Ionic effects of NaCl counter osmotic inhibition of germination and seedling growth of *Scorzonera hispanica* and subsequent plantlet growth is not affected by salt

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Abstract: *Scorzonera* (*Scorzonera hispanica*) is experiencing range reduction in Portugal while its distribution in the Iberian Peninsula largely coincides with areas affected by salinization. Thus two experiments were conducted to evaluate the response of scorzonera to salinity. In the first experiment, seed germination and seedling growth were investigated under osmotic potentials down to -1.21 MPa using NaCl or iso-osmotic polyethylene glycol (PEG) solutions. Results of PEG treatments show that osmotic potential alone reduces germination and seedling growth while on NaCl reductions were much lower. Ionic effects of NaCl are stimulatory and clearly counteract osmotic effects. Most seeds completed germination after being transferred from PEG to distilled water. Conversely, on NaCl recovery results essentially from more time allowed for seeds to complete germination. In the second experiment, young plantlets were treated with NaCl solutions up to 250 mM. In general plantlets were insensitive to NaCl except for a concentration-dependent increase of relative chlorophyll content suggesting that scorzonera tolerance to NaCl might depend upon nitrogen availability. Altogether, results support the conclusion that scorzonera may be a naturally salt tolerant species able to accumulate and sequester NaCl, its tolerance increasing as plants grow older.

Key words: continuance of germination, recovery of germination, relative chlorophyll content, polyethylene glycol, salinity, sodium chloride.

Introduction

Scorzonera, Spanish salsify or black salsify (*Scorzonera hispanica* L., Asteraceae) is a herbaceous perennial plant propagated by cypselas, hereafter referred to as seeds, distributed across central and southern Europe and in southern regions of the former USSR but absent from

Sicily, Greece, north-western Africa and southwest Asia. The major reason for its cultivation lies in the taproot, milky and white inside, blackish outside (Vulsteke and Calus 1990; Nuez and Bermejo 1994) and rich in inulin (Vulsteke and Calus 1990; Dolota and Dąbrowska 2004), which is the second most abundant storage carbohydrate in nature (Singh and Singh 2010) and especially suited for diabetic diets (Nuez and Bermejo 1994).

It is labeled a rare, threatened species highly susceptible to habitat fragmentation (Münzbergová 2006; Münzbergová and Plačková 2010) and data presented previously (Pereira et al. 2014) show that its distribution has been drastically reduced in Portugal during the last century from being relatively frequent in the hinterland in the early 1900's to being only spotted nowadays in semi-arid southern Portugal in very few sites and all of them in close vicinity.

Agronomic, cultivation and quality aspects of scorzonera production were extensively investigated in the last decade in east European conditions (Dolota and Dąbrowska 2004; Błażewicz-Woźniak and Konopiński 2011; Konopiński and Ferens 2011), but research on the biology and ecophysiology of the early stages of the scorzonera life cycle is lacking. To fill this gap we set out a research programme aimed at identifying early life-stage constraints to scorzonera replantation and cultivation starting with the investigation of effects of constant and alternating temperatures, pH, mineral deficiencies and growth substrates on germination and seedling- and early plantlet-growth (Dias et al. 2013; Pereira et al. 2014).

Soil salinity is known to be one of the major abiotic stresses suffered by plants and despite the fact that the soil solution of saline soils is composed of a variety of salts, sodium chloride is widely viewed as the most prevalent salt (Tavakkoli et al. 2010) affecting plant growth and crop productivity through the single or joint action of osmotic and ionic stresses (Munns and Tester 2008).

Currently, soils salinization is not a pressing issue in Portugal, affecting relatively small

areas, most of them near the sea or strongly influenced by it. However, in a broader context, increasingly larger areas of the Iberian Peninsula are affected by NaCl, in great part coincident with the natural area of distribution of scorzonera (Guardia and Blanca 1987; Tóth et al. 2008). Thus we set out to investigate the effects (if any) of sodium chloride on germination and early seedling- and plantlet-growth of scorzonera. Because germination and heterotrophic early growth are generally considered the phases of plant life history most sensitive to salt stress, halophytes included (Khan and Rizvi 1994; Debez et al. 2004; Zhou and Xiao 2010) we investigated the effects of NaCl on germination and seedling growth separately from its effects on plantlet growth. Also we designed the experiments of germination and early seedling growth so that ionic and osmotic effects could be evaluated separately and true persistence of effects after removal of NaCl or of the osmotic agent could be quantified.

Materials and methods

Plant material

Papus-free seeds of scorzonera cv. Géant Noire de Russie (Semillas Batlle) were purchased at a specialized seed provider. Seeds of this cultivar are relatively large; the volume ranges between 5.476 mm³ and 32.299 mm³, and the mass between 6 mg and 23 mg (Pereira et al. 2014). Germination is affected by temperature occurring between 10 °C and 25 °C but not by its regime (constant or alternate), whereas seedling growth is affected by both (Dias et al. 2013). Conversely, germination of this cultivar is essentially insensitive to pH in the range 5-10. Young plantlets are able to rapidly accumulate mineral reserves and grow during at least two weeks in the absence of several elements tested individually, but are highly sensitive to the absence of iron

(Pereira et al. 2014).

