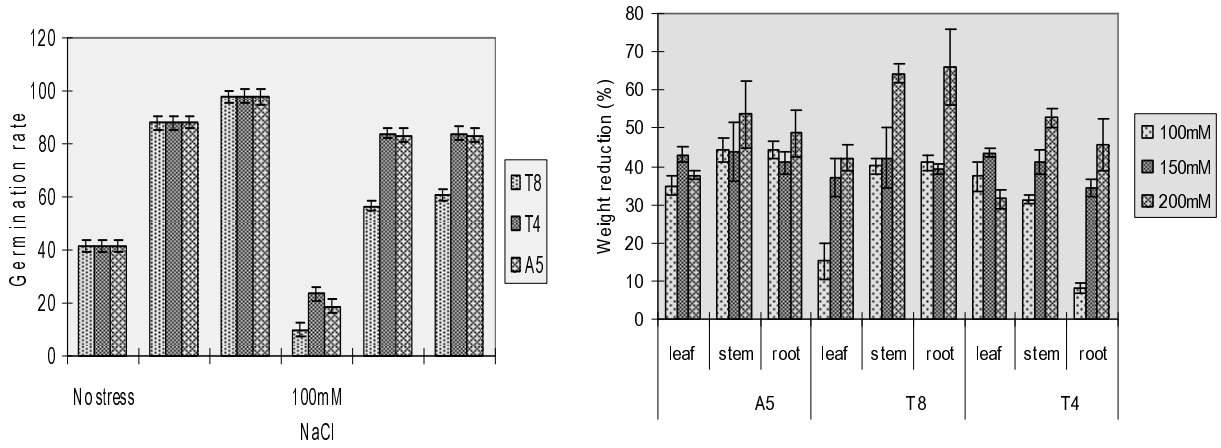

Figure 1: The germination and plant growth of the three cultivars under or no salt stress. The SE means the error of the three replicates

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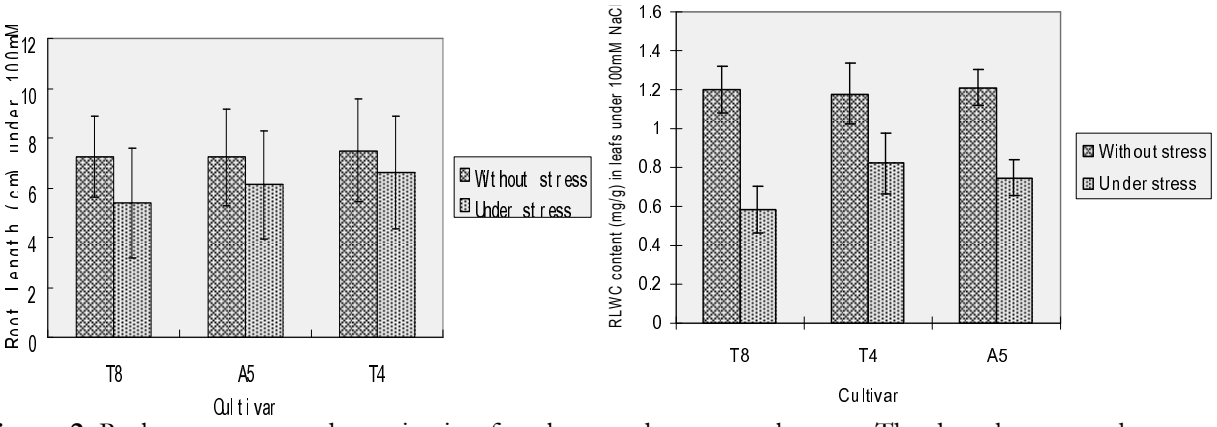
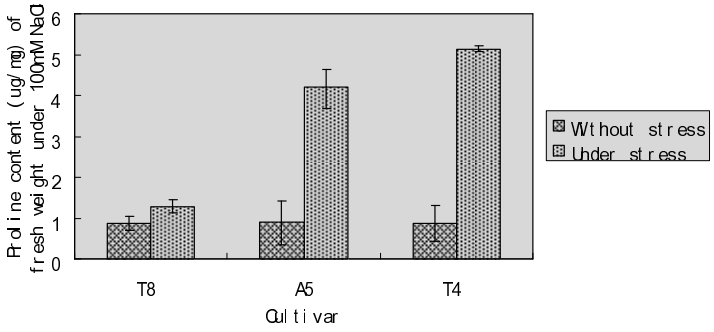


Figure 2: Real water content determination for plants under or no salt stress. The data shown are the means with S.E. (n = 3).

