RESEARCH ARTICLE

Physiological changes of pepper accessions in response to salinity and water stress

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Abstract

New sources of water stress and salinity tolerances are needed for crops grown in marginal lands. Pepper is considered one of the most important crops in the world. Many varieties belong to the genus *Capsicum* spp., and display wide variability in tolerance/sensitivity terms in response to drought and salinity stress. The objective was to screen seven salt/drought-tolerant pepper accessions to breed new cultivars that could overcome abiotic stresses, or be used as new crops in land with water and salinity stress. Fast and effective physiological traits were measured to achieve the objective. The present study showed wide variability of the seven pepper accessions in response to both stresses. Photosynthesis, stomatal conductance and transpiration reduced mainly under salinity due to stomatal and non-stomatal (Na^+ accumulation) constraints and, to a lesser extent, in the accessions grown under water stress. A positive relationship between CO_2 fixation and fresh weight generation was observed for both stresses. Decreases in Ψ_s and Ψ_w and increased proline were observed only when accessions were grown under salinity. However, these factors were not enough to alleviate salt effects and an inverse relation was noted between plant salt tolerance and proline accumulation. Under water stress, A31 was the least affected and A34 showed the best tolerance to salinity in terms of photosynthesis and biomass.

Additional keywords: osmotic potential; photosynthesis; proline; salinity ions; water potential.

Abbreviations used: A_N (maximum net CO_2 fixation rate); A_N/C_i (instantaneous carboxylation efficiency); C_i (substomatal CO_2 concentration); DW (dry weight); E (transpiration); ETc (evapotranspiration); FW (fresh weight); g_s (stomatal conductance to water vapour); Ψ_s (osmotic potential); Ψ_w (water potential).

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Introduction

With the global scarcity of water resources and increased of salinity in water and soil, these abiotic stresses constitute major limiting factors in plant growth and, consequently, agriculture productivity is decreasing (Bray *et al.*, 2000).

Plant responses to water and salinity stresses are complex and involve adaptive changes and/or deleterious effects (De Oliveira *et al.*, 2013). The outcomes of both stress types on plant performance are diverse, but have some points in common. The main effect when plants start becoming stressed is the reduced water content in their tissue, and therefore the closure of leaf stomatal complexes takes place. Consequently, transpiration (Bray *et al.*, 2000) and/or photosynthesis may decrease through reduced osmotic potential in the soil solution, which involves reduced water potential (Bojórquez-Quintal *et al.*, 2014; Penella *et al.*, 2014a, 2015, 2016). If salinity stress occurs, a specific ionic effect appears, mediated by the accumulation of toxic ions in cellular tissues (De Pascale *et al.*, 2003) with imbalances between nutrients (Hasanuzzaman *et al.*, 2013). All these factors have adverse effects on both plant growth and development at physiological and biochemical levels (Munns & James, 2003).

Plants have evolved mechanisms to overcome salinity and water deficit that allow them to perceive incoming

stresses and to regulate their metabolic functions. In general, one of the important pathways to enhance water stress and salt tolerance is through osmotic adjustment (OA), in which leaf turgor remains necessary for stomatal opening and, thus, sustains photosynthesis and growth (Huang *et al.*, 2010; Nio *et al.*, 2011). Besides, various types of compatible solutes accumulate, such as sugars, proline, gycinebetaine or potassium, among others (Munns *et al.*, 1979; Morgan, 1992; Nio *et al.*, 2011), and can increase. These compounds can be added to the list of the non-enzymatic antioxidants that plants need to counteract the inhibitory metabolic effects of reactive oxygen species (ROS) provoked by stress (Gill & Tuteja, 2010; Penella *et al.*, 2014a, 2016). They also play a role in both the stabilisation of enzymes and proteins and the protection of membrane integrity (Patade *et al.*, 2012). Salt tolerance may arise from the ability to tolerate osmotic stress, from mechanisms of salt exclusion or from intercellular ion compartmentalisation (Munns & Tester, 2008). These mechanisms are not normally exclusive, so plants can combine some of these strategies at the same time (Chaves *et al.*, 2003). Furthermore, tolerance levels may vary between species (Munns, 2002) and within cultivars of the same species (Chartzoulakis & Klapaki, 2000).

Understanding the tolerance mechanisms that occur at the whole plant level has implications for screening and distinguishes plants that are tolerant to salinity and water stress (Munns, 2002). In the climate change scenario, new sources of salt and water stress tolerance are needed for the crops grown in areas with salinity and scarcity water problems. This available genotypic variability in terms of tolerance to abiotic stresses can provide plant species with a breeding opportunity to obtain better yields and production, and good fruit quality.

