

## Specific Root Length and Diameter of Hydroponically-grown Tomato Plants under Salinity

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**Abstract:** Salinity causes great growth and yield reduction on crops in many parts of the world. In order to study the impact of salinity on root systems the aim of this experiment was to investigate how root features of tomato plants cultivated in hydroponics are modified under salt stress conditions. Tomato plants underwent three salinity levels: 2.2, 10 and 15 dS m<sup>-1</sup>, through NaCl addition, corresponding to 0, 100 and 150 mM NaCl. In hydroponically grown tomato plants it was measured a reduction in total weight and length of the root system as salinity increased and a shallower root system in the more severe treatment. An increase of specific root length was found in the middle section of the root system under high salinity and it corresponded to a significant increase of roots belonging to the lowest diametric class in the more severe salt treatment. It can be considered as an important evidence of an adaptation to salinity in conditions where soil modifications due to salinity do not interfere with direct plant responses.

**Key words:** *Lycopersicon esculentum* L. Mill, root growth, root system, root architecture, salt stress

### INTRODUCTION

Soil salinity is a great problem in many areas of the world where causes significant reductions of crop yield (Tavakkoli *et al.*, 2011; Hajiboland *et al.*, 2009). Thus, improving crop salt tolerance is a key global agricultural goal (Ghanem *et al.*, 2011b). The increase of salts in the root zone causes inhibition of growth and reduction of productivity of many crops (Maas, 1993; Paranychianakis and Chartzoulakis, 2005). Plant response to salts takes place in two phases (Munns, 1993): the first is linked to osmotic stress (days to weeks) and the second (toxic phase; weeks to months) is due to the high salt accumulation in leaf tissues. In crops, shoot growth is generally more sensitive than root growth to salinity (Munns and Termaat, 1986; Lauchli and Epstein, 1990) and for this reason when soil salinity dramatically increases we usually observe higher root/shoot ratio (Snapp and Shennan, 1992; Zadeh and Naeini, 2007; Albacete *et al.*, 2008; Lovelli *et al.*, 2011). Moreover, salinity causes root growth rate reduction and anatomy root modifications such as lower formation of endodermal and exodermal suberization close to the root apex (Enstone *et al.*, 2002; Shannon *et al.*, 1994; Reinhardt and Rost, 1995a, b).

The great importance of the root system and its role in mediating shoot responses to environmental stress

such as salinity, were recently emphasised (Ghanem *et al.*, 2011a). Root traits such as root system architecture should be deeper investigated to improve plant development under environmental stress conditions (Ghanem *et al.*, 2011a).

Tomato is a widespread crop in the Mediterranean area where soil salinization is currently a serious problem (Paranychianakis and Chartzoulakis, 2005). Tomato is "moderately sensitive" to salinity (Foolad, 2004). On tomato, Snapp and Shennan (1992) observed no inhibition of root growth in hydroponically-grown tomato plants under salinity. Recently, we showed the high root-to-shoot ratio under salinity in tomato and the close relationship to high Abscisic Acid (ABA) root concentration (Lovelli *et al.*, 2011). In tomato under advanced salinization, the high ABA tissue concentration could control organ adaptation processes, such as dry matter partitioning (Albacete *et al.*, 2008) and functional modifications of the root/shoot ratio (Maggio *et al.*, 2007).

As regards to root morphology and tissue differentiation tomato does not normally produce a hypodermis with an exodermis (Reinhardt and Rost, 1995a, b; Peyrano *et al.*, 1997), the latter being a special type of hypodermis between the epidermis and cortical cells (Peterson, 1988) with Casparian bands in the anticlinal walls of its cells (Peterson and Perumalla, 1990). Exodermis in tomato only develops under stress such as salinity

(Peyrano *et al.*, 1997; Reinhardt and Rost, 1995a, b), where it works as a barrier to the apoplastic flux of ions at root apex level, while when it is absent or not yet formed ions reach cortical walls (Enstone *et al.*, 2002). However, some authors (Peyrano *et al.*, 1997) showed that salts (100 mM NaCl) did not modify the amount of lignin or suberin in the roots (both in endodermis and exodermis) in tomato, even if the hydraulic conductance of the salinized plants was deeply reduced. It was also supposed that modification in aquaporin function was the responsible for the reduced hydraulic conductance observed (Enstone *et al.*, 2002; Peyrano *et al.*, 1997).

Since there is still insufficient information about root system architecture of hydroponically-grown plants under salinity, the aim of this experiment was to investigate how root features of tomato plants cultivated in hydroponics is modified under salt stress conditions.

