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

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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.

Keywords: grafting; pepper; photosynthesis; proline; salt ions; water relations

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 (Gomiero, 2016). 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,b, 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 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,b) 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 20ºC: 8.1; EC 1:5 at 25ºC: 0.289 dS/m. 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 20ºC: 7.8; EC 1:5 at 25ºC: 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 20ºC: 7.8;

EC 1:5 at 25ºC: 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 9-10º C for April and 28-29 º 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 location.

2.3. Biomass and ion determination

Plants were harvested after 140 DAT for n=8 samples of each treatment and plant combination. Afterward, leaf area was measured with a LI-COR 3000 A (LI-COR, Nebraska, USA). For the dry weight (DW) determinations, leaves and roots were dried at 70°C for 72 h in a laboratory oven and were then weighed. Leaves and roots were digested in a mixture at 70% HNO_3-$HClO_3$ (2:1). Na^+ concentrations 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).

2.4. Water relations

The midday leaf water potential (Ψ_w) was determined in a Scholander pressure chamber (PMS Instruments, Albany, USA). In the same leaves, the osmotic potential of leaf sap (Ψ_s) was measured by a Digital osmometer (Wescor, Logan, USA). To this end, five leaves from each treatment and plant combination were frozen at -70 ºC. Samples were thawed and centrifuged in Eppendorf tubes at 8000 g for 10 min to obtain sap (modified from Callister et al., 2006). The mmol kg^{-1} of osmolytes were converted into MPa by the Van't Hoff equation. The turgor potential (Ψ_p) was determined as the difference between the leaf water potential and the osmotic potential. The water, osmotic and turgor potentials were determined at 80, 110 and 140 DAT.

2.5. Gas exchange measurements

The net CO_2 fixation rate (A_N, μmol CO_2 m^{-2} s^{-1}), stomatal conductance (g_s, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), and substomatal CO_2 concentration (C_i, μmol CO_2 mol^{-1} (air)), and water use efficiency (WUE= A_N/E) were determined on fully expanded leaves (3rd-4th leaf from the apex) in the steady state under saturating light conditions (1000 μmol m^{-2} s^{-1}) and with 400 ppm CO_2 by a LI-6400 (LI-COR, Nebraska, USA). Gas exchange measurements were performed from 9 am to 11 am (GMT), and 6 determinations were conducted in different plants for each plant combination and location at 80, 110 and 140 DAT.

2.6. Proline determination

Proline content was determined as described by Bates et al., (1973). Leaf pepper tissue (20 mg) was ground in 3% sulfosalicylic acid, the homogenate was filtered, and glacial acetic acid and ninhydrin reagent were added to an aliquot of the filtrate. The reaction mixture was boiled for 1 h, and readings were taken in a spectrophotometer at a wavelength of 546 nm. Proline concentration was measured for $n \geq 4$ leaves of each treatment, plant combination and location at 80, 110 and 140 DAT.

2.7. Statistical analyses

To statistically compare the means between plant combinations under each treatment condition and for each studied time, a one-way analysis of variance (ANOVA) was used (Statgraphics Centurion for Windows, Statistical Graphics Corp.). Data were tested first for homogeneity of variance and normaly of distribution by Barlett's test. Mean comparisons were made by Fisher's least significance difference (LSD) test at $P \leq 0.05$.

3. Results

3.1. Fruit yield

Under control conditions, Adige grafted onto ANT and A25 rootstocks increased marketable yield (Fig. 1) when compared to the ungrafted plants (44 and 40 % more, respectively).

Water and salt stress reduced the amount of marketable fruits. It is noteworthy that the A/ANT combination showed the highest reduction under both stress conditions. Under drought stress, A/25 gave the best marketable fruit responses, displaying the lowest reduction when compared to control conditions (Fig. 1). A/C12 and A/B14 combinations gave intermediate production under water limitation. Minimal production was displayed by the ungrafted plants and A/ANT combination under salinity (Fig. 1). Higher amount of marketable fruits were produced when grafted onto C12, B14 and A25 under salt stress.

