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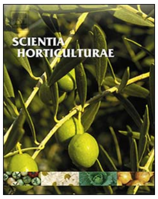

Research Paper

Grafting pepper onto tolerant rootstocks: An environmental-friendly technique overcome water and salt stress

Consuelo Penella[a], Sergio G. Nebauer[b], Salvador López-Galarza[b], Ana Quiñones[a], Alberto San Bautista[b], Ángeles Calatayud[a,*]

[a] Instituto Valenciano de Investigaciones Agrarias (IVIA), Departamento de Horticultural, Ctra. Moncada-Naquera km. 4.5. 46113 – Moncada, Valencia, Spain
[b] Universitat Politècnica de València, Departamento de Producción Vegetal, Camino de Vera 14, 46020 Valencia, Spain

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ABSTRACT

Salinity and water shortages are two of the biggest environmental constraints that crops have to face in the climate change scenario. A fast and efficient way to overcome these stresses under the prism of a sustainable crop management is the use of grafting, combining the desired cultivar with the rootstock providing tolerances to abiotic stresses. Our aim was to validate three accessions previously selected for their tolerances to salt and water stresses (A25, B14 and C12) as rootstocks, in real field conditions. The physiological and productive behavior of the commercial pepper 'Adige' (A) grafted onto these accessions was compared along the growing cycle with this cultivar grafted onto the commercial rootstock 'Antinema' (ANT) and with the ungrafted pepper plant (A).

Under water and salt stress, grafted plants onto the selected accessions, gave higher marketable yields than ungrafted plants or that plants grafted onto ANT, particularly the A25 accession. This rootstock was able to maintain high photosynthesis levels under stressing conditions through different adjustments made in the physiological processes, such as proline accumulation. The ANT rootstock showed comparable yields to A25 in control conditions. Under salt stress, Na^+ and Cl^- were equally accumulated in A/A25 plants and the ungrafted ones, but A/ANT, A/B14 and A/C12 were more restrictive in their absorption along the growing season. These results reinforce the idea that the use of tolerant pepper rootstocks is a good adaptation strategy for abiotic stressing conditions. The results also suggest that the abiotic stress was alleviated by the lack of negative effects mainly on photosynthesis, which maintained plant growth and the marketable yield.

1. Introduction

Nowadays, nearly 82% of potential crop yields are lost yearly due to abiotic stress (Hirt and Shinozaki, 2004). In addition, the amount of variable productive arable lands continues to decrease worldwide, which forces farming to move to marginal areas where the incidence of salinity and water stresses increases (Chaves and Davies, 2010). The need of obtaining crops being productive under abiotic stresses is enhanced by global warming, and by the increasing food demand for a growing world population (Thiry et al., 2016).

Among vegetables, peppers (*Capsicum* spp., mainly *C. annuum* L.) are economically and socially important crops grown in most countries around the world, and have been described as being very susceptible species to salt and water stresses (Delfine et al., 2000; Fernández et al., 2005; Pascale et al., 2003; Ashraf, 2004; Foolad, 1996; Munns et al., 2006; Russo, 2012). As a result, fruit disorders, such as blossom-end rot (BER) and cracking, diminish their marketable productivity (Penella et al., 2013).

The effect on pepper plants of both salt and water stresses has been described as a wide range of physiological, metabolic and genomic changes that provoke alterations in photosynthesis, carbohydrate partitioning, respiration, production of reactive oxygen species (ROS), and an imbalance uptake of other nutrients (Bethke and Drew, 1992; Lee et al., 2004; Navarro et al., 2003; Penella et al., 2014a, 2014b, 2015, 2016). Overall, the physiological alterations induced by abiotic stresses correspond to stunted plant growth and poor yields.

For many years breeding and biotechnological programs have been implemented to develop tolerant crops capable of producing economic yields under saline or drought conditions (Cuartero et al., 2006). However, the genetically complex nature of such stress tolerance makes this task an extremely difficult one (Ashraf and Foolad, 2007).

