Journal of Plant Physiology 193 (2016) 1–11

Contents lists available at ScienceDirect

Journal of Plant Physiology

journal homepage: www.elsevier.com/locate/jplph

Physiology

# Salt-tolerant rootstock increases yield of pepper under salinity through maintenance of photosynthetic performance and sinks strength

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ARTICLE INFO

*Article history:*
Received 22 September 2015
Received in revised form 1 February 2016
Accepted 3 February 2016
Available online 22 February 2016

*Keywords:*
Antioxidant systems
*Capsicum annuum*
Chlorophyll fluorescence
Grafting
NaCl stress
Proline

ABSTRACT

The performance of a salt-tolerant pepper (*Capsicum annuum* L.) accession (A25) utilized as a rootstock was assessed in two experiments. In a first field experiment under natural salinity conditions, we observed a larger amount of marketable fruit (+75%) and lower Blossom-end Root incidence (−31%) in commercial pepper cultivar Adige (A) grafted onto A25 (A/A25) when compared with ungrafted plants. In order to understand this behavior a second greenhouse experiment was conducted to determine growth, mineral partitioning, gas exchange and chlorophyll *a* fluorescence parameters, antioxidant systems and proline content in A and A/A25 plants under salinity conditions (80 mM NaCl for 14 days). Salt stress induced significantly stunted growth of A plants (−40.6% of leaf dry weight) compared to the control conditions, while no alterations were observed in A/A25 at the end of the experiment. Accumulation of $Na^+$ and $Cl^-$ in leaves and roots was similar in either grafted or ungrafted plants. Despite the activation of protective mechanisms (increment of superoxide dismutase, catalase, ascorbate peroxidase activity and non-photochemical quenching), A plants showed severely reduced photosynthetic $CO_2$ assimilation (−45.6% of $A_{N390}$) and substantial buildup of malondialdehyde (MDA) by-product, suggesting the inability to counteract salt-triggered damage. In contrast, A/A25 plants, which had a constitutive enhanced root apparatus, were able to maintain the shoot and root growth under salinity conditions by supporting the maintained photosynthetic performance. No increases in catalase and ascorbate peroxidase activities were observed in response to salinity, and MDA levels increased only slightly; indicating that alleviation of oxidative stress did not occur in A/A25 plants. In these plants the increased proline levels could protect enzymatic stability from salt-triggered damage, preserving the photosynthetic performance. The results could indicate that salt stress was vanished by the lack of negative effects on photosynthesis that support the maintained plant growth and increased marketable yield of the grafted plants.


## 1. Introduction

Nowadays, about 7% of the world's land area and 20% of irrigated land are affected by salinity (Ferreira-Silva et al., 2010). In general terms, effects of salinity on plants are the result of both water stress (due to a higher osmotic potential in soil as compared to plant tissues) and a toxic effect caused by the influx of ions mainly $Na^+$ and $Cl^-$ into plant tissues (Tuteja, 2007; Munns and Tester, 2008). The result of these effects is a wide range of physiological, metabolic and genomic changes that provoke alterations in photosynthesis, carbohydrate partition, respiration, increased reactive

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http://dx.doi.org/10.1016/j.jplph.2016.02.007
0176-1617/

oxygen species (ROS) production, and an unbalanced uptake of other nutrients (Parida and Das, 2005; Hu and Schmidhalter, 2005; Chaves et al., 2009). Overall, the physiological changes induced by salinity correspond to diminished plant growth and yields.

In spite of these deleterious effects, plants present different degrees of tolerance to salinity, conferred by biochemical pathways, which can alleviate the negative effect of salt toxicity; amongst them: (I) retention and acquisition of water mediated by osmotically-active metabolites (mainly proline, glycine-betaine or sugars) (Singh et al., 2014); (II) maintenance of ion homeostasis (Rivero et al., 2014; Razzaghi et al., 2015); (III) induction of antioxidant systems (Ashraf et al., 2012; Hu et al., 2012; Wang et al., 2012; Fini et al., 2014); (IV) over production of hormones (Krasensky and Jonak, 2012; Yoshida et al., 2014) or (V) synthesis of specific stress-associated molecules such as heat-shock proteins (Wang et al., 2004; Krasenski and Jonak, 2012; Pérez-Salamó et al., 2014) and late embryogenesis abundant proteins (Parida and Das, 2005; Radić et al., 2013). In view of the complexity of salinity tolerance, differences on salt sensitivity occur not only among species, but sometimes even genotypes belonging to the same species perform differently under salinity (Shabala and Munns, 2012).

