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Some rootstocks improve pepper tolerance to mild salinity through ionic regulation

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ABSTRACT

Grafting has been proposed as an interesting strategy that improves the responses of crops under salinity. In pepper, we reported increased fruit yield of the commercial 'Adige' cultivar under salinity when grafted onto accessions *Capsicum chinense* Jacq. 'ECU-973′ (12) and *Capsicum baccatum* L. var. *pendulum* 'BOL-58′ (14), whereas no effect was observed when grafted onto accession *Capsicum annuum* L var. 'Serrano' (5). We also analysed the physiological and biochemical mechanisms related to the tolerance conferred by these rootstocks. Responses to salinity (40 mM NaCl) were studied in the different plant combinations for 30 days by determining water relations, mineral content, proline accumulation, photosynthetic parameters, nitrate reductase activity and antioxidant capacity. Higher salt tolerance was achieved when the 'Adige' cultivar was grafted onto the 12 genotype, which allowed not only lower Na^+ and Cl^- accumulation in the scion, but also ion selectivity maintenance, particularly Na^+/K^+ discrimination. These traits led to a minor negative impact on photosynthesis, nitrate reductase activity and lipid peroxidation in grafted scion leaves. This work suggests that using tolerant pepper rootstocks that maintain the scion's ion homeostasis is a promising strategy to provide salinity tolerance and can consequently improve crop yield.

1. Introduction

Sweet pepper is one of the most important vegetable crops in arid and semiarid regions with salinity problems, and is considered sensitive to salinity [1,2], even though salt tolerance can vary between pepper genotypes [3]. Maas [4] reported a salinity resistance threshold of 1.5 dS m^{-1}, below which no effect on growth and a 14% decrease in biomass production for every additional 1 dS m^{-1} were observed. Thresholds ranging from 0 to 2 dS m^{-1} and slopes of salinity response curves ranging from 8% to 15% have been reported for greenhouse peppers [5,6]. By way of example, the use of irrigation water of 4.4 dS m^{-1} [7] resulted in reductions of 46% in pepper dry biomass and of 25% in marketable pepper fruits. In pepper plants, the negative effects of salinity on yield have been mainly described as a result of increased salt in leaves, which can lead to salt toxicity and may result in reduced total photosynthesis, which modifies the carbon balance required to maintain growth [2]. The results of the salt ions responsible for such inhibition in pepper plants are controversial. Accordingly, Na^+ or Cl^- can lead to inhibition [8,9], an increase in Na^+ accumulation in leaves can be responsible [10], or increased Cl^- may be the cause of disturbance in the plant [5].

Grafting plants onto tolerant rootstocks is one of several approaches that can cushion the impact of salinity [11] and is a common agronomic practice in tomato and melon. Several studies have been conducted in these species to elucidate the mechanisms involved in increased salinity tolerance of grafted plants. This increased tolerance of grafted plants is generally associated with their capacity to exclude or retain and/or accumulate toxic ions, Na^+ and Cl^- in rootstock roots, thus limiting their transport to leaves rather than through the synthesis of osmotically active metabolites or the induction of antioxidant systems [12–14]. Other authors have indicated that influence of rootstock on the salt tolerance of the scion is due to a more efficient control of stomatal functions (changes in stomatal regulation and water relations), which indicate that the grafting incision may alter hormonal signalling between roots and shoots [15]. In other cases, this raised tolerance

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has been explained by the re-establishment of ionic homeostasis [16].

Nevertheless, the mechanism of resistance against salinity in grafted plants displays great complexity in association with specific rootstock/scion interactions [17,18], and can vary among species. As far as we know, very few studies of this type have been conducted in pepper to elucidate whether or not salt tolerance conferred by rootstocks is also due to exclusion and/or retention mechanisms, as in tomato or melon given their better capacity to alleviate the toxic effects of salts or other processes; e.g., maintenance or water relations or antioxidant capacity. Giuffrida et al. [19] found that stunted growth due to salinity was attenuated in pepper-grafted plants when compared to non-grafted plants associated primarily with reduced uptake of salt ions and, therefore, with a lower concentration of these ions in the grafted plants instead of maintaining leaf turgor by osmotic adjustments.

In previous experiments we selected three pepper accessions with different degrees of salinity tolerance [20] under mild salt stress. In this study, we used these accessions as rootstocks and we identified different behaviours in response to salinity for fruit yield. In order to identify the reason for this disparity, the second step was to study the physiological responses to salinity stress involved in increased tolerance of some pepper-grafted plants and to test the hypothesis that tolerance might be related to the role of rootstocks in altering the stress perception by the scion. To fulfil these objectives, we discussed differences in pepper-grafted plants adaptation mechanisms in response to mild salt stress by comparing some physiological parameters: photosynthesis; lipid peroxidation levels; relative water content (RWC); proline concentration; osmotic potential (Ψ_S); ions concentration and nitrate reductase activity (NR). We present evidence that grafting plants onto appropriate (tolerant) rootstocks is a good tool against salinity stress, which is mediated mainly by reducing ionic toxicity to the scion, and it improves yield.