One environmental-friendly technique used to avoid or reduce

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[*] Corresponding author.
E-mail address: calatayud_ang@gva.es (Á. Calatayud).

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losses in commercial yields caused by abiotic stress conditions is to graft susceptible commercial cultivars onto rootstocks capable of reducing the negative effect of external stress on shoots (Colla et al., 2010; Penella et al., 2015, 2016; Rivero et al., 2003; Savvas et al., 2010; Schwarz et al., 2010). The improved tolerance to salinity of grafted plants is generally associated with their capacity to exclude or retain and/or accumulate toxic ions, mainly Na^+ and Cl^- in rootstock roots, which thus limits their transport to leaves rather than through the synthesis of osmotically active metabolites or the induction or antioxidant systems (Edelstein et al., 2011; Estañ et al., 2005). Other authors have indicated that the influence of rootstock on a scion's salt and water stress tolerance is due to: a more efficient control of stoma functions (changes in stomatal regulation and water relations); maintenance of photosynthesis; or using a larger and vigorous root systems capable of absorbing water and nutrients much more efficiently (Aloni et al., 2010; Penella et al., 2016). In other cases, such raised tolerance has been explained by the re-establishment of ionic homeostasis (Martinez-Rodriguez et al., 2008). Grafting to overcome water stress has been mostly studied in melon, cucumber (Rouphael et al., 2012) and tomato (Nilsen et al., 2014; Sánchez-Rodríguez et al., 2013) by focusing on the growth effects of grafting, and also on its physiological effects, mainly on water relations and photosynthesis traits.

The grafting technique is widely used in a commercial scale in several crops, i.e. watermelon, tomato and cucumber in several countries. I.e. in Spain, one of the main producers of fresh vegetables for export, almost 100% of watermelon is produced by grafted plants, as it is the greenhouse tomato, which is near 90%. In pepper, there are not yet rootstocks robust enough to be economically interesting. That's the reason why it is necessary to search for genotypes that in poor growing conditions give both extra yields and quality, able to face the extra cost of the technique, both seed and labor.

To date, pepper grafting has been less exploited to overcome abiotic stresses, basically because pepper genotypes tolerant to these stresses to be used as rootstocks are still not available. Studies about physiological-agronomical responses in pepper-grafted plants are scarce, and their behavior when subjected to water deficit and salinity has been insufficiently tested. To tackle these problems, the first step would be to select appropriate rootstocks by searching for tolerances in wild pepper types, which is crucial to amplify genetic diversity (Naegele et al., 2014). The second step would involve knowing how grafting alleviates abiotic stress as this would be essential for performing more phenotypical screenings of different rootstock-scion combinations. In our previous experiments, we selected three pepper accessions with different degrees of salinity and drought tolerance (C12, B14 and A25) (Penella et al., 2013, 2014b, 2015). These accessions have been tested under highly controlled conditions, and their physiological behavior has been studied (Penella et al., 2014a, 2015, 2016). However, their behavior under real field conditions is still unknown. To date, research that has compared several pepper graft combinations under control, water and salinity stress conditions cannot be found. Such studies would be extremely useful to know plant resilience under stressing conditions in order to face the climate change scenario.

From the results observed in the selection process we hypothesize that using theses wild pepper accessions as rootstocks represents a promising strategy to provide salinity and water stress tolerances, which can consequently improve crop yields under stress conditions. To evaluate the behavior of these accessions as rootstocks under real field conditions, different physiological and agronomical parameters were measured, under salinity, drought stress and control conditions, by comparing commercial rootstocks and ungrafted plants.

2. Material and methods

2.1. Plant material

Based on previous studies, we selected three Capsicum accessions with increased water (Penella et al., 2014a, 2014b) and salt stress (Penella et al., 2013, 2015, 2016) tolerance to be used as rootstocks from the COMAV Gene bank at the UPV university (Valencia, east Spain): one from *Capsicum chinense* Jacq. (code C12), one from *Capsicum baccatum* L. var. pendulum (code B14), and one from *Capsicum annuum* L. (code A25). In general terms, when a sensitive scion was grafted onto these accessions under abiotic stress, physiological and biochemical traits such as higher net photosynthesis rate, higher nitrate reductase activity, biomass maintenance, among other characters were observed (Penella et al., 2014a, 2015).