Overall, these data indicate that pepper plants displayed improved yield performance under all tested conditions when grafted onto the A25 rootstock.

3.2. Water relations

A statistical analysis on the leaf water potential (Ψ_w) confirmed the significant impact of the graft combination for the stressing treatment at 80 and 140 DAT (Table 1). The Ψ_w values lowered (became more negative) with time for all the plant combinations and treatments, and the average decrease was greater for the plants grown under the water stress condition by the end of the experiment (140 DAT) ($P \leq 0.05$).

At the end of the experiment, no differences were observed among combinations under control conditions. However, under both stress conditions the plants grafted onto accession A25 maintained less negative Ψ_w values, followed by A/B14 under water deficit, and then by A/C12 and A/B14 under salt stress.

Similar values were found for the leaf osmotic potential (Ψ_s) in the plants under the control and salt stress conditions at any time (Table 1). Under water stress at 140 DAT, significant differences were observed in the ungrafted plants compared the plants grafted onto B14, C12 and A25, which had less negative Ψ_s values.

The turgor potential, Ψ_p, (Table 1) under water stress for the 80 DAT A/C12 and A/B14 graft combinations obtained the lowest values. At 110 DAT only the Ψ_p values of the ungrafted plants were negative. However at the end of the experiment (140 DAT), only plants A/A25 and A/B14 maintained positive values. Under salt stress at 80 DAT, the highest values with significant differences were for the ungrafted A/ANT and A/A25 plants, but all the plant combinations were negative at the end of the experiment.

3.3. Ion partitioning

No significant changes of sodium and chloride were observed during the experiment under the control and water stress conditions (data not shown), with values below 5 mg g^{-1} DW for Cl^- and 3 mg g^{-1} DW for Na^+.

Overall, sodium and chloride content increased with time ($P \leq 0.05$) in the roots of all combinations tested (Fig. 2). Chloride content was highest in the ungrafted and A/25 plants. Furthermore, lowest sodium content at the end of the experiment was determined in A/C12 plants.

In leaves, chloride also accumulated in all combinations with time ($P \leq 0.05$). Highest values were observed at the end of the experiment in ungrafted plants, followed by A/ANT and A/A25, being lowest in A/B14 and A/C12 (Fig. 2).

3.4. Proline content

The statistical analysis of proline accumulation confirmed the significant impact of the studied plant combinations, treatment and time (Fig. 3). Under control conditions, plants grafted onto C12, B14 and A25 displayed slightly higher proline content at the end of the experiment. It is noteworthy that higher levels of proline were accumulated in A/B14 and A/A25 combinations under both water and salt stress in all measured dates ($P \leq 0.05$). Ungrafted plants had lower proline concentration at 140 DAT, whereas A/ANT and A/C12 plants displayed intermediate levels (Fig. 3). A/ANT combination accumulated lower proline content under salinity at the end of the experiment.

3.5. Gas exchange

As shown in Table 2, the net assimilation of CO_2 (A_N), the intercellular CO_2 concentration (C_i) and instantaneous WUE parameter did not show any statistical differences during the experiment under control conditions. Drought provoked a progressive decrease in the net assimilation rate of CO_2, although A/A25 and A/C12 showed highest rates at the end of the experiment (Table 2). In addition, stomatal conductance also decreased during the experiment, especially at 140 DAT. At this time point, A/A25 plants displayed higher values of g_s, At the end of the experiment, this stomatal closure was related to a decrease in Ci ($P \leq 0.05$) in all plant combinations excepting A/A25 suggesting stomatal limitations to photosynthesis.

Under salinity, although some decrease in the net photosynthetic rate was provoked in all combinations, the ungrafted plants showed significantly lower values at 140 DAT (Table 2). However at this time, stomatal closure was significant in the ungrafted and A/ANT plants, being higher in A/B14 and A/A25 combinations. From 110 DAT onwards, A/A25 showed increased WUE values (Table 2).