4.2 Relative leaf water content (RLWC): NaCl stress had significant effect on leaf RWC for both T8 and T4 at day 30 (fig 2). The T4 keep a high LRWC in comparison the both cultivars.

4.3 Changes in soluble osmolyte contents and level of lipid peroxidation: Different chemical compounds are known to accumulate in response to different abiotic stresses such drought, cold, flooding and salt. Some of these accumulate under salt stress like proline, soluble sugars, sugar alcohols and quaternary ammonium compounds. Therefore, the changes were analyzed in level of some of these biochemical markers in plants under salt stress conditions. Proline contents increased in plants subjected to salt stress (Fig. 3). But the proline contents of T4 plants were significantly higher than the two other cultivars T8 and A5. Proline contents of T8 were lower than both of T4 and A5 plants. Soluble sugars were higher in T4 plants than both cultivars under unstressed conditions. Under stress, soluble sugar contents increased in all the plants; this increase was more in T4 plants followed by A5 plants under both stresses (Fig.3). Malondialdehyde (MDA) contents were determined in stressed and unstressed plants as a measure of lipid hydro-peroxidation. MDA contents were increased in all plants under stress conditions. But this increase was higher in T8 plants followed by A5 (Fig. 3). This distinctive change in MDA contents in different plants lines indicates that the T4 has less oxidative damage.



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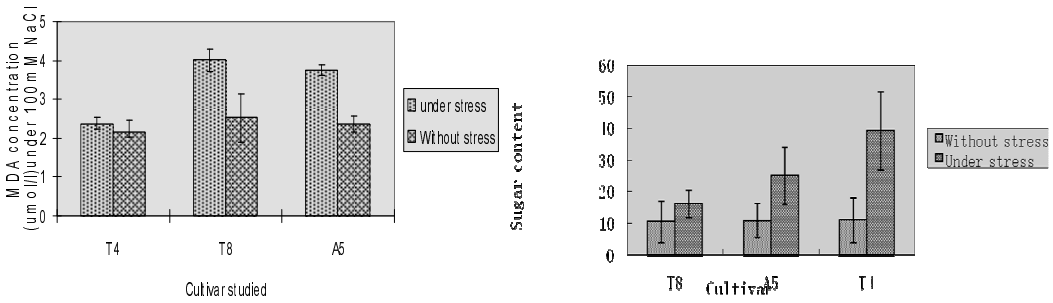
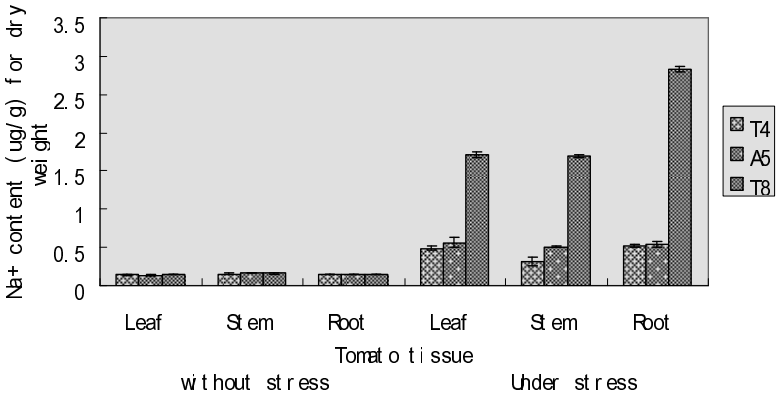


Figure 3: Changes in proline, sugars and MDA contents of the cultivars under salt stress conditions, MDA content for the cultivars under or no salt stress, Proline content for the plants under or no stress, the total soluble sugar for the plants under or no salt stress. Vertical bars means SE for three replicates.

4.4 Na+ and K+ content: Total Na+ content was higher in NaCl treated than untreated plants in three cultivars. After salt treatments level of Na+ in stem & leaf as well as roots in the cultivars increased significantly. The highest amount of Na+ in stem & leaf was observed for the A8 cultivar, while roots showed the great amount of Na+ for this cultivars, the T4 showed the lowest rate followed by T5. Evaluation of K+ content in stem, leaf & Root showed a significant decrease in all the cultivars, but the high rate was for the cultivar A8 followed by T5 and then T4 which showed the lowest decreasing rate. The original level (untreated) of K+ content in stem & leaf and roots of the three cultivars was higher nearly the same. For Na+/K+ rate, the cultivar T8 show an important rate especially in root, meanwhile the cultivar T4 show the lowest rate (fig 4).