In order to test the behavior of these accessions, pepper cultivar 'Adige' (A) (Lamuyo type, Sakata Seeds, Japan) was grafted onto them. A commercial rootstock, cv. Antinema (Sakata) (code ANT), was also used as control rootstock.

Seeds were sown in 104-hole seed trays filled with an enriched substrate for germination at the end of December. After 2 months, plants were grafted by the tube-grafting method (Penella et al., 2015). The ungrafted 'Adige' (A) plants were sown 2 weeks later to obtain plants with a similar biomass to that of the grafted plants at the time of transplantation (10–12 true leaves). Five plant combinations were studied: ungrafted Adige plants (A), Adige grafted onto Antinema (A/ANT), Adige grafted onto accession C12 (A/C12), Adige grafted onto accession B14 (A/B14) and Adige grafted onto accession A25 (A/A25).

2.2. Soil-field experiment

The experiment was conducted in spring/early summer at three different locations. An unstressed control was carried out in Moncada (Valencia, Spain; Latitude: 39.58951793357715, Longitude: −0.3955507278442383), in the IVIA experimental fields. Irrigation of control plants satisfied 100% evapotranspiration (ETc), as described in Penella et al. (2014b). The electrical conductivity of the nutrition solution was 1.16 $dS\ m^{-1}$ at pH 7.5. The soil characteristic were sandy clay loam soil (clay: 21.2%; silt: 11.8%; 67%); Organic matter: 0.61%; pH1/5 at 20 °C: 8.1; EC 1:5 at 25 °C: 0.289 $dS\ m^{-1}$. The water stress assay was conducted in the ANECOOP experimental station field located in Museros (Valencia, Spain; 39.57736296452871, −0.36434054374694824), 4 Km away from the IVIA station and sharing similar soil conditions (sandy loam soil (clay 16.72%; silt 18%; sand 65.28%); Organic matter 1.48%; pH1/5 at 20 °C: 7.8; EC 1:5 at 25 °C: 0.344 $dS\ m^{-1}$). The water stressed treated plants were irrigated to satisfy 60% of ETc by modifying the number of irrigations and maintaining the volume constant for each irrigation event. The electrical conductivity of the irrigation water was 1.03 $dS\ m^{-1}$ at pH 7.5. For the salt condition, a field near Valencia (El Perelló; 39.28159975375096, −0.28244733810424805) with a salinity problem was used. The soil in this field had a moderate salt concentration (sandy loam soil (clay 20%; silt 6%; sand 74%); Organic matter 3.31%; pH 1/5 at 20 °C: 7.8; EC 1:5 at 25 °C: 1.44 $dS\ m^{-1}$) and the electrical conductivity and pH of the irrigation water in this area were 7.5 $dS\ m^{-1}$ and 7.6, respectively, with 57.5 mM of Na^+ and 71.2 mM of Cl^-.

The average range of minimum and maximum temperatures was 9–10 °C for April and 28 − 29 °C for July in all locations.

During the trial experiments, seedlings were transplanted in April at a density of 2.5 plants m^{-2} in a polyethylene greenhouse, in lines 1 m apart and 0.4 m between plants. The three experiments were laid out according to a complete randomized block design with three replicates. Each replicate consisted in 40 plants. Fertilizers were applied at a rate of 200 N, 50 P_2O_5, 250 K_2O, 110 CaO, and 35 MgO all in kg ha^{-1}, as recommended by Maroto (2002).

Ripe fruits were harvested from the end of May to the end of July, and marketable and unmarketable fruits, mainly due to BER, were weighed. All the physiological parameters were measured 80, 110 and 140 days after transplanting (DAT). No significant differences were observed among replicates in all the studied parameters at each studied