Pepper is a member of the family *Solanaceae* and is considered one of the most important crops in the Mediterranean area, where water shortage and salinity are major problems that limit productivity (Penella *et al.*, 2013, 2014b). Many crops belong to the genus *Capsicum* spp., and display wide genetic variability (Aktas *et al.*, 2006). Pepper has been classified from moderately sensitive to sensitive under salinity and water stress conditions (Tanji & Kielen, 2002; Penella *et al.*, 2015). In fact, some studies have reported reduced seedling growth with 50 mM concentration of NaCl (Chartzoulakis & Klapaki, 2000; De Pascale *et al.*, 2003). Sometimes pepper has been described as one of the most susceptible crops to water stress, mainly due to its large transpiring leaf surface and high stomatal conductance of water vapour (Alvino *et al.*, 1994; Delfine *et al.*, 2002). Consequently, pepper plants are particularly sensitive to water stress at flowering and fruit setting (Bosland & Votava, 2000). However, not all *Capsicum* genus cultivars have the same sensitivity to abiotic stresses (Penella *et al.*, 2013, 2014b; Aktas *et al.*, 2006). Therefore, the study and identification of the tolerance level and mechanisms of different pepper genotypes are immensely important to breed new cultivars that can overcome abiotic stresses, or be used as new crops in land with drought and salinity problems to help extend the cultivated property. For pepper, very rare information about genotype variability in terms of its behaviour under salinity and water stress is available.

Different physiological markers have been proposed as key traits to select salt and water stress tolerance. Our most recent works evaluated several pepper accessions. We selected some of them as a source of tolerance to salinity and water stress (Penella *et al.*, 2013, 2014b) using gas exchange as a useful technique to differentiate tolerance and susceptibility to these stresses.

In the present study, we tested new accessions of *Capsicum annuum* L. for them being the most economically important species from the *Capsicum* genus in the Mediterranean climate. Accessions selection was made according to previous results (Penella *et al.*, 2013; 2014b). To evaluate their behaviour under salinity and water stresses, we studied the physiological mechanisms that underlie tolerance strategies using efficient parameters to identify which pepper accessions are tolerant to salt and/or water stress to be used in marginal areas and/or in breeding programmes. Further, we describe the physiological parameters roles and discussing the possibility of using them as selection criteria for cast salt and water stress genotypes with tolerance. As predictive screening parameters to salinity and water stresses in these seven new pepper accessions, we measured photosynthesis (A_N), stomatal conductance (g_s), inner carbon (C_i), water potential (Ψ_w), osmotic potential (Ψ_s), proline content, ion concentrations and biomass and their relationships.

Material and methods

Plant material

The *C. annuum* accessions used herein were Numex X (A31), Numex sandia type 2 (A32), Numex conquistador type 2 (A33), BGV-11814 (A34), BGV-4349 (A35), SIURIYA 600 (A36) and KAPIYA UV (A37). A numerical code for each accession is indicated in brackets. All the accessions used in the present study belong to the COMAV Institute collection (Universitat Politècnica de València, Valencia, Spain). Seeds

were germinated in moistened perlite at 28ºC under greenhouse conditions. Seedlings were transferred to 15 L pots that contained coconut coir fibre (Cocopeat, Projar Co., Spain) in a heated polyethylene greenhouse on 10 April 2016 in the Instituto Valenciano de Investigaciones Agrarias (IVIA, Valencia, Spain). Plants were drip–irrigated with Hoagland's No. 2 nutrient solution containing (all in mM): 14 NO_3^-, 1.0 $H_2PO_4^-$, 2.0 SO_4^{2-}, 1.0 NH_4^+, 16.0 K^+, 4.0 Ca^{2+} and 2.0 Mg^{2+}. Micronutrients were also provided (all in µLM): 15 Fe^{2+}, 10 Mn^{2+}, 5 Zn^{2+}, 30 B^{3+}, 0.75 Cu^{2+} and 0.6 Mo^{6+}) (Maynard & Hochmuth, 2007). The electrical conductivity (EC) of the nutrient solution was 1.4 dS/m and pH 6.1. The greenhouse conditions in this period varied between 16ºC and 25ºC and from 50% to 70% of relative humidity.

After 15 days in pots, plants were divided into three groups for the control, saline and water stress treatments. Salinity treatment began by adding NaCl (60 mM) to the irrigation solution to reach an EC of 6.8 dS/m. Drip irrigation was applied based on estimations of weekly crop evapotranspiration (ETc) (Allen *et al.*,1998), even though the nutrient saline solution was allowed to drain freely from pots and the control drainage was controlled from 10% to 20% depending on solar radiation. Water stress treatment began by reducing the volume of irrigation water to 60% of the control. The volume of each irrigation and the number of irrigations were scheduled to maintain drainage between 10% and 20% (depending on solar radiation).

Eight plants per accession were used in each treatment. Physiological measurements were taken 1 month after the salinity and water deficit treatments began on fully expanded mature leaves (third or fourth leaf from the shoot apex) and completed in 1 day.