Pepper is one of the most important crops in Mediterranean area, which is usually classified as a salt-sensitive species (Kurunc et al., 2011; del Amor and Cuadra-Crespo, 2011), even though Aktas et al. (2006) observed that salt tolerance can vary amongst pepper genotypes. A promising perspective to improve pepper resistance to salinity is the use of grafting of commercial cultivars onto salt-tolerant rootstocks (Penella et al. 2013, 2015). The main general objective of using rootstocks is to increase scion growth and development rate, yield and fruit quality (Venema et al., 2008). Tomato and melon are the two commonest herbaceous species in which the grafting practice has been efficiently applied to obtain salt-tolerant plants (Estañ et al., 2005; Edelstein et al., 2011; Orsini et al., 2013). In melon, the favorable effects of grafting on plant growth cannot be ascribed to a more efficient exclusion of $Na^+$ or enhanced nutrient uptake but they were associated with a more efficient control of stomatal functions (changes in stomatal index and water relations), which may indicate that the rootstock may alter hormonal signalling between root and shoot (Orsini et al., 2013). As far as we know, very few studies on grafted pepper plants have been conducted to elucidate whether or not salt tolerance might be conferred by rootstocks.

Given the poor genetic basis of cultivated pepper accessions, the screening of wild pepper accessions has been performed in previous works to assess naturally-occurring genetic variation to salinity in order to select salt-tolerant accessions to be used as rootstocks (Penella et al., 2014). In a previous work, a wild-type pepper accession (code A25) was select as high salt tolerant. Now, in this study, we used a valid commercial cultivar Adige either ungrafted (A) or grafted onto the rootstock A25 (A/A25) and we found an increased fruit yield under salinity conditions as compared with ungrafted plants. To gain insight into the mechanisms by which the grafting improved plant's yield, we address the question whether or not the increase of the production in these plants was associated with the maintenance of their photosynthetic capacity, ion homeostasis, osmotic regulation and/or water relations under 80 mM NaCl for 14 days. Gas exchange and chlorophyll fluorescence parameters, antioxidant systems, hydric and osmotic relations, and $Na^+$ and $Cl^-$ partitioning were assessed to this aim.

## 2. Materials and methods

### 2.1. Plant material

Based on previous studies, a pepper accession of *Capsicum annuum* L. from the COMAV Genebank at the UPV university (Valencia, east Spain) was selected, which was tolerant to salinity (code A25). This accession was chosen to be used as a rootstock and pepper cultivar 'Adige' (A) (Lamuyo type, Sakata Seeds, Japan) was the scion. Seeds of A25 were sown in 96-hole seed trays filled with an enriched substrate for germination. After two months, A plants were grafted onto A25 (A/A25). The graft was performed by the tube-grafting method (Penella et al., 2015). The ungrafted 'Adige' (A) plants were sown two weeks later to obtain plants with a similar biomass to that of the grafted plants at the time of transplantation (10–12 true leaves). The plants obtained by the aforementioned procedure were utilized for both field and greenhouse experiments.

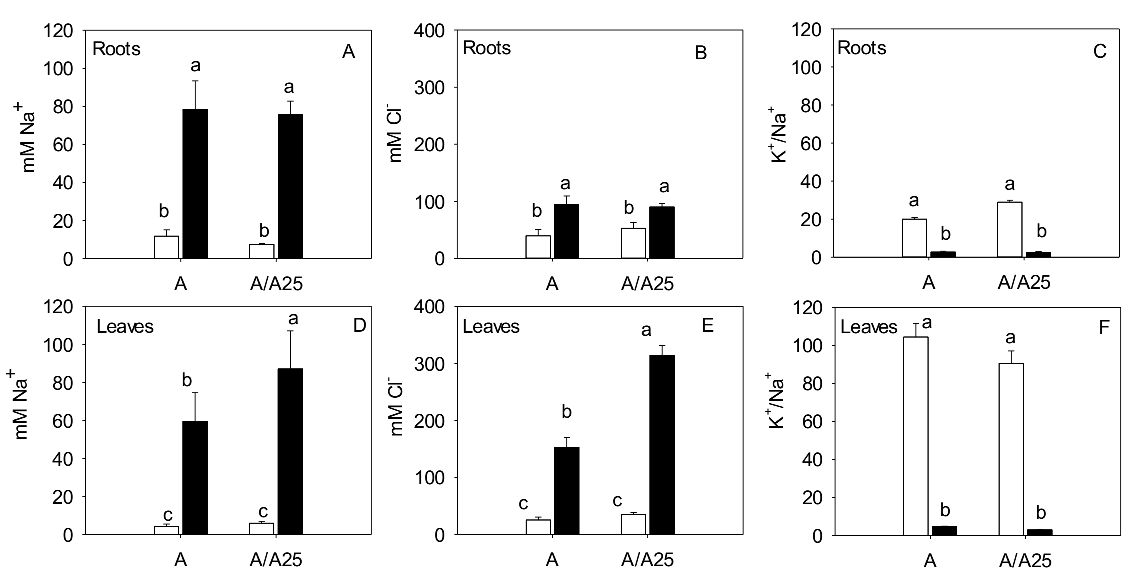


**Fig. 1.** Mineral content (on a DW basis) in the roots and leaves of the control (white bars) and salt-treated plants (black bars) of pepper cultivar Adige, ungrafted (A) or grafted onto the A25 genotype (A/A25). Means ($n = 6$; ±SE) with different letters being significantly different at $P \leq 0.05$ according to a two-way ANOVA, with salt treatment and plant type as the variability factors.