3.6. Plant Growth

At the end of the experiment (140 DAT), A/A25 plants grown in control conditions showed higher shoot biomass than the ungrafted and A/ANT plants (Fig. 4A). No significant differences were found among combinations in the root biomass in the control experiment.

Interestingly, similar results were obtained in the shoot biomass both under water and salt stress conditions (Fig. 4B,C). In addition, A/A25 plants developed bigger roots under stress conditions when compared to both the ungrafted and A/ANT plants.

The leaf area of the ungrafted plants was significantly smaller than the other combinations at 140 DAT ($P \leq 0.05$) (Fig. 5), when plants were grown under water (Fig. 5B) and salt stress (Fig. 5C) conditions.

4. Discussion

The selection of salt and water stress tolerant accessions to be used as rootstocks could be a promising approach to ameliorate the negative effects of abiotic stresses on pepper productivity (Penella et al., 2013, 2014a,b, 2015, 2016). In the present study, the results showed that Adige peppers grafted onto tolerant accessions under real field stressing conditions were less sensitive to salt and water stress than their ungrafted counterparts were. This finding was noticed at the end of the experiment by the major yields obtained mainly for Adige grafted onto A25.

As expected, the growth of the ungrafted pepper plants in our experiment decreased for all the studied times under both the saline and water stress conditions. In line with other authors, this indicates that pepper plants behave as salt and drought-sensitive species (Delfine et al., 2000; Pascale et al., 2003; Rubio et al., 2010). In this sense, different authors have demonstrated that sweet pepper irrigated with water salinity (3.4 dS m^{-1}) reduced the total yield (35%), although this salinity concentration was far respect to our salinity conditions (7.5 dS m^{-1}); however improved some fruit quality like total sugar was observed (Alharbi et al. 2014). Increased levels of salinity produced a high level of salt accumulation in pepper root zone inducing decrease in vegetative growth, biomass production and yield but this effect was depend of growing season and of an appropriate irrigation regime (Rameshwarean et al., 2016) and the cultivar (Chartzoulakis and Klapaki 2000). Deficit irrigation at 60% (identical decrease to our water stress) mainly during the period between flowering and fruit development caused significant reductions in pepper yield in comparison with full irrigation (Nagaz et al., 2012). These constraints could be coping with the use of tolerant root system that it could help overcome stress to the scion. Root biomass production was higher in the A/A25 plants than in ungrafted plants or in plants grafted onto ANT under stress conditions. This is in agreement with in a previous work in a controlled short-term experiment (Penella et al., 2016).

Regarding aerial parts, according to Yetişir et al. (2007) and Colla et al., 2010, all the grafted plants of watermelon showed a larger number of leaves and higher dry weight values than the non-grafted control plants. Similar results have also been reported in tomato (Borgognone et al., 2013; He et al., 2009). According to our results, the ungrafted plants showed a reduced leaf area when grown under stress, which remained unchanged for all the grafted pepper plant combinations. These effects can be ameliorated by grafting, as our results evidenced. Photosynthetic activity was higher in all grafted combinations under salinity at the end of the experiment. Under drought, A/A25 and A/C12 maintained highest photosynthetic rate. Similar results were obtained in our previous studies (Penella et al., 2014a, 2016). It has to be pointed out that only A/A25 combination maintained high photosynthetic rates under both stresses, in accordance with the larger amount of marketable fruits obtained.

In general terms, stomatal conductance markedly decreased with the water deficit treatment for all the plant combinations; on the contrary, the decline was more attenuated under our salinity conditions. Notably at the end of the experiment (140DAT), along with the CO_2 assimilation rate, the plants grafted onto accession A25 had higher stomatal conductance values under salinity and drought. The decrease in Ci with g_s suggested stomatal limitations in the other plant combinations under drought. Under salinity conditions, A/A25 plants showed higher WUE. These results coincide with previous findings, which have highlighted the use of tolerant rootstocks to improve the photosynthesis performance of the scion under abiotic stress conditions (He et al. 2009; Orsini et al. 2013, Penella et al. 2014a).