Biomass

All the plants were harvested immediately after physiological parameters were measured. Aerial parts and roots were separated and their fresh weight (FW) was recorded. They were dried at 70°C for 72 h in a laboratory oven and then weighed for the dry weight (DW) determinations. Salt and water tolerance efficiencies (Fischer & Wood, 1981) were calculated according to the formulae: (DWstress/DWcontrol)*100, where DWstress and DWcontrol are total dry weight (aerial and root) of each genotype under the stress (water or salinity) or control conditions.

Photosynthesis measurements

Maximum net CO_2 fixation rate (A_N, µmol CO_2/m²·s), stomatal conductance to water vapour (g_s, mol H_2O/m²·s) transpiration rate (E, mmol H_2O/m²·s) and substomatal CO_2 concentration (C_i, µmol CO_2/mol (air)) were measured at the steady state under conditions of saturating light (1000 µmol/m²·s), 400 ppm CO_2 and 23-25ºC leaf temperature cuvette with a LI-6400 (LI-COR, Nebraska, USA). Parameter A_N/C_i was calculated as instantaneous carboxylation efficiency. Gas exchange measurements were taken on the third or fourth leaf from the shoot apex from 9 am to 11 am (GMT). One measurement per plant was taken, and eight different plants were used (n=8) for each treatment and accession.

Water relations

The osmotic potential of leaf sap (Ψ_s in MPa) was measured by an osmometer (Digital osmometer Vapro 5520, Wescor, USA). Leaves were tightly wrapped in aluminium foil, frozen in liquid nitrogen and stored at -80ºC. After thawing, sap was collected by centrifuging at 8,000 rpm at 4ºC and placed in the osmometer (modified from Callister *et al.*, 2006). Osmolyte content (mmol/kg) was converted into MPa using the Van't Hoff equation (Penella *et al.*, 2014a). Leaf water potential (Ψ_w in MPa) was measured on the leaves sampled with a Schlolander pressure chamber (Wescor Model 600, PMS Instruments, Albany, USA). Two independent determinations were made on each replicate and plant, obtained from six plants per treatment and combination for Ψ_s and Ψ_w.

Proline determination

Proline content (mg proline/g DW) was determined as described by Bates *et al.* (1973). Leaf and root dried pepper tissue (0.02 g) was ground in 3% sulphosalicylic acid, the homogenate was centrifuged at 8,000 rpm for 5 min, filtered, and 0.60 mL of glacial acetic acid and 0.70 mL of ninhydrin reagent (2.5 g ninhydrin in 600 mL glacial acetic acid and 40 mL 6 N phosphoric acid) were added to an aliquot of the supernatant. The reaction mixture was boiled for 1 h at 100ºC, and readings were recorded at a wavelength of 520 nm in a spectrophotometer. Proline determination was made for n=4 for each treatment and accession.

Sodium and chloride ions analysis

The leaves and roots collected for n=4 samples of each treatment and accession were dried at 70ºC for 4 days. Dried samples (0.1-0.2 g) were burnt in a muffle furnace for 12 h at 550ºC. Ions were extracted with 2% nitric acid in an ultrasonic bath for 30 min at 4ºC. Na^+ concentration was measured by an atomic absorption spectrometer (A Analyst 200, Perkin Elmer).

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 titration with $AgNO_3$ in a chloride analyzer (Sherwood, MKII 926).

The results for both ions were expressed as $[Na^+]$ or $[Cl^-]$ salt stress/$[Na^+]$ or $[Cl^-]$ control for n=6 independent samples in leaves and roots.

Statistical analysis

The layouts of the experiments took a completely randomised design. Data of each accession for treatments (control, water and salinity stress) were subjected to one-way ANOVA`s. The mean comparisons were made using Fisher's least significance difference (LSD) test at $p<0.05$. The data of (DWstress/DWcontrol)*100 (Arcsin $X^{½}$ transformation) and $[Na^+]$ or $[Cl^-]$ salt stress/$[Na^+]$ or $[Cl^-]$ were subjected to a one-way ANOVA with genotype as the variability factor, and the means comparisons Fisher's least significance difference (LSD) test at $p<0.05$ was applied.

The data obtained in some measurement parameters were subjected to linear regression and analyses to identify the relationships between the physiological parameters.

Results

Biomass

The first step in this experiment was to detect the phenotypical variations regarding water and salinity tolerance in these seven pepper accessions. The pepper accessions grown under stress and control conditions showed significant differences in DW (Fig. 1A). A31 and A32 were the accessions with the highest DW values under the control treatment. Both stresses significantly decreased parameter DW in all the accessions. Salinity generated the lowest DW biomass in all the accessions. Under drought, A31 showed the minor decrease and under salinity A34 stood out. The salt and drought tolerance indices were also determined to distinguish between sensitive or tolerant accessions (Fig. 1B). Under water stress, A31 stood out with 72% and A36 obtained the lowest value with 46% inhibition, while the other accessions displayed similar values. Regarding salinity, A34 showed the higher percentage (53% compared to its control), and the rest exhibited values of around 24-39%.