## MATERIALS AND METHODS

**Plant material and growth conditions:** The experiment was carried out at the University of Basilicata, Italy (40°N, 15°E) in a temperature-controlled glasshouse in rounded plastic pots (1 m diameter x 0.38 m height; volume of 250 L). On the 20th of May 2010, processing tomato plants (cv Perfectpeel), at the stage of two fully expanded leaves, were transplanted in plastic pots filled with aerated Hoagland nutrient solution (EC = 2.5 dS m<sup>-1</sup>; pH 6.0) (Hoagland and Arnon, 1950) containing the following nutrients as mmol L<sup>-1</sup>: NO<sub>3</sub><sup>-</sup> 13.5; NH<sub>4</sub><sup>+</sup> 1.5; PO<sub>4</sub><sup>-3</sup> 1.0; K<sup>+</sup> 6.0; Ca<sup>2+</sup> 5.0; Mg<sup>2+</sup> 2.0; SO<sub>4</sub><sup>2-</sup> 2.0. Loss of nutrient solution was compensated weekly. Salinization treatments began 10 days after transplantation. An automated heating system started working each time air temperature dropped under 18°C, while the greenhouse roof opened as the temperature exceeded 25°C. During the whole experimental period, meteorological data were recorded by an automatic weather station placed in the greenhouse.

Tomato plants underwent three salinity levels: 2.2, 10 and 15 dS m<sup>-1</sup>, through NaCl addition (commercial salt), corresponding to 0, 100 and 150 mM NaCl. Each experimental treatment was replicated twice arranging the pots according to a randomized block scheme. In each pot, there were 6 plants giving a total of 12 plants per experimental treatment. Salt addition to the nutrient solution occurred 7 days after transplant to avoid osmotic shock to plants. Nutrient solution pH was daily adjusted to 6.0, for all the treatments.

Aboveground growth and hormonal relations were reported in a separate paper (Lovelli *et al.*, 2011).

**Root parameters:** Plants were harvested 37 days after transplant. Root depth was measured and 3 whole root

systems per treatment were collected. The root systems were divided horizontally in three section corresponding to three depths of equal length (top, mid and bottom). For each depth a subsample corresponding to about 1/3 of the total root was collected, weighed and used for length and diameter determinations with the Win-Rhizo image analysis software (Win-Rhizo Pro (Regent Instruments Inc. Canada). Root length was determined on the whole subsample and separately on 10 diameter classes starting from a class of 0 to 0.5 mm and proceeding in 0.5 mm increments. The remaining roots were weighed fresh and after oven-drying at 65°C until constant weight. The root percent water content was then calculated as:

$$\text{Root water (\%)} = \frac{\text{Root fresh weight} - \text{Root dry weight}}{\text{Root fresh weight}} \times 100 \quad (1)$$

The dry weight of the subsample was calculated based on root water content and added to the dry weight of the remaining root to give total root dry weight. Root length from the subsample was then converted to root length per plant by multiplying it for the ratio of the total root dry weight to the dry weight of the subsample. Specific root length was calculated dividing root length by the corresponding weight.

**Statistical analysis:** Experimental results underwent Variance Analysis (ANOVA) by JMP software 8.01 (SAS, 2008). Significant differences were pointed out by Duncan's Test with 5% significance.

## RESULTS AND DISCUSSIONS

In the experiment total root weight and length were on average lower in more salinized treatment (S15; Fig. 1, 2) and depth of the S15 roots was 60 cm maximum, versus 90 cm of the roots of less saline treatments. Conversely, a large increase in specific root length (SRL) compared to the control was found in this treatment (S15) (Table 1).

Table 1: Specific root length measured in the three different treatments

	Specific root length (mg <sup>-1</sup> )		
	Salinity level		
Depth	S0	S10	S15
Top	58 <sup>c</sup>	42 <sup>c</sup>	48 <sup>c</sup>
Mid	73 <sup>bc</sup>	53 <sup>c</sup>	36 <sup>7a</sup>
Bottom	108 <sup>b</sup>	108 <sup>b</sup>	70 <sup>bc</sup>

Mean values within a column followed by different letters are significantly different at p<0.05 according to Duncan's test

