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REGULAR ARTICLE

PHYSIOLOGICAL RESPONSES OF TOMATO PLANTS TO THE COMBINED EFFECT OF ROOT HYPOXIA AND NACL-SALINITY

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SUMMARY

Flooding and salinity are important environmental factors restricting plant growth and productivity throughout the world because these two stresses frequently coexist. The objective in this work was to investigate the interactive effects of salinity and hypoxia on the physiological responses of tomato (*Solanum lycopersicum* L.) plants. To this end, growth, photosynthesis, stomatal conductance and organic solute accumulation was determined in hydroponically grown plants exposed for 4 weeks to hypoxia, salinity (100 mM) or to the combination of salinity and hypoxia. Obtained results showed that plants exposed to salinity, either alone or in combination to hypoxia showed decreased root and shoot biomass production. However, root and shoot water contents were decreased only for plants exposed to the combination of the two stresses. Concomitantly, leaf area, leaf mass per area, and K^+ and sugar contents were significantly decreased in comparison with control (normoxia, 0 mM NaCl) plants. Na^+ and proline significantly accumulated in roots and leaves of plants exposed to salinity, either alone or in combination to hypoxia. Taken together, these results suggest that tomato plants are strongly sensitive to the combination of hypoxia and salinity stresses. This is most probably due to a low K^+-uptake selectivity, a strong Na^+ absorption, and the disturbance of K^+ translocation towards shoots and the loss of its use efficiency for biomass production.

Key words: Tomato, hypoxia, salinity, growth, ions, solute accumulation

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1. Introduction

Plants grow in a dynamic environment, which frequently imposes constraints on growth and development. Among the adverse environmental factors commonly encountered by land plants, flooding and salinity are two of the most significant abiotic stresses (Ashraf, 1994; Blom and Voesenek, 1996).

Soil salinity is considered a major factor threatening crop production in arid and semi-arid regions, where soil salt content is naturally high and precipitation can be insufficient for leaching (Zhao *et al*., 2007). Moreover, irrigation of poor-quality water without taking into account soil characteristics during irrigation tends to worsen the situation (Hafsi *et al*., 2010). In Tunisia, like many regions of the world, soil salinity is a major factor limiting plant growth and development. In fact, 1.5 million ha (10 % of the whole territory and 18 % of the arable lands) are affected by salinity (Hachicha *et al*., 1994). The deleterious effects of salinity on plant growth are associated with (i) osmotic stress, (ii) nutrient deficiencies, (iii) specific ion toxicities, or (iv) a combination of these factors (Ashraf, 1994). Salinity is known to inhibit photosynthesis in a number of plant species as a consequence of stomatal closure, thereby limiting CO_2

diffusion into chloroplasts (Centritto *et al*., 2003; Degl'Innocenti *et al*., 2009).

Although all higher plants require access to free water, excess of water in the root environment of land plants can be injurious or even lethal because it blocks the transfer of oxygen and other gases between the soil and the atmosphere (Blom and Voesenek, 1996). With transient flooding, or irrigation followed by slow drainage, or in natural wetlands, plant roots can become oxygen deficient because of slow transfer of dissolved oxygen in the water-filled pore space of the soil. Oxygen deficiency is thought to be a major determinant in the adverse effects of flooding on crops and other plant species (Mommer *et al*., 2004). In Tunisia, like many regions of the world, soil flooding is a major factor limiting plant growth and development. In fact, 1 million ha (6.6 % of the whole territory and 25 % of the arable lands). Due to hypoxia in the rhizosphere, waterlogging can severely impair the performance of terrestrial plants and, thus, has a great impact on the distribution of wild plant species in many parts of the world (Vartapetian and Jackson, 1997). Some of the most important effects of flooding include a reduction in water and nutrient uptake and a decrease in metabolism (Gibbs and Greenway, 2003; Greenway and Gibbs, 2003; Ricard *et al*., 2006; Horchani *et al*., 2008; 2009; 2010).