Photosynthetic parameters

The leaf CO_2 assimilation rate (Fig. 2A), stomatal conductance (Fig. 2B) and transpiration (Fig. 2C) were strongly reduced in pepper accessions exposed
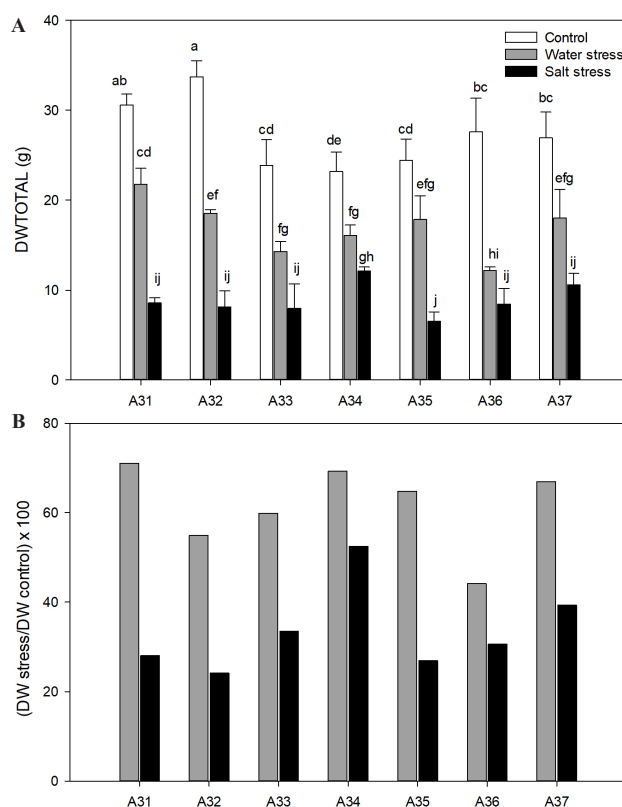

Figure 1. Total dry weight (A) and salt and water tolerance efficiencies (B) calculated as (DWstress/DWcontrol)*100 of pepper accessions after 1 month under water stress and under NaCl (60 mM) supplied to the nutrient solution. Different letters in (A) indicate significant differences at $p<0.05$ (LSD test) according to ANOVA, with treatments and accessions as the variability factors. In (B) for each histogram bar, the value is significantly different at $p<0.05$ (LSD test) to its control. Data are the mean for n=8 plants and SE.

to salinity and in minor extends under water stress compared to controls. Under water stress A31 obtained the higher values of A_N, g_S and E without significant differences with its control; A36 gave the lowest values. The accession A34 showed the minor decrease of gas exchange parameters compared with the rest of pepper accessions under salinity but showed significant differences with its control.

Relation between photosynthesis and fresh weigh

The data showed a positive relationship between A_N and FW (A_N= 0.0392FW + 8.914; r^2= 0.678; $p<0.05$) for all the values (Fig. 3A). In the water stress group, A31 obtained the highest FW and photosynthesis values, and A36 gave the lowest ones. In the salinity group, A34 presented the highest biomass and the greatest increase in photosynthesis, while A35 displayed the worst behaviour.
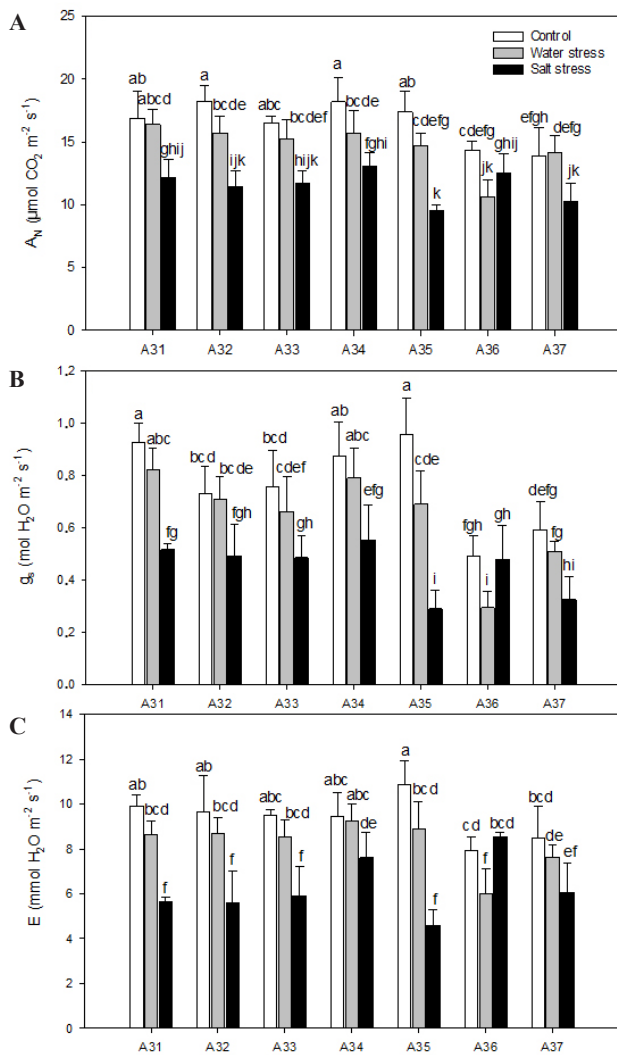
Figure 2. Leaf CO_2 assimilation (A_N) (A), stomatal conductance (g_S) (B) and transpiration (E) (C) in pepper accessions after 1 month under water and salt stress. Data are the mean values for n=8 plants ± SE. Different letters indicate significant differences at *p*<0.05 (LSD test).