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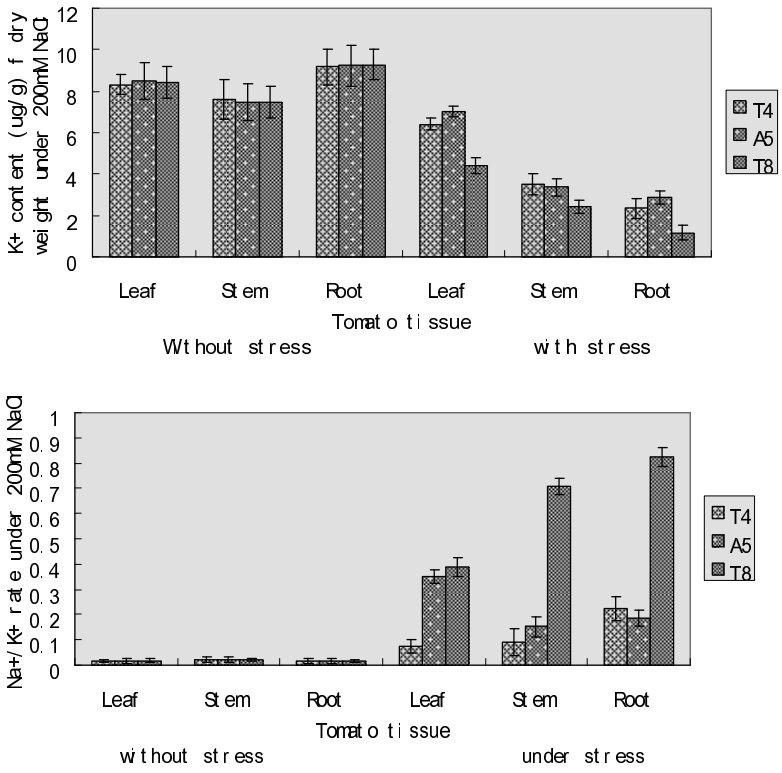

Figure 4: Effects of salinity on sodium (Na+), potassium (K+) contents and Na+/K+ ratio in different tomato genotypes under or without salt stress, the bars means the error of three replicates.

4.5 Leaf chlorophyll fluorescence: Salt tolerance was analyzed of the different cultivars. Four week plants of different cultivars were watered with 200mM NaCl weekly and observed for phenotypic differences. T8 cultivars showed more chlorosis compared to T4. Chlorophyll fluorescence of the three cultivars was recorded to unveil any change in the efficiency of the photochemical system II. Results indicate that maximal quantum yield of PSII (Fv/Fm) start changing after the third weeks under salt stress. The T4 cultivars retained maximal quantum yield of PSII (Fv/Fm) but considerable decrease was recorded in A5 and T8 cultivar (Fig. 5). Therefore, we analyzed other parameters of chlorophyll fluorescence. Efficiency factor of PSII or photochemical quenching (qP) as well as PSII operating efficiency (ΦPSII) were significantly higher in T4 as compared to other cultivars (Fig. 5). Non-photochemical quenching (NPQ) was also high inT4 when compared with T5 and A8 (Fig. 5). Operating efficiency of open PSII (F'v/F'm) was not significantly affected (Fig. 5E). Also we checked the chlorophyll content on the mature leafs under NaCl stress, for the result the cultivar T8 was the more affected under high NaCl concentration (Figure 6).

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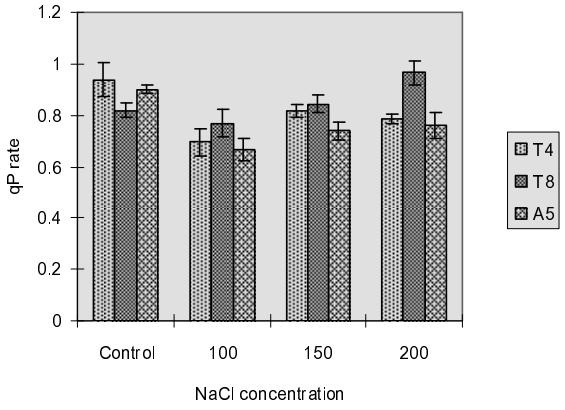