Many of the habitats occupied by some plant species are not only saline, but also are prone to flooding. Previous reviews of terrestrial halophytes (Barrett-Lenard, 2003; Flowers and Colmer, 2008) have hardly touched upon the effects of combined salinity and flooding although the adverse interactive effects of these stresses combined are now regarded as crucial in determining the failure of crops in many saline soils, and need also to be considered in the design of re-vegetation programmes for salt-affected lands (Flowers and Colmer, 2008). In Tunisia, like many regions of the world, salinity and flooding stresses can coexist. This situation seems to be more frequent in the north of the country where a wide variety of saline-sodic soils characterizes depressions and main sebkhas (Hypoxic and saline areas), and where agriculture is based mainly on the culture of tomato (Riahi, 2003). Moreover, in irrigated areas, the bad quality of irrigation water charged with dissolved salts has resulted unfortunately in soil secondary salinization. The determination of species-specific salt and waterlogging tolerance and the responsible acclimation mechanisms will contribute to understanding the common patterns of colonization and zonation of the plants occurrences and the dynamics in saline environments. The experiments reported here represent a contribution to this approach.

In the literature, the effects of root hypoxia (Germain *et al*., 1997; Horchani *et al*., 2008; 2010) or salinity (Ben Ahmed *et al*., 2008) on tomato growth has been extensively studied. However, data on the combined effects of these two stresses on the growth of these plant species are scarce. Therefore, the aim of the present study was to investigate, for tomato seedlings grown under hydroponic conditions, whether combination of hypoxia with NaCl-salinity was related to growth, water relations, photosynthetic parameters and solute accumulation.

2. Material and Methods

Plant material and growth conditions

Tomato (*Solanum lycopersicum* L. cv. Rio Grande) seeds were germinated on filter paper moistened with distilled water for 1 week at 23°C in the dark, and then grown hydroponically as in Horchani et al; (2008). The nutrient solution contained macronutrients: 2.25 mM KNO_3, 0.25 mM $Ca(NO_3)_2$, 0.35 mM KH_2PO_4, and 0.075 mM $MgSO_4$, micronutrients: 268.6 µM EDTA-Fe, 8.9 µM $MnSO_4$, 24.1 µM H_3BO_3, 1.7 µM $ZnSO_4$ and 3.9 µM $CuSO_4$.

Hypoxic treatment was applied at the second leaf stage (one week after transplanting), by stopping air bubbling for four weeks (up the emergence of the first floral buds), whereas control plants were continuously aerated. At this time, 100 mM NaCl was applied. The nutrient solutions were weekly renewed, and pH was controlled daily and restored to 5.8 as in Horchani *et al.* (2010).

Vegetative growth analysis

Growth parameters were evaluated at the end of the experiment (4 weeks after salt and hypoxia treatment application). Plants were harvested and divided into roots and shoots. Roots were washed in distilled water. Fresh weights (FW) were immediately determined for roots and shoots. Dry weights (DW) were obtained by weighing the plant material after drying at 80 °C until a constant mass was reached. Water content (WC) was calculated as (FW-DW) / DW. Leaf area (LA) was measured as in Horchani *et al*. (2008), and leaf mass per area (LMA) was calculated as DW/LA ratio.

Chlorophyll and photosynthesis parameters analysis

Photosynthesis, stomatal conductance and transpiration rate were analyzed using an infrared CO_2 analyzer (LCA3-Analytical Development Corporation, Hoddeson, UK) following the recommendation of the manufacturer. Chlorophyll measurement was performed according to Wintermans and Mots (1965), and total chlorophyll concentration was calculated as in Horchani *et al*. (2008).

Ions, sugar and proline determination

Ions were extracted from dried plant material (50 mg DW) in an acid mixture (HNO_3:$HClO_4$, 3/1, v/v). K^+ was assayed by flame emission photometry (Corning, UK). Na^+, Ca^{2+}, and Mg^{2+} were determined by atomic absorption spectrophotometry (Perkin Elmer, Courtaboeuf, France).

Total soluble carbohydrates were determined in roots and leaves as in Horchani *et al*. (2009). Free proline was quantified spectrophotometrically by the ninhydrin method according to Bates *et al.* (1973). The plant material was homogenized in 3 % (w/v) aqueous sulfosalicylic acid and the homogenate was centrifuged at 14,000 rpm. The supernatant was used for the estimation of the proline concentration. The reaction mixture consisted of 2 ml of ninhydrin acid and 2 ml of glacial acetic acid, which was boiled at 100 °C for 1h. After termination of reaction in ice bath, the reaction mixture was extracted with 4 ml of toluene, and absorbance was read at 520 nm.