Figure 3. Relation between total fresh weight (FW TOTAL) and CO_2 fixation (μmol CO_2/m²·s) under the control and the water and salinity stresses at the end of the experiment (A). Each point is the value mean for n=8 plants in each accession. Instantaneous carboxylation efficiency (A_N/C_i) for pepper accessions after 1 month under the control and water and salinity stresses (B). Data are the mean values for n=8 plants ± SE. Different letters indicate significant differences at *p*<0.05 (LSD test).

Instantaneous carboxylation efficiency

Under control conditions, instantaneous carboxylation efficiency, expressed as A_N/C_i (Fig. 3B), displayed the highest values, while the lowest values were obtained under the salinity treatment for all the studied pepper accessions. Under salinity, A37 was the accession with the greatest decrease in this parameter following A35 and A32. Under water stress, A36 gave the lowest A_N/C_i value with significant differences with the rest of the accessions under drought and control plants.

Water and osmotic potential

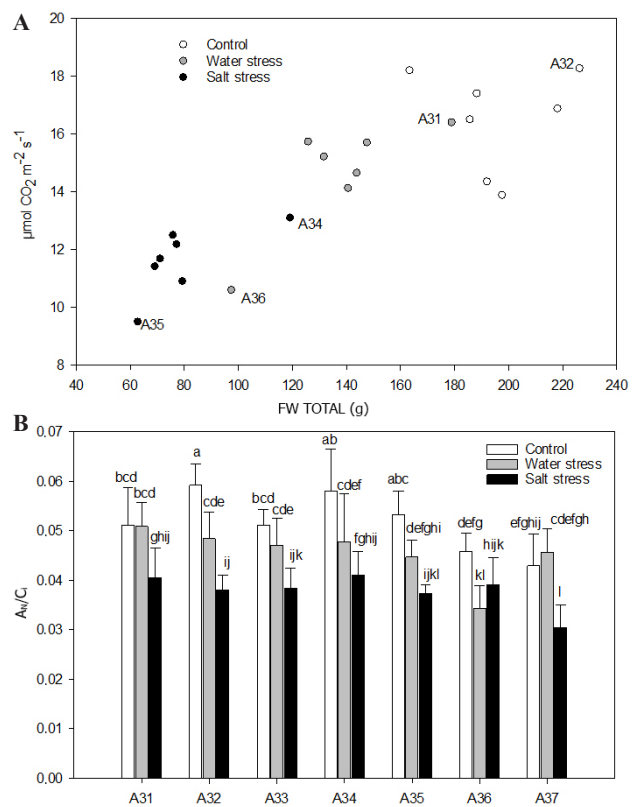Leaf Ψ_w drastically lowered in response to salinity and led to large significant differences in A32, A33,

A34 and A35 compared to their controls (*p*<0.05) (Fig. 4A). During the drought period, Ψ_w remained higher for all the accessions than those exposed to salinity. It was noteworthy that A37 obtained significantly different Ψ_w values compared to its control, but not contrary to salt stress, where values were the lowest compared to the other accessions.

Leaf Ψ_s decreased in response to salt treatment, but not under water stress (Fig. 4B). The salt-induced decrease in Ψ_s was more pronounced in A35, followed by A34 and A36, compared to the rest, and Ψ_s was not modified under water stress, except in A31 and A32 where Ψ_s values were biggest compared to their controls.