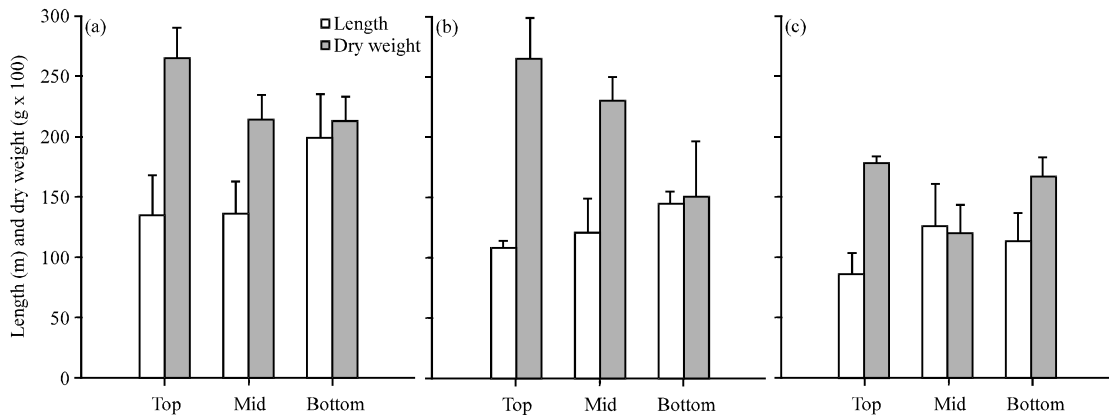


Fig. 1(a-c): Root length and dry weight measured at three different depths (top, mid and bottom) in the three treatments (a) S0, (b) S10 and (c) S15. Vertical bars are standard error of the mean

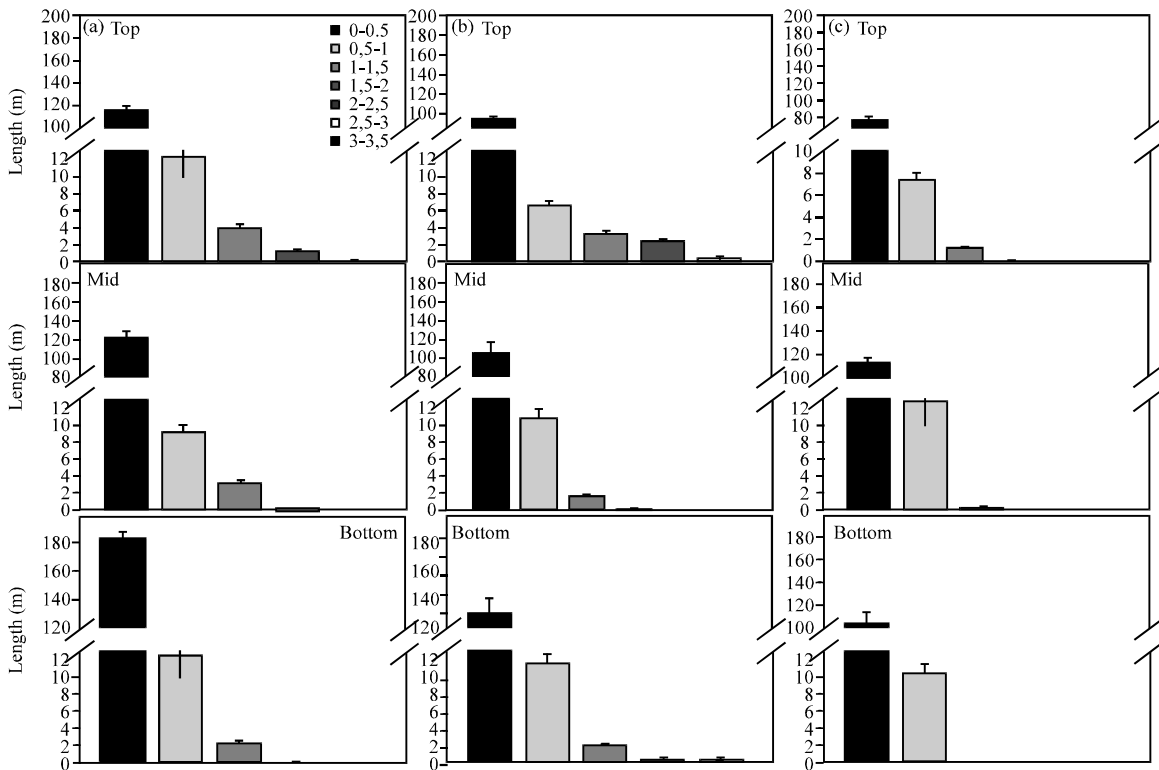


Fig. 2(a-c): Length of roots belonging to different diameter classes in the three treatments (a) S0, (b) S10 and (c) S15 and at three different depths (top, mid and bottom). Vertical bars are standard error of the mean

Snapp and Shennan observed no inhibition of root length density in hydroponically-grown tomato plants under salinity. However, these authors used a lower salt level (75 mM) than it was used in this experiment (150 mM NaCl in the more salinized treatment). Recently, both a root fresh weight reduction (30%) was observed on tomato after three weeks under saline conditions (Albacete *et al.*,

2008) and a root dry matter reduction under salinity together with a root/shoot increase (Lovelli *et al.*, 2011). According to Cuartero and Fernandez-Munoz (1999) salinity negatively affects root biomass of tomato but other authors (Abrisqueta *et al.*, 1991) showed that tomato root biomass grown with 135 mM NaCl (about 13 dS m<sup>-1</sup>), have a 20 day delay in reaching a depth

of 80 cm and the end root length density is a quarter than in control plants. Considering its function in absorbing water and nutrients, the root is the main part of the plant to meet soil salinity (Ouyang *et al.*, 2007). In this sense root system likely plays an important role to cope with salts and how salts affects root growth and architecture is of great importance to understand plant adaptation process to this abiotic stress. The reduction of root growth traits with increasing of nutrient solution electrical conductivity agree with the results of several authors (Schwarz and Grosch, 2003; Kafkafi, 1996).