Statistics

Statistical data analysis was made using the Student's t-test. The results are given as means with standard errors of at least six replicates per treatment. The significance of differences between the control and the treatment mean values was determined at the significance level of $p<0.05$. Experiments were replicated two to three times.

3. Results

Vegetative growth analysis

Hypoxia and NaCl-salinity (100 mM) were applied, in the present study, at the second leaf stage. At this moment, the initial root and shoot dry weights were 0.04 ± 0.01g and 0.13 ± 0.02g, respectively. The changes in the root and shoot dry weights (DW), shoot/root ratio, water content (WC), leaf area (LA) and leaf mass per area (LMA) were investigated after a 4-week period of combined hypoxia and NaCl-salinity treatment.

Our results showed that both root hypoxia (H-0) and NaCl-salinity (N-100) treatments, applied separately, decreased root dry weight (DW) by about 22%, compared to control plants (N-0). Combined application of root hypoxia and NaCl-salinity (H-100) leaded to a 70% decrease in root biomass production (Fig. 1A). Shoot DW was decreased by about 25% in salt treated as compared to control plants. Oxygen deficient plants produced more shoot biomass than control plants. When applied together, root hypoxia and salinity stresses leaded to an 80% decrease in shoot DW (Fig. 1B). No obvious effects were observed in root and shoot water contents (WC) when root hypoxia and salinity treatments were applied separately, whereas the combination of these two stresses leaded to a 60 and 67% decreases in root and shoot WC, respectively (Fig. 2).

Fig. 1. Root (A) and shoot (B) dry weight of control (N-0), hypoxically treated (H-0), salt treated (N-100), and hypoxically and salt treated (H-100) tomato plants for 4 weeks. Values are the mean ± S.D. from six measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$

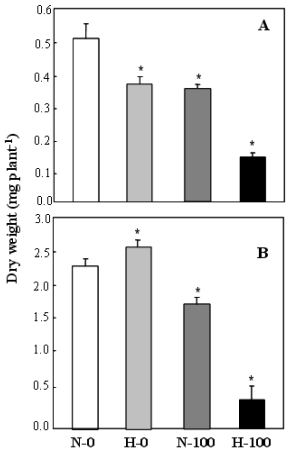


Fig. 2. Root (A) and shoot (B) water content of control (N-0), hypoxically treated (H-0), salt treated (N-100), and hypoxically and salt treated (H-100) tomato plants for 4 weeks. Values are the mean ± S.D. from six measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$

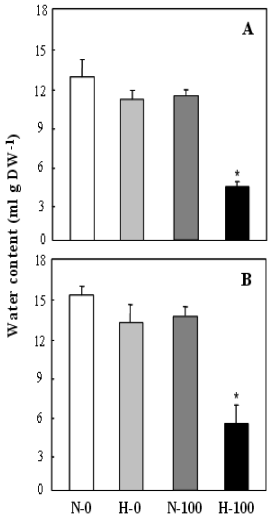
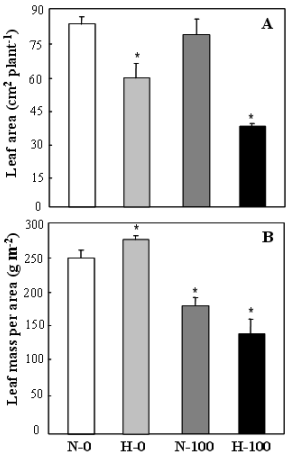


Fig. 3. Leaf area (A) and leaf mass per area (B) of tomato plants grown for 4 weeks under control (N-0, normoxia and 0 mM NaCl), hypoxic (H-0, hypoxia and 0 mM NaCl), saline (N-100, normoxia and 100 mM NaCl), and hypoxic and saline (H-100, hypoxia and 100 mM NaCl) conditions. Values are the mean ± S.D. from six measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$



Leaf area (LA) and leaf mass per area (LMA) were decreased by 58 and 45% in H-100 treated plants, respectively, compared to control plants. Salt treatment had no effect on LA, whereas LMA was significantly decreased (Fig. 3). The decrease in LA (Fig. 3A) and the increase in leaf DW (Fig. 1B) of H-0 plants led to a significant increase in their LMA (Fig. 3B) which indicates an increase in leaf thickness under flooded conditions. This was already observed in the H63-5 tomato variety submitted to hypoxia (Horchani *et al*., 2008).