Proline concentration in leaves and roots

Proline leaf accumulation occurred under salt stress in all the accessions (Fig. 5A), but was not observed under water stress. The greatest increase was observed for A37, followed by A33 and A35.
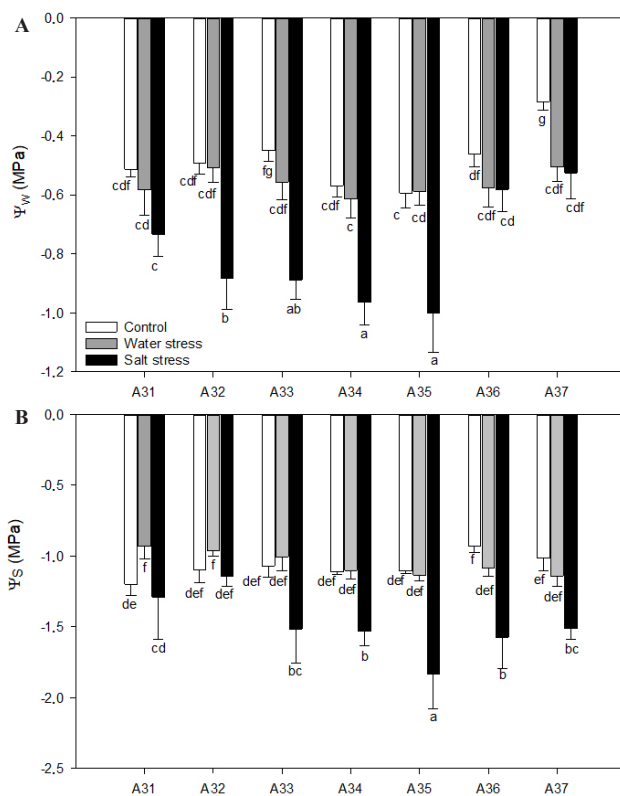
Figure 4. Water potential (Ψ_w, MPa) (A) and osmotic potential (Ψ_s, MPa) (B) in the leaves of pepper accessions under the control, water stress and salinity at the end of the experiment. Data are the mean values for n=6 ± SE for each treatment and accession. Different letters indicate significant differences at $p<0.05$ (LSD test).

For a given population, the proline concentration in roots was lower compared to leaves (Fig. 5B). Erratic proline behaviour in this organ was observed, where accession A33 showed the most marked increase in proline content under salinity stress, whereas A35 and A36 displayed a decrease. Under water stress, accessions maintained similar values to their controls, except for A37 for which a decrease was noted (Fig. 5B).

Sodium and chloride analysis

Increases in Na^+ and Cl^- were observed in all the pepper accessions under the salinity condition (Fig. 6). Under water stress, the values were similar to the control (data not shown). In roots, Na^+ increased between 2-3.2-times were observed compared to its control values (Fig. 6A). In leaves, Na^+ increased less compared with root levels in all the pepper accessions, except for A35 where the Na^+ leaf increase was 4.5-times than its control. Compared to the control, chloride accumulation (Fig 6B) was higher in leaves than in roots in some accessions (A34, A35 and A37), while the accession values for the rest were similar between roots and leaves.

For both ions and organs, an increase in each accession showed significant differences compared to its control.

Discussion

The pepper accessions shown in this experiment exhibited physiological differences in response to drought and salinity stress. In particular, we obtained photosynthesis values connected to biomass, which indicated the ability to cope with these stresses. Therefore, these accessions would be suitable to be grown in semi-arid or salinity lands and/or to be used in breeding programmes as a source of tolerance.

It is well-known that water and salinity stress reduces plant growth and that there are differences among cultivars with peppers (Aktas *et al.*, 2006; Penella *et al.*, 2013, 2014b). According to our results, both stresses significantly suppressed the growth of pepper plants in dry weight terms, although their stress responses depended on the accession. It should be noted that among all accessions, A34 for salinity and A31 for water stress showed minor growth reduction; nevertheless, A31 under salinity experienced an important biomass
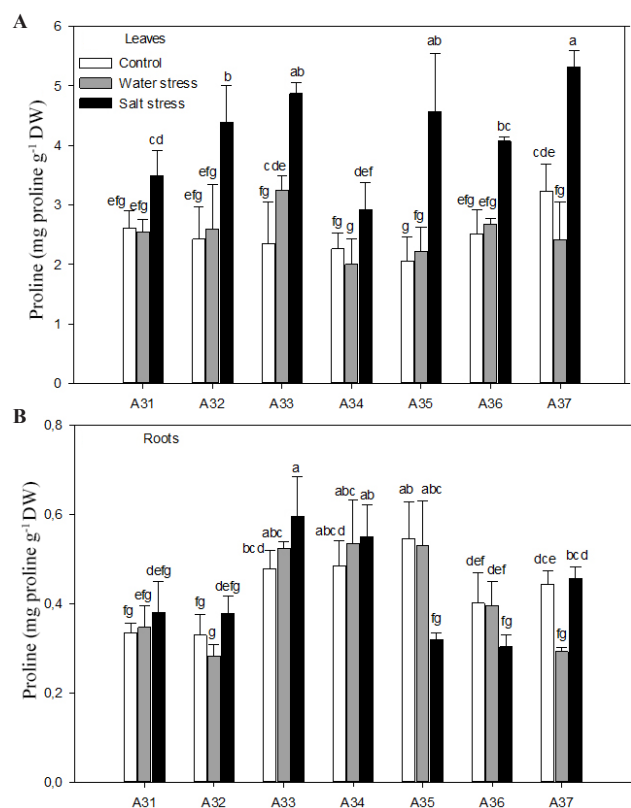