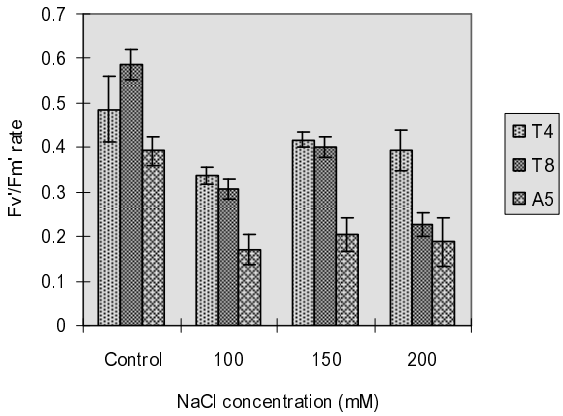

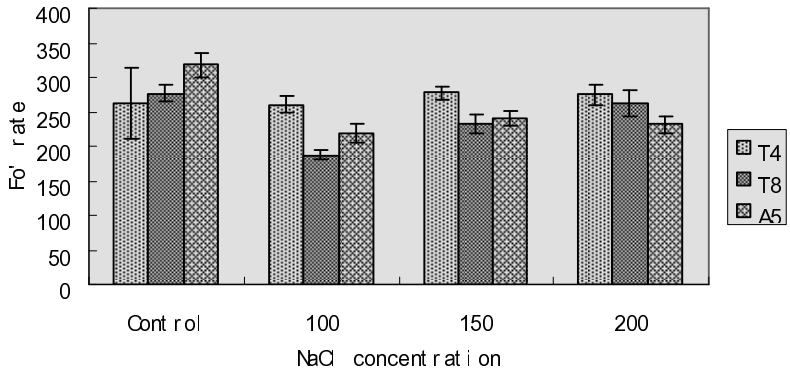

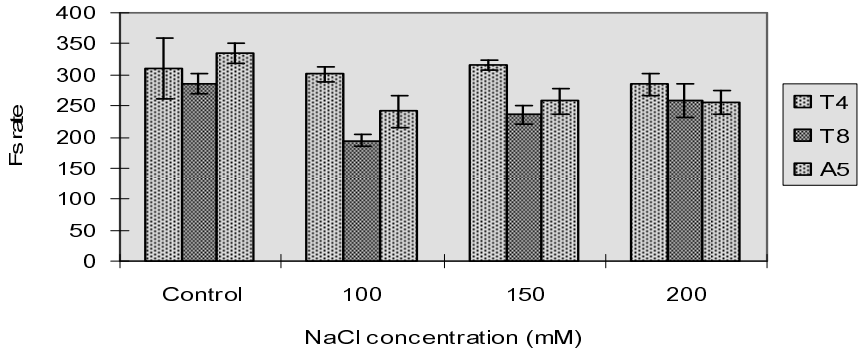

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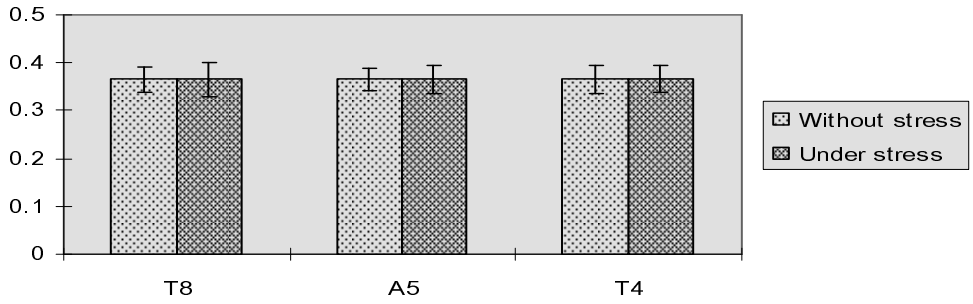
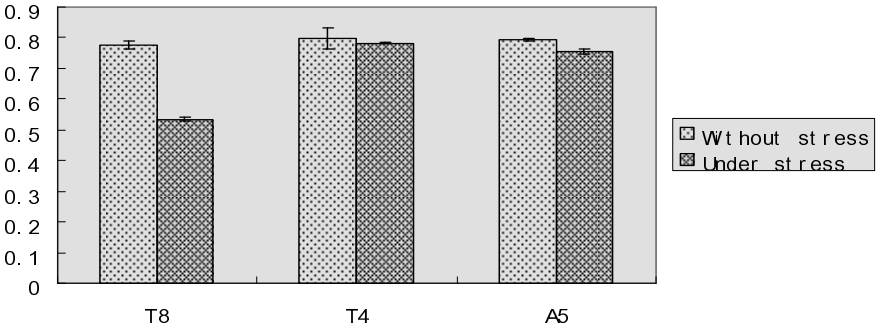


ΦPSII



Fv/Fm

Figure 5: Evaluation of salt tolerance of cultivars. Relative leaf water contents of unstressed and stressed plants. Maximal quantum yield of PSII (Fv/Fm), photochemical quenching (qP), operating efficiency of PSII (ΦPSII), Non-photochemical quenching and operating efficiency (F'v/F'm). Vertical bars mean the error of three replicates.