Proline accumulation is a well-known adaptive mechanism in plants to fight against abiotic stress conditions. Several studies have attributed multiple roles to proline: compatible osmolite, a signaling molecule that influences defense pathways, regulation of complex metabolic and development processes, and a protective compound (Szabados and Savouré, 2010; Verslues and Sharma, 2010). Proline synthesis in leaves significantly increased in plants A/A25 and A/B14 for both salinity and water stresses, and for all the studied times. Our results suggest that the proline accumulation observed in our experiments was more related with their protective role than with its role in maintaining the osmotic potential, as its contribution was less than 0.1 MPa. However, the reported protective role in the photosynthetic process (Szabados and Savouré, 2010) seems to be clear in A/A25 plants, as under both stressing conditions photosynthesis was maintained, which agrees with other studies conducted in tomato genotypes (Amini and Ehsanpour, 2005; Patanè et al., 2016). Nevertheless, that was not observed in A/B14 plants, at least in water stress conditions.

A specific attribute of salinity conditions was ion toxic effects on plants (Bartels and Sunkar, 2005; Munns et al., 2002). Grafting has been described to increase salt tolerance by excluding or restricting toxic ion accumulation in shoots (Colla et al., 2013). In fact we previously reported this mechanism in pepper plants grafted onto accessions B14 and C12 during a short-term experiment (Penella et al., 2015). With the present long-term experiment performed under field conditions, we corroborate that less Cl^- was transported to the leaves of these accessions used as a rootstock compared to the ungrafted and A/ANT and A/A25 plants. Regarding Na^+ concentration, less Na+ was allocated to A/B14 and A/ANT plant leaves under the field conditions. Contrarily, the A/A25 plant tissues accumulated high concentrations of toxic ions, as in previous studies (Penella et al., 2016), and as reported by He et al., 2009 in salt-tolerant grafted tomato plants. Despite the continuous salt ions uptake, the buffer capacity of the A/A25 plants was not superseded, as witnessed by the unaffected biomass production, even at 140 DAT. In view of the high Na^+ and Cl^- accumulations, their probable compartmentalization in the vacuole and/or apoplastic space to preserve the cytosol from ionic toxic effects could occur (Penella et al. 2016).

Despite the negative effect on plant growth that derived from its toxic effect, accumulation of salt ions could help maintain the turgor pressure of plants (Blum et al., 1996; Navarro et al., 2003), and could occur in the A/25 plants grown under salinity conditions, which was the only combination that maintained a positive Ψ_p. Regarding long-term water stress, A/A25, followed by A/B14, conserved their Ψ_p, and consequently their leaf cells remained turgid.

5. Conclusion

The A/A25 plants were highly tolerant to salt and water stresses presumably given the adjustments made in the physiological processes, and they obtained larger marketable yields, even under the control conditions. C12 and B14 showed intermediate behaviors with minor yields for both stress situations, a better capacity to control the entry of salt ions to plants. The ANT rootstock gave similar yields than A25 under control conditions but its behavior was greatly reduced in abiotic stressing conditions.

Our results show that pepper grafting on suitable rootstocks from *Capsicum* spp. has positive effects on cultivation performance. Specifically, the accession A25 is a priceless plant material to be used as a rootstock, which can be further improved by breeding programs.

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Figure legends

Fig. 1. Marketable fruit yield (kg m^{-2}) of cultivar Adige (A), ungrafted, Adige grafted onto commercial rootstock Antinema (A/ANT), Adige grafted onto the C12 genotype (A/C12), Adige grafted onto B14 (A/B14), and Adige grafted onto A25 (A/A25) under control conditions (A), water stress conditions (B) and salt stress (C). Values are the mean of n= 120 plants for each treatment±SE. Different letters in each column indicate significant differences at P≤0.05 using the LSD test, following a one-way ANOVA for each treatment, with plant combinations as the variability factor.