2. Material and methods

2.1. Plant material

Based on previous studies, we selected three pepper accessions (wild types) with a different salinity tolerance [20]: 'ECU-973′ of *Capsicum chinense* Jacq. (code 12) as being tolerant; 'BOL-58′ of *Capsicum baccatum* L. var. *pendulum* (code 14) as being moderately tolerant; and 'Serrano' of *Capsicum annuum* L. (code 5) as being less tolerant. These accessions were chosen as rootstocks and the pepper cultivar 'Adige' (Lamuyo type, Sakata Seeds, Japan) was grafted onto these three pepper accessions in this study. The pepper seeds for grafting were sown on 1 December in 100-cell polystyrene trays filled with peat-based substrate and kept in a Venlo-type glasshouse. The graft was performed on 12 February using the tube-grafting method (cutting the growing tip of the rootstock at a 45° angle below the cotyledons, attaching to the scion, previously cut at a 45° angle above the cotyledons, and fixing the rootstock and scion with a clip). The grafted combinations (cultivar/rootstock) were labelled A/5, A/12 and A/14. Ungrafted 'Adige' plants were sown 2 weeks later to obtain plants with a similar biomass to grafted plants at the time of transplantation (10–12 true leaves).

2.2. Soil-field experiment

One month after grafting and for 2 consecutive years, grafted and ungrafted plants were transplanted in a sweet pepper-producing area in Valencia (east Spain) with salinity problems in soil and water. Plant density was 2.1 plants m^{-2} in sandy soil (pH = 8.0; EC as saturated past was 6.64 dS m^{-1}; Sand = 76%) in polyethylene greenhouses. The electrical conductivity and pH of the irrigation water were 4.5 dS m^{-1} and 7.60, respectively, with 32 meq l^{-1} of Na^+ and 41 meq l^{-1} of Cl. Fertilisers were applied at a rate of 200 UF N, 50 UF P_2O_5, 250 UF K_2O, 110 UF CaO and 35 UF MgO [20]. A randomised complete block design was used with three replicates, each consisting of 25 plants/year. Fruit was harvested from the end of May to the end of July and marketable fruits were weighed.

2.3. Hydroponic greenhouse experiment

One month after grafting, the root system of the plants was washed to clean the substrate and plants were placed in 5 l polyethylene pots covered with aluminium sheets. Pots were filled with a standard nutrient solution for pepper [21]. The electrical conductivity (EC) and pH of this nutrient solution was 1.7 dS m^{-1} and 6.5, respectively. Nutrient solution was added daily to compensate for uptake. After 7 days of leaving seedling plants to acclimatise to pots, salinity treatment was initiated by adding NaCl (40 mM) to the nutrient solution to reach an EC of 5.2 dS m^{-1} NaCl, similarly to that used in the soil-field experiment.

Treatments were defined by two salinity levels (0 and 40 mM NaCl) and four plant combinations: the cultivar 'Adige' grafted onto rootstock accessions 5, 12 and 14, and ungrafted 'Adige' plants were used as the controls. The layout was completely randomised with three replications per combination and six plants per replication.

During the culture, plants were grown in a Venlo-type greenhouse under natural light conditions (610–870 μmol m^{-2} s^{-1}), temperature ranges were 21–24 °C, and relative humidity was 52–72%.

All the physiological measurements were taken on 14 (*T*1) and 28 (*T*2) days after NaCl addition on fully expanded mature leaves (third or fourth leaf from the shoot apex).

2.3.1. Water relations

The osmotic potential of leaf sap (Ψ_S in MPa) was measured with an osmometer (digital osmometer, Wescor, Logan, USA). Two independent determinations were made on each replicate and plant combination, obtained from six plants per treatment and combination at *T*1 and *T*2.

Leaves were tightly wrapped in aluminium foil, frozen in liquid nitrogen and stored at −80 °C. After thawing, sap was collected from syringes at 25 °C and placed in the osmometer. Osmolyte content (mmol kg^{-1}) was converted into MPa using the Van't Hoff equation [22].

Six other similar leaves from two independent plants of each plant combination, salinity treatment and replicate were collected to determine the (RWC) as $(FW - DW)/(TW - DW) \times 100$, where FW is fresh weight, DW is dry weight, and TW is turgid weight [22].

2.3.2. Ion analysis

The leaves and roots collected at *T*1 and *T*2 for $n \geq 5$ samples of each treatment and plant combination were dried at 70 °C for 4 days. Dried samples were digested in a mixture at 70% of HNO_3–$HClO_3$ (2:1). Macronutrients (K^+, Ca^{2+}, Mg^{2+} and Na^+) were measured by ICP emission spectrometry (iCAP 6000, Thermo Scientific. Cambridge, United Kingdom).

The chloride concentration (Cl^-) in the dry plant material was extracted with 0.1 N HNO_3 in 10% (v/v) acetic acid and was determined by potentiometric tritation with $AgNO_3$ in a chloride analyzer (Sherwood, MKII 926). The results were expressed as mg g^{-1} DW.