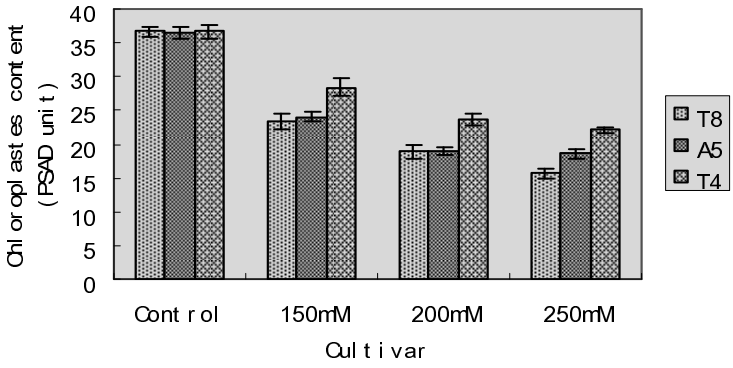


Figure 6: The chloroplasts content in tomato leafs under or without NaCl stress, the SE is mean of three replicate

Abbreviation: FW: fresh weight; DW: dry weight; RWC: relative water content; Fm: maximal fluorescence in dark-adapted leaves; Fm': maximal fluorescence in light-adapted leaves; Fo: minimal fluorescence in dark-adapted leaves; Fo',: minimal fluorescence in light-adapted leaves; Fs: steady-state fluorescence; Fv: maximal variable fluorescence in dark-adapted leaves; Fv': maximal variable fluorescence in light-adapted leaves; Fv/Fm: maximal efficiency of PSII photochemistry; Fv'/Fm': efficiency of excitation energy capture by open PSII reaction centers; ΦPSII, the quantum yield of PSII electron transport; qP: photochemical quenching coefficient; NPQ, non-photochemical quenching; PSII: photosystem II; ROS: reactive oxygen species.

5 DISCUSSION

Salinity induced in the form of NaCl had a pronounced effect on tomato cultivar growth, resulting in a considerable decrease in plant height and dry weights of roots and stem in the three cultivars with a significant difference. In this present study T8 was severely injured and A5 show a moderate reduction, while the T4 showed an important stress tolerance Total leaf weight, stem and roots were markedly reduced in 150 and 200mM, in 100mM NaCl tomato plant grow without showing significant reductions. Also it was observed that the leaf number was less or not affected at all by salt stress than plant height. This inhibition of growth may due to two reasons: water-deficit and salt-specific or ion-excess effects (Munns et al, 2006). Rahman et al. (2008) reported that the seedling growth inhibition of cultivars is by slow or less mobilization of reserve foods, suspending the cell division, enlarging and injuring hypocotyls. The root in A5 showed a small decrease and this may attribute to the tolerant characteristics of this cultivar under salinity conditions, since the root is essential for plant to absorb water and nutrition (Munns and Tester, 2008). Several researchers have shown that shoot growth is more sensitive to salinity than root growth (Shalhevet et al, 1995; Azevedo Neto and Tabosa, 2000), which is compatible with our results.

Limitation of plant growth by salt stress cannot be assigned to a single physiological process, but the dominant one is photosynthesis, the growth inhibition observed in many plants subjected to salinity is often associated with a decrease in their photosynthetic capacity (Lu et al, 2003; He et al, 2009). In sensitive species, salt stress can negatively affect photosynthetic electron transport and inhibit PSII activity as a consequence of the accumulation of salts (Sudhir and Murthy, 2004). In addition, salinity reduces net photosynthetic rate (Stępień and Klbus, 2006; Zhang et al, 2009). Indeed, photosynthetic activity decreases as water potential of leaves decreases which can result in stomatal closure (Lawlor, 2002). In recent years, chlorophyll fluorescence analysis is one of methods of studies in photosynthesis, which is one of the most powerful and widespread techniques. PSII is the membrane protein complex found in oxygenic photosynthetic organisms, which harnesses light energy to split H2O into O2, protons and electrons, and is believed to play an important role in the adaptation of leaf photosynthesis to environmental stresses (Baker, 1991). The decrease of ΦPSII might cause excess light energy, which would increase the excitation pressure on PSII, raising the probability of reactive oxygen species (ROS) generation and the photoinhibition of PSII (Müller et al, 2001). However, the excess light energy could be partly dissipated via non-photochemical quenching (NPQ). This may explain why Fv/Fm, Fv'/Fm', ΦPSII, and qP decreased, whereas NPQ increased in the T8 under 200mM NaCl (Chen et al, 2007), the chloroplasts being the most sensitive organelle to salt stress (Demetriou et al. 2007), in sensible cultivar rather then the tolerant one as in our case for A5. These results may confirm that salt stress might change the construction of thylakoids, destroying the PSII reaction centre and the chloroplast apparatus. These results are in accordance with those of Tiwari et al. (1997) and Murata et al. (2007).