Chlorophyll content, stomatal conductance and photosynthesis

Compared to control plants, total leaf chlorophyll content was decreased by 23, 26 and 47% in H-0, N-100 and H-100 plants, respectively. Stomatal conductance and photosynthetic activity were significantly reduced in H-0 and N-100, compared to N-0 plants. A more significant decrease was observed in H-100 plants (Table 1).

Table 1. Total leaf chlorophyll content, photosynthetic activity and stomatal conductance of tomato plants grown for 4 weeks under control (N-0, normoxia and 0 mM NaCl), hypoxic (H-0, hypoxia and 0 mM NaCl), saline (N-100, normoxia and 100 mM NaCl), and hypoxic and saline (H-100, hypoxia and 100 mM NaCl) conditions.

		NaCl (mM)	
	Treatment	0	100
Total chlorophyll (mg g^{-1} FW)	N	0.81 ± 0.10	0.51 ± 0.05*
	H	0.54 ± 0.07*	0.43 ± 0.09*
Photosynthesis (pmol CO_2 m^{-2} s^{-1})	N	34.5 ± 2.3	25.4 ± 1.8*
	H	23.1 ± 1.3*	18.0 ± 2.1
Stomatal conductance (mol m^{-2} s^{-1})	N	25.1 ± 0.9	20.2 ± 1.5*
	H	19.7 ± 0.6*	16.4 ± 1.3*

Values are the mean ± S.D. from six measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$

Ions and organic solutes concentrations

Roots and leaves were analysed for their Na^+, K^+, Ca^{2+} and Mg^{2+} (Fig. 4). For hypoxically treated plants, Na^+ content was not changed in comparison to control plants. As for salt treated plants, leaf and root Na^+ content was significantly increased in plants submitted to the combination of hypoxia and NaCl-salinity. Leaf and root K^+ contents were significantly decreased by root hypoxia, NaCl-salinity and by the combination of the two stresses. No obvious effects of root hypoxia and NaCl-salinity were observed in root and leaf Ca^{2+} and Mg^{2+} contents (Fig. 4).

Fig. 4. Changes in Na^+, K^+, Ca^{2+} and Mg^{2+} contents in roots (A) and leaves (B) of tomato plants grown for 4 weeks under control (N-0, normoxia and 0 mM NaCl), hypoxic (H-0, hypoxia and 0 mM NaCl), saline (N-100, normoxia and 100 mM NaCl), and hypoxic and saline (H-100, hypoxia and 100 mM NaCl) conditions. Values are the mean ± S.D. from six measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$

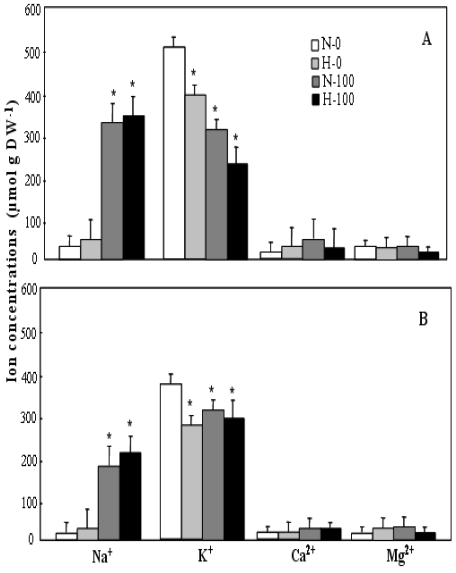


Fig. 5. Changes in soluble sugar contents in roots (A) and leaves (B) of tomato plants grown for 4 weeks under control (N-0, normoxia and 0 mM NaCl), hypoxic (H-0, hypoxia and 0 mM NaCl), saline (N-100, normoxia and 100 mM NaCl), and hypoxic and saline (H-100, hypoxia and 100 mM NaCl) conditions. Values are the mean ± S.D. from six measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$