Figure 5. Changes in proline concentration (mg proline/g DW) in the leaves (A) and roots (B) of pepper accessions under the control and the water and salinity stresses. Data are the mean for n= 4 ± SE for each treatment and accession. Different letters indicate significant differences at $p<0.05$ (LSD test).
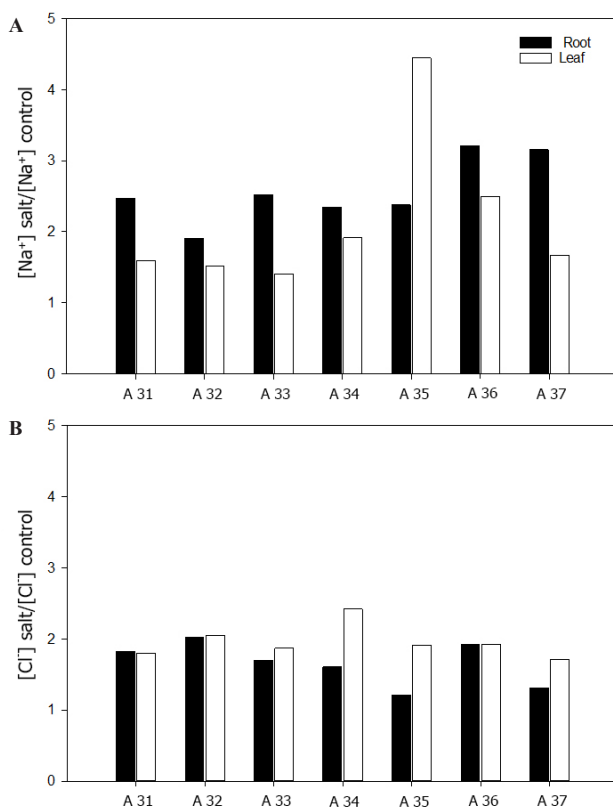
Figure 6. Ratio of the Na^+ (A) and Cl^- (B) concentration in the salt treatment and the control in roots and leaves for each pepper accession at the end of the experiment. For each histogram bar, the value is significantly different compared to its control after the LSD test at $p<0.05$. Data are the mean for n=6 independent samples in leaves and roots.

reduction. However, the physiological, biochemical and genetics mechanisms involved in growth inhibition have not yet been well characterised (Misra *et al.*, 2002; Munns & Tester, 2008; Noreen *et al.*, 2010). Knowledge of plant certain capacities of cope with stress could be essential for characterising stress tolerance.

This differential growth of the seven pepper accessions under both stresses may have been due to a differential regulation of the distinct physiological attributes involved in growth processes. Previous studies have demonstrated a positive relationship between photosynthetic capacity and growth in the plants grown under both salinity (Praxedes *et al.*, 2010; Saleem *et al.*, 2011; Penella *et al.*, 2015; 2016) and water stress (Chaves *et al.*, 2002; Abbad *et al.*, 2004; Hassine *et al.*, 2008; Del Amor *et al.*, 2010). In spite of considerable reduction of carbon assimilation rate and biomass under both stresses for all accessions studied the genotype A31 under water stress showed the minor decrease for both physiological parameters showing not significant differences in A_N and a biomass reduction of 29% respect its control plants. Under salinity stress,

among all accessions, the genotype A34 exhibited the higher photosynthesis rate and its biomass experienced the minor reduction respect to the rest of accessions.

Reduced photosynthesis can be caused by stomatal closure and/or non-stomatal inhibition, and the latter is associated with damage in photosynthetic machinery (Flexas *et al.*, 2004). In our experiment, a high correlation between A_N and g_s was observed for all data ($A_N = 13.304\ g_s + 6.63$, $r^2 = 0.8579$; $p<0.05$). This apparent linearity indicates that g_s and A_N reduced in a coordinated relation. This finding agrees with the interpretations under water and salinity stress conditions made by several researchers (Cowan & Farquhar, 1977; Delfine *et al.*, 2002; Filippou *et al.*, 2014; Penella *et al.*, 2015). The equation of the relationship between both parameters (A_N *vs.* g_s) did not pass through the origin. This fact indicates that stomatal closure occurred earlier than CO_2 fixation (Delfine *et al.*, 2002), although A_N reduction was due mainly to stomata closure, the partial inhibition of mesophyll conductance and/or photochemical efficiency cannot be ruled out.