Under NaCl stress, plants are subjected to low water potential and excess Na+ and Cl- contents. NaCl stress changes water relations in leaves leading to decrease in cell turgor and perturbation of Calvin Cycle (Flexas et al, 2004). Moreover, an excess accumulation of Na+ and Cl- is toxic and may disrupt integrity of photosynthetic apparatus (Bethke and Drew, 1992). In this present study, lower accumulation of Na+ and higher relative water content in the T4 and A5 were observed and this may partly explain why these salt tolerant cultivars had higher photosynthetic capacity.

It is well known that plants may respond to low water potential induced by salt stress by

Abbreviation: FW: fresh weight; DW: dry weight; RWC: relative water content; Fm: maximal fluorescence in dark-adapted leaves; Fm': maximal fluorescence in light-adapted leaves; Fo: minimal fluorescence in dark-adapted leaves; Fo',: minimal fluorescence in light-adapted leaves; Fs: steady-state fluorescence; Fv: maximal variable fluorescence in dark-adapted leaves; Fv': maximal variable fluorescence in light-adapted leaves; Fv/Fm: maximal efficiency of PSII photochemistry; Fv'/Fm': efficiency of excitation energy capture by open PSII reaction centers; ΦPSII, the quantum yield of PSII electron transport; qP: photochemical quenching coefficient; NPQ, non-photochemical quenching; PSII: photosystem II; ROS: reactive oxygen species.

accumulation of some organic solutes (e.g. soluble sugar, proline) and inorganic ions (e.g. Na+, Cl-, K+) (Munns and Tester, 2008). A4 cultivar accumulated Cl- for osmotic adjustment, it was reported that Na+ rather than Cl- is the primary cause of salt damage in tomato, which seems to the reason why A4 accumulated lots of Cl- rather Na+ (Trajkova et al, 2006). This study showed an increase in tissue Na+ and Cl− when salinity increased. However, this increase was more conspicuous in leaves than in roots. The stabilization in Na+ and Cl− contents verified in moderate and high salinity in the roots suggests saturation in the sodium and chloride retention mechanism in this organ. Higher Na+ and Cl− accumulation in roots than shoot has been considered as a physiological trait indicator of salt tolerance in plants. Although salinity did not affect K+ content, increases of Na+ content in leaves and roots substantially raised Na+/K+ ratio in these organs. High Na+ concentration can induce K+ deficiency inhibiting the activity of enzymes that require K+. Thus, the interaction between relative K+ and Na+ concentration has been considered a key factor in determining salt tolerance in plants. To elucidate more data for the possible salt stress tolerance of the T4 cultivars, lipid peroxidation level was determined (MDA). The data showed that T8 accumulated more MDA than the other cultivars and less remarked was for T4 cultivars. Osmotic stress damaged the membranes more in T8 cultivar as it was noticed from important level of MDA. Kotchoni (et al. 2006) showed that the reduced MDA accumulation was correlated with increased stress tolerance, possibly because of membrane integrity in the tolerant lines (Srinivasan et al. 2009). Also the proline and soluble sugar contents were assayed under normal and stress conditions. The data showed that T4 accumulated more soluble sugars and proline than other cultivar under stressed conditions.

Data regarding salt tolerance of the three cultivars under investigation show that cultivar T4 is most tolerant to salinity stress. On the basis of the tolerance to salts the cultivars can be arranged as follows: T4 > A5 > T8 in which the T8 are the most sensible.

6 CONCLUSION

Referring to previous work and this recent result more vigorous cultivars T4 could be considered as plant materials which are useful to breeders of a salt tolerant cultivars. Therefore, the reduced marketable yield of moderately salinized plants may have been possibly compensated by an enhanced quality of tomato fruits. Overall, saline water up to approximately 100mM NaCl can be used for tomato production, in the specific environment considered, without affecting the plant witch can applied on this cultivar T4.