Also in other crops there are contrasting results: in soil-grown faba bean plants root length density and root mass density are significantly reduced as a result of application of salinity (Abdelhamid *et al.*, 2010), while on soil-grown alfalfa some authors (Vaughan *et al.*, 2002) showed that root production was stimulated by salinity. These contrasting results may depend also on heterogeneous growing conditions. When plants are grown under salinity soil compaction could affect root growth (Tu and Tan, 1991; Grzesiak, 2006) by causing increased resistance to root penetration and the resulting different mechanisms of salt damage may be very different according to the system under which the plants were grown (Tavakkoli *et al.*, 2011). For this reason it is important to separate salt stress from other soil abiotic stress and this can be done only growing plant in hydroponics.

In this experiment on hydroponically-grown plants analyzing root length density along the depth we found a significant interaction between salinity and root depth on this parameter (Table 1). A root system with a high SRL in high salinity conditions could be considered an adaptive response that allows plants to thoroughly explore the soil volume (Bazzaz and Morse, 1991; Snapp and Shennan, 1992) and to delay toxic ions accumulation in plant shoots (Maggio *et al.*, 2007). Moreover on other crops some authors pointed out that differential rooting was greatest in the upper half of the root zone on alfalfa soil grown plants and that high fibrous rooting in alfalfa is a trait with potential usefulness as a salinity stress avoidance mechanism (Vaughan *et al.*, 2002). Anyway root architecture modification under salinity seems to be a crucial aspect of crop response to salt stress on whose significance there are still many aspect to clarify.

In tomato it was observed that salt or other abiotic stresses may affect different roots to a different extent (Cuartero and Fernandez-Munoz, 1999). Other researches showed that under stress conditions the tomato usually develops numerous small lateral feeder roots, which are not present in tomato plants growth in non stress

conditions (Zobel, 1975). Moreover, tomato, like other typically non-exodermal plants (Perumalla *et al.*, 1990) may develop exodermis tissue at the base of the root under high salinity (Peyrano *et al.*, 1997; Reinhardt and Rost, 1995b).

As showed in a previous paper high level of Abscisic Acid (ABA) synthesized in roots play an important role as root-to-shoot signal in biomass partitioning (Lovelli *et al.*, 2011). In addition some authors showed that the exodermis developed under salinity could play an important role in keeping Abscisic Acid (ABA) within the apoplast of the root cortex (Hose *et al.*, 2001). As showed on other crop such as cotton, the exodermis formation induced by salinity may protect roots from water loss and leakage of ions useful for osmotic adjustment (Reinhardt and Rost, 1995b).

Moreover, in this experiment a higher SRL corresponds to a different root diameter distribution. Under severe salt stress (S15) it was measured a significant amount of tomato roots belonging to the lower diametric class (0-0.5 cm; Fig. 2). Increased Specific Root Length (SRL) is usually accompanied by low average root diameters (Schwarz *et al.*, 1995; Schwarz and Grosch, 2003). These results are in agreement with other authors (Kurth *et al.*, 1986; Sharp *et al.*, 1990) that observed thinner roots in cotton and maize, respectively, under high level of salinity. In general the increase of Specific Root Length (SRL) under salinity reflects differences in diameter distribution and may be used as an indicator of plant response to management (Basirat *et al.*, 2011) or environmental change (Ostonen *et al.*, 2007).

Changes in the root class diameter distribution may be considered as a mechanism of adaptation to salinity, thinner roots allow osmotic adjustment without having to change the amount of carbon partitioned to root tips (Snapp and Shennan, 1992).

## CONCLUSION

There is a great interest on root architecture and salinity interactions for its impact on plant environmental adaptability. The role of root architecture in tomato response to salinity is still unravelled but together with anatomy alterations it likely plays an important role. In hydroponically grown tomato plants under high salinity it was measured a decrease in root weight, depth and length density but an increase in specific root length, corresponding to an increase in roots belonging to the lowest diameter class (Fig. 2, Table 1) in the middle part of the root system. This may be considered as an important evidence of an adaptation to salinity in conditions where the absence of the soil effect, since we

are in hydroponics, do not interfere with direct plant root responses. Salt stimulates a morphogenic response giving greater root surface area to uptake nutrients and water.

#### ACKNOWLEDGMENTS

This study was funded by CLIMESCO Italian Project, contract No. 286 of 20/Feb/2006 (Ministry of University and Research). We thank the support of all members in our lab, including Mercurio G. and Trupo G.

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