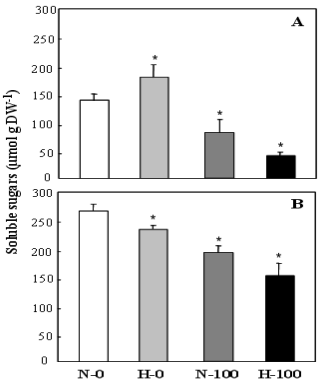
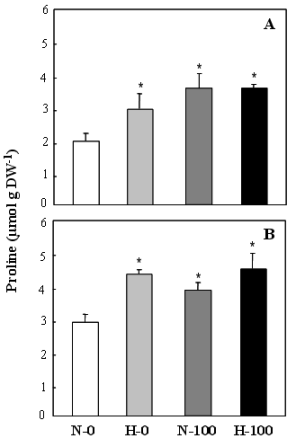


Fig. 6. Changes in proline contents in roots (A) and leaves (B) of tomato plants grown for 4 weeks under control (N-0, normoxia and 0 mM NaCl), hypoxic (H-0, hypoxia and 0 mM NaCl), saline (N-100, normoxia and 100 mM NaCl), and hypoxic and saline (H-100, hypoxia and 100 mM NaCl) conditions. Values are the mean ± S.D. from six measurements. *The significance of differences between the control and the treatment mean values was determined by the Student's t-test at the significance level of $p<0.05$



As already reported for other tomato cultivars (Gharbi *et al*., 2007; Horchani *et al*., 2010), root hypoxia leaded to an increase in root carbohydrate content (Fig. 5A), whereas leaf sugar content was slightly decreased (Fig. 5B). Root and leaf carbohydrate contents were significantly decreased in plants submitted to NaCl-salinity and to the combination of hypoxia and salinity (Fig. 5).

As shown in fig. 6, root and leaf proline contents were significantly in plants submitted to hypoxia, salinity and to the combination of the two stresses.

4. Discussion

Hypoxia and salinity are two major environmental stresses of estuarine ecosystems. The ability to over come multiple and simultaneous stresses is of great importance for the plant growth and survival in such environments (Lichtenthaler, 1996). This study evaluated the physiological response of tomato plants grown under interactive effects of salinity and hypoxia in nutrient solution.

Our results showed that salinity and hypoxia combination is highly stressful, not only for roots, but also for the aerial part of tomato plants. These negative effects included epinasty and chlorosis (data not shown), a decrease in total chlorophyll (Table 1) and carbohydrate (Fig. 4) contents. Stomatal conductance decrease (Table 1) prevented the excessive water loss by transpiration. This reduction has been related to a decrease in root permeability and root hydraulic conductivity under stressful conditions (Pezeshki, 2001) and has been demonstrated to occur even without detectable changes in leaf water potential (Pezeshki, 2001). The production of abscissic acid in the roots and subsequent transport to leaves are argued as the main mechanisms that induce stomatal closure, which is considered as a mechanism that enhance survival rate under stressful conditions (Pezeshki, 2001).

Salt tolerance in plants is associated usually with the ability to restrict the uptake and/or transport of saline ions from roots to shoots (Hajibagheri et al., 1987). Our data show that tomato plants had high Na^+ and low K^+ concentrations in roots and leaves under combined hypoxia and salinity treatment. This may be related to a low

K^+/Na^+ selectivity ratios when exposed to NaCl-salinity and hypoxia conditions.

Osmotic adjustment can be achieved by the accumulation of inorganic ions and/or organic substances, to permit the maintenance of turgor (Tezara et al., 2002). Reduced tissue hydration was accompanied by an increase in root carbohydrate content (Fig. 5) and a decrease in leaf carbohydrate content (Fig. 4). The decrease in tissue water content of tomato plants under salt stress has been interpreted as a mechanism that concentrates solutes in the cell sap, thereby lowering the osmotic potential and contributing to osmotic adjustment (Lissner et al., 1999).

Proline in the cytoplasm plays an important role in balancing the osmotic potential with that in vacuoles; it also protects enzymes when plants are under salt stress (Ashraf, 2004). Our results show that leaf concentration of this amino acid increased under stressful conditions. This is in agreement with finding of Pagter et al. (2009), who reported that leaf proline concentration increased under saline conditions.

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