Fig. 2. The Cl^- and Na^+ concentrations in leaves (A, C) , B) and roots (B, D) measured under salt field conditions at 80, 110 and 140 days after transplanting (DAT) in ungrafted cultivar Adige (A), and grafted onto commercial rootstock Antinema (A/ANT), Adige grafted onto the C12 genotype (A/C12), Adige grafted onto B14 (A/B14) and Adige grafted onto A25 (A/A25), represented from white to black, as shown in the legend. Data are the mean values±SE for n=5. For each studied time, different letters indicate significant differences at P≤0.05 (LSD test), following a one-way ANOVA test with plant combination as the variability factor.

Fig. 3. Changes in the proline concentration at 80, 110 and 140 days after transplanted (DAT) from ungrafted cultivar Adige (A), and grafted onto commercial rootstock Antinema (A/ANT), Adige grafted onto the C12 genotype (A/C12), Adige grafted onto B14 (A/B14) and Adige grafted onto A25 (A/A25), under the control conditions (A), water stress conditions (B) and salt stress (C). Data are the mean values±SE for n=4. For each studied time, different letters

indicate significant differences at P≤0.05 (LSD test), following a one-way ANOVA test for each treatment, with plant combinations as the variability factor.

Fig. 4. Dry weight (DW) for leaves and roots 140 days after transplanting from ungrafted cultivar Adige (A), and grafted onto commercial rootstock Antinema (A/ANT), Adige grafted onto the C12 genotype (A/C12), Adige grafted onto B14 (A/B14) and Adige grafted onto A25 (A/A25), under the control conditions (white bar), water stress conditions (gray bar) and salt stress (black bar). Data are the mean values±SE for n=8. In each treatment, different letters indicate significant differences at P≤0.05 (LSD test), following a one-way ANOVA test with plant combinations as the variability factor.

Fig. 5. Leaf area (cm^2) 140 days after transplanting from ungrafted cultivar Adige (A), and grafted onto commercial rootstock Antinema (A/ANT), Adige grafted onto the C12 genotype (A/C12), Adige grafted onto B14 (A/B14) and Adige grafted onto A25 (A/A25), under the control conditions (A), water stress conditions (B) and salt stress (C). Data are the mean values±SE for n=8. In each studied treatment, different letters indicate significant differences at P≤0.05 (LSD test), following a one-way ANOVA test with plant combinations as the variability factor.

Table 1. Water potential Ψ_w(MPa), osmotic potential Ψ_s (MPa) and turgor potential Ψ_p (MPa) of the leaves from the ungrafted cultivar Adige (A), and grafted onto commercial rootstock Antinema (A/ANT), Adige grafted onto the C12 genotype (A/C12), Adige grafted onto B14 (A/B14) and Adige grafted onto A25 (A/A25), under the control conditions, water stress conditions and salt stress, measured 80, 110 and 140 days after transplanting (DAT). Data are the

mean values±SE for n=5. For each studied time and treatment, different letters indicate significant differences at $P \leq 0.05$ (LSD test), following a one-way ANOVA test with plant combinations as the variability factor for each treatment.

Table 2. Gas exchange parameters of the ungrafted cultivar Adige (A) and grafted onto commercial rootstock Antinema (A/ANT), Adige grafted onto the C12 genotype (A/C12), Adige grafted onto B14 (A/B14) and Adige grafted onto A25 (A/A25), under the control conditions, water stress conditions and salt stress, measured 80, 110 and 140 days after transplanting (DAT). The net CO_2 fixation rate (A_N, μmol CO^2 m^{-2} s^{-1}), stomatal conductance to water vapor (g_s, mol H_2O m^{-2} s^{-1}), transpiration rate (E, mmol H_2O m^{-2} s^{-1}), and substomatal CO_2 concentration (C_i, μmol CO_2 mol^{-1} (air)), and water use efficiency ($WUE= A_N/E$) were measured on fully expanded leaves in the steady state under conditions of saturating light (1000 μmol m^{-2} s^{-1}) and 400 ppm CO_2. Data are the mean values±SE for n=6. For each studied time and treatment, different letters indicate significant differences at $P \leq 0.05$ (LSD test), following a one-way ANOVA test with plant combinations as the variability factor for each treatment.