Decrease in A_N/C_i indicates that both stresses affected the photosynthesis by metabolic limitations (Da Silva *et al.*, 2011; Penella *et al.*, 2015). The marked drop in A_N/C_i occurred more drastically under the salt-stressed pepper accessions. Excessive Na^+ and Cl^- accumulation is harmful and may disrupt the integrity of chloroplasts and decrease photosynthetic capacity (Munns & Tester, 2008; Chaves *et al.*, 2009; Rouphael *et al.*, 2012; Penella *et al.*, 2015). We observed how the accumulation of mainly Na^+, and of Cl^- to a lesser extent, occurred in the roots and leaves of all the pepper accessions. However, A35 stood out with the greatest Na^+ accumulation in its leaves, affecting directivity to photosynthetic apparatus, which could cause the lowest A_N, g_s, E and FW (and DW) values. Under water stress, A_N/C_i decreased to a lesser extent, which supports the notion that the major photosynthesis inhibition was mainly resulted from stomatal closure, and that A36 was the most affected genotype with the lowest growth and CO_2 fixation.

Under the osmotic stress provoked by water and salt stress in the root medium, plants lowered leaf Ψ_s in an attempt to maintain water uptake (decrease Ψ_w) with a positive turgor, which is indispensable for cell growth and maintaining photosynthetic performance (Yadollahi *et al.*, 2011; Penella *et al.*, 2015, 2016). In order to face water loss, plants accumulate many compatible (organic) metabolites to increase tolerance against tissue dehydration (Yoshiba *et al.*, 1997; Patakas *et al.*, 2002). Proline accumulation is believed to be one of the most important metabolites that are implied in osmotic adjustment. Moreover, several studies have attributed multiple roles to proline, such as signalling molecule that influences defence pathways, complex metabolic

regulation and development processes, and protective compounds (see Szabados & Savouré, 2010). In our experiment, proline content increased considerably under the salinity conditions (from 29% for A34 to 64% for A37 compared with their controls), but not under water stress. When proline was taken as an osmolyte, the role it played to contribute to lower Ψ_s did not suffice under salinity (between 0.06 and 0.1 MPa) to generate osmotic pressure (Smirnoff & Cumbes, 1989; Penella *et al.*, 2015). Therefore, the increase in proline under salt stress was unable to explain the observed decrease in Ψ_s, while the relationship between them was very weak ($r^2 = 0.027$). Nevertheless, the largest proline amount observed in all the pepper accessions, except A34, was related with the greater salt sensitivity of these genotypes. These findings are consistent with the research reported for pepper into higher leaf proline in salt-sensitive genotypes (Penella *et al.*, 2015), or for other species, such as wheat (Colmer *et al.*, 2005), barley (Chen *et al.*, 2007), or rice (Lutts & Guerrier, 1995). Moreover, proline or other compatible solutes may protect plants by scavenging the oxygen-free radicals caused by salt stress (Huang *et al.*, 2010; Penella *et al.*, 2016), a role as signalling molecule, implicated in regulation and developmental processes, and should be considered a protective compound (see Szabados & Savouré, 2010). Under water stress, no changes were observed in proline content respect to controls due to there were not differences in Ψ_w and Ψ_s was observed between treatments.

The adjustment of Ψ_s through inorganic salt-ion uptake is a strategy that implies a much lower energy cost for cells compared with the organic molecules synthesised in cells (Munns, 2002). Pepper accessions showed a better correlation between osmotic potential and Na^+ levels in leaves ($\Psi_s = -0.361 [Na^+] - 0.9288$; $r^2 = 0.666$), but not for Cl^- accumulation (data not shown). The decreased in Ψ_s in the pepper accession leaves subjected to salt stress was largely the result of strong Na^+ accumulation. This result was also observed by Chen *et al.* (2007) in barley genotypes, Abideen *et al.* (2014) in *Phragmite karka*, or Navarro *et al.* (2003) and Penella *et al.* (2015) in pepper plants.

Overall, all the analysed physiological parameters, photosynthesis and stomatal conductance, can be reliable indicators of biomass under water and salinity stress. Both salinity and water stress lead to reduce photosynthesis, stomatal conductance and growth. The present study evidences a wide variability of the seven pepper accessions in response to both stresses, and in both drought and salinity treatments. Under our conditions, growth inhibition occurred under water stress, provoked mainly by stomatal closure, where A31 was the less affected, and A36 was the most sensitive one and also correlated with minor CO_2

fixation and biomass. Under the salinity conditions applied in this experiment, our results showed that damage was greater compared with water stress. In this case, photosynthesis inhibition was due to the stomatal and non-stomatal effects caused by osmotic stress and toxic salt ion accumulation. Even with increased proline synthesis, the reduction in Ψ_s and Ψ_w was not enough to alleviate the salt effects. A34 was the most interesting accession due to its better tolerance to salinity with a major photosynthesis capacity, minor growth inhibition, but it had a lower proline concentration compared with A35, which suffered the worst adaptation. This genetic variation in response to both stresses can be exploited in pepper crops so they can be grown in marginal areas and/or in breeding programmes.

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