7 ACKNOWLEDGMENTS

This work was supported by grants from the Ministry of Science and Technology of Morocco, and thanks to the Université Ibn Tofaïl. Faculté des Sciences. Laboratoire de Botanique et de Protection des Plantes

8 REFERENCES

Azevedo N, Tabosa, AD: 2000. Estresse salino em plˆantulas de milho: Parte I An´alise do crescimento. Rev. Bras. Eng. Agr. Amb. 1, 159–164.

Baker N.R, 2008 Chlorophyll fluorescence: A probe of photosynthesis in vivo. Annu. Rev. Plant

Abbreviation: FW: fresh weight; DW: dry weight; RWC: relative water content; Fm: maximal fluorescence in dark-adapted leaves; Fm': maximal fluorescence in light-adapted leaves; Fo: minimal fluorescence in dark-adapted leaves; Fo',: minimal fluorescence in light-adapted leaves; Fs: steady-state fluorescence; Fv: maximal variable fluorescence in dark-adapted leaves; Fv': maximal variable fluorescence in light-adapted leaves; Fv/Fm: maximal efficiency of PSII photochemistry; Fv'/Fm': efficiency of excitation energy capture by open PSII reaction centers; ΦPSII, the quantum yield of PSII electron transport; qP: photochemical quenching coefficient; NPQ, non-photochemical quenching; PSII: photosystem II; ROS: reactive oxygen species.

Biol. 59: 89-113.

Bethke P.C, Drew M.C, 1992. Stomatal and non-stomatal components to inhibition of photosynthesis in leaves of Capsicum annum during progressive exposure to NaCl salinity. Plant Physiol. 99, 219–226.

Bravo L.A, Zúñiga G.E, Alberdi M and Corcuera LJ.:1998 The role of ABA in freezing tolerance and cold acclimation in barley. Physiol. Plant. 103: 17-23.

Chen, Z, Shabala, S, Mendham, N, Newman, I, Zhang, G.P, and Zhou, M.X. (2008) Combining ability of salinity tolerance on the basis of NaCl-induced K+ flux from roots of barley. Crop Sci. 48: 1382 – 1388.

Demetriou G, Neonaki C, Navakoudis E, Kotzabasis K: 2007: Salt stress impact on the molecular structure and function of the photosynthetic apparatus-The protective role of polyamines. – Biochem. biophysics. Acta 1767: 272-280.

Ehsan Tavakkoli, Pichu Rengasamy and Glenn K. McDonald: 2010. High concentrations of Na+ and Cl– ions in soil solution have simultaneous detrimental effects on growth of faba bean under salinity stress. Journal of Experimental Botany

Estañ MT, Martinez-Rodriguez MM, Perez-Alfocea F, Flowers TJ, Bolarin MC, 2005. Grafting raises the salt tolerance of tomato through limiting the transport of sodium and chloride to the shoot. J. Exp. Bot. 56, 703-712.

Flexas J, Bota J, Loreto F, Cornic G, Sharkey TD: 2004. Diffusive and metabolic limitations to photosynthesis under drought and salinity in C3 plants. Plant Biol. 6, 269-279.

He Y, Zhu ZJ, Yang J, Ni X.L, Zhu B: 2009. Grafting increases the salt tolerance of tomato by improvement of photosynthesis and enhancement of antioxidant enzymes activity. Environ. Exp. Bot. 66, 270-278.

Hodges DM, DeLong JM, Forney CF, and Prange RK: 1999. Improving the thiobarbituric acid-reactive-substances assay for estimating lipid peroxidation in plant tissues containing anthocyanin and other interfering compounds. Planta 207: 604-611.

Kotchoni SO, Kuhns C, Ditzer A, Kirch H.H. and Bartels D: 2006. Over expression of different aldehyde dehydrogenase genes in Arabidopsis thaliana confers tolerance to abiotic stress and protects plants against lipid peroxidation and oxidative stress. Plant Cell Environ. 29: 1033-1048.

Lawlor DW and Cornic G: 2002. Photosynthetic carbon assimilation and associated metabolism in relation to water deficits in higher plants. Plant Cell Environ. 25: 275-294.

Lu C, Qiu N, Wang B, Zhang J: 2003. Salinity treatment shows no effects on photosystem II photochemistry, but increases the resistance of photosystem II to heat stress in halophyte Suaeda salsa. J. Exp. Bot. 54, 851-860.

Mark T, Romola D: 2003. Na+ tolerance and Na+ transport in higher plants. Annals of botany 91: 503-527.

Müller P, Li X.P, Niyogi K.K, 2001. Non-photochemical quenching. A response to excess light energy. Plant Physiol. 125, 1558-1566.

Munns R, James R.A, L¨auchli A: 2006. Approaches to increasing the salt tolerance of wheat and other cereals. J. Exp. Bot. 57, 1025–1043.

Murata N, Takahashi S, Nishiyama Y, Allakhverdiev S.I: 2007. Photoinhibition of photosystem 2 under environmental stress. − Biochem. biophys. Acta 1767: 414-421.

Romero A.R, Soria T, Cuartero J.J: 2002. Greenhouse mist improves yield of tomato plants grown under saline conditions. J. Am. Soc. Hortic. Sci. 127, 644–648.

Shalhevet J, Huck M.G, Schroeder B.P, 1995. Root

Abbreviation: FW: fresh weight; DW: dry weight; RWC: relative water content; Fm: maximal fluorescence in dark-adapted leaves; Fm': maximal fluorescence in light-adapted leaves; Fo: minimal fluorescence in dark-adapted leaves; Fo',: minimal fluorescence in light-adapted leaves; Fs: steady-state fluorescence; Fv: maximal variable fluorescence in dark-adapted leaves; Fv': maximal variable fluorescence in light-adapted leaves; Fv/Fm: maximal efficiency of PSII photochemistry; Fv'/Fm': efficiency of excitation energy capture by open PSII reaction centers; ΦPSII, the quantum yield of PSII electron transport; qP: photochemical quenching coefficient; NPQ, non-photochemical quenching; PSII: photosystem II; ROS: reactive oxygen species.

and shoot growth responses to salinity in maize and soybean. Agron. J. 87, 512–516.

Tiwari B.S, Bose A, Ghosh B: 1997. Photosynthesis in rice under a salt stress. – Photosynthetica 34: 303-306,

Trajkova F, Papadantonakis N, Savvas D: 2006. Comparative effects of NaCl and CaCl2 salinity on cucumber grown in a closed hydroponic system. HortScience 41, 437-441.

Pertala E knapp S. and Spooner D.M: 2005. New species of wild tomato (Solanum section *Lycopersicon Solanaceae*) from Northern Peru. Systematic bot, 30: 424-434

Pichu Rengasamy: 2006. World salinization with emphasis on Australia. Journal of Experimental Botany, Vol. 57, No. 5, pp. 1017–1023, 2006

Rahman M, Soomro U, Zahoor M, Gul S: 2008. World J. of Agricultural Sciences 4(3), 398-403.

Stępień P, Klbus G, 2006. Water relations and photosynthesis in *Cucumis sativus* L. leaves under salt stress. Biol Plant. 50, 610-616.

Sudhir P, Murthy S.D.S, 2004. Effects of salt stress on basic processes of photosynthesis Photosynthetica 42, 481-486.

Martinez-Rodriguez M.M, Estañ M.T, Moyano E, Garcia-Abellan J.O, Flores F.B, Campos J.F, Al-Azzawi M.J, Flowers T.J, Borarín M.C, 2008. The effectiveness of grafting to improve salt tolerance in tomato when an "excluder" genotype is used as scion. Environ. Exp. Bot. 63, 392-401.

Munns R, Tester M, 2008. Mechanisms of salinity tolerance. Annu. Rev. Plant Biol. 59, 651-681.

Srinivasan T, Kumar K.R.R. and Kirti P.B. 2009. Constitutive expression of a trypsin protease inhibitor confers multiple stress tolerance in transgenic tobacco. Plant Cell Physiol. 50(3): 541-553.

Abbreviation: FW: fresh weight; DW: dry weight; RWC: relative water content; Fm: maximal fluorescence in dark-adapted leaves; Fm': maximal fluorescence in light-adapted leaves; Fo: minimal fluorescence in dark-adapted leaves; Fo',: minimal fluorescence in light-adapted leaves; Fs: steady-state fluorescence; Fv: maximal variable fluorescence in dark-adapted leaves; Fv': maximal variable fluorescence in light-adapted leaves; Fv/Fm: maximal efficiency of PSII photochemistry; Fv'/Fm': efficiency of excitation energy capture by open PSII reaction centers; ΦPSII, the quantum yield of PSII electron transport; qP: photochemical quenching coefficient; NPQ, non-photochemical quenching; PSII: photosystem II; ROS: reactive oxygen species.