Salt and osmotic effects on seed germination and seedling growth of scorzonera

An exploratory experiment showed no differences or slightly better results for dark germination with seeds being checked briefly under natural light compared with germination under natural illumination (data not shown). Therefore germination bioassays were conducted under dark conditions. Four 10 cm glass Petri plates per treatment were fitted with Whatman No. 1 filter paper, each sown with 25 seeds, wetted with 5 mL of distilled water or with 5mL of NaCl (Merck) 50 mM, 100 mM, 150 mM, 200 mM and 250 mM corresponding at 25 °C, the temperature set for the experiment, to water potentials of 0 MPa, -0.25 MPa, -0.49 MPa, -0.73 MPa, -0.97 MPa and -1.21 MPa, respectively (Lang 1967). Iso-osmotic solutions of PEG 6000 (Merck) were prepared according to Michel and Kaufmann (1973) and a parallel set of glass Petri plates prepared to control the effect of osmotic potential alone. After the onset of the experiment further additions of NaCl or PEG solutions were done whenever deemed necessary. Seeds were incubated under dark and continuous 25 °C. Seeds were checked twice a day during eight days for those that had completed germination. Because previous results (Dias et al. 2013; Pereira et al. 2014) presented no evidences that green light has to be used when checking for completion of germination of scorzonera seeds incubated under dark conditions, we always examined seeds briefly under white light. Seeds were considered to have completed germination when the embryo, generally the root, protruded from the seed covers.

Seeds that had completed germination were then transferred to newly prepared glass Petri plates fitted with Whatman No. 1 filter paper, wetted with distilled water, NaCl or PEG solutions as required, kept at the same temperature conditions as before and seedlings allowed to grow

under a 16 h photoperiod supplied by three white light cool fluorescent lamps (Philips Fluotone TLD 18W/840; PAR $\sim 110 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$). Change of relative precision of means calculated from parallel experiments according to Penas et al. (2002) for root and hypocotyl length with sample sizes between $n=2$ and $n=88$ (data sorted by descending value of root length) showed that $n=15$ was an adequate sampling effort for seedling growth evaluation (data not shown). Thus five days after sowing, root and hypocotyl length of the 15 plants with the longest root were measured to the nearest mm.

To evaluate the recovery of germination completion, eight days after sowing, replicates of each NaCl and PEG treatments were divided in two groups maximizing the chance that the two groups had approximately the same number of seeds that did not complete germination. Therefore one group comprised the replicates with the highest and the lowest total germination completion; the other group included the two remaining replicates which had intermediate values of total germination completion. Seeds of the first group were separately transferred to new 10 cm diameter glass Petri plates fitted with Whatman No. 1 filter paper and wetted with 5 mL of distilled water (recovery of germination completion group) while seeds of the second group were separately transferred to new 10 cm glass Petri plates fitted with Whatman No. 1 filter paper and wetted with 5 mL of their original NaCl or PEG treatment (continuance of germination completion group). The assignment of seeds to the recovery- or to the continuance-group was random. Seeds were incubated during eight more days in the dark under continuous 25 °C, seeds checked twice a day and those seeds that had completed germination recorded and discarded.

Salt effects on early growth of scorzonera plantlets

Forty three 10×13×7 cm plastic containers with 10 drainage holes in the bottom per

treatment were filled with white sand and sown with 10 seeds at 1-cm depth, kept indoors under dim light and watered daily with distilled water. After four days most of the plantlets had emerged and supplementary light was provided by two sodium and mercury vapour lamps (500 W; PAR 270–350 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$; 14 h photoperiod). Twelve days after sowing 7 containers were randomly assigned to each treatment, 0, 50, 100, 150, 200, 250 mM NaCl (Merck) and the plantlets of the remaining container were measured to assess growth at the beginning of the experiment. Starting from the 12th day after sowing each treatment was watered every other day with 37 mL of complete nutrient solution (98.0 $\text{mg}\cdot\text{L}^{-1}$ NO_3 ; 12.8 $\text{mg}\cdot\text{L}^{-1}$ NH_4 ; 117.0 $\text{mg}\cdot\text{L}^{-1}$ K; 106.4 $\text{mg}\cdot\text{L}^{-1}$ Ca; 47.1 $\text{mg}\cdot\text{L}^{-1}$ Cl; 24.4 $\text{mg}\cdot\text{L}^{-1}$ Mg; 14.1 $\text{mg}\cdot\text{L}^{-1}$ P; 34.5 $\text{mg}\cdot\text{L}^{-1}$ S; 4.0 $\text{mg}\cdot\text{L}^{-1}$ Fe; 503.6 $\mu\text{g}\cdot\text{L}^{-1}$ B; 499.7 $\mu\text{g}\cdot\text{L}^{-1}$ Mn; 53.3 $\mu\text{g}\cdot\text{L}^{-1}$ Mo; 115.1 $\mu\text{g}\cdot\text{L}^{-1}$ Zn; 29.8 $\mu\text{g}\cdot\text{L}^{-1}$ Cu) supplemented with the appropriate NaCl concentration. Throughout the experiment room temperature ranged between 14 °C and 21 °C, the relative humidity of the air was between 45% and 65%.

Three weeks after the beginning of salt treatments, four containers per treatment were randomly selected and growth parameters of all plantlets registered. Tuberos roots and leaves were counted, roots and shoots measured to the nearest mm, the largest diameter of roots and the diameter at the base of the stem measured to the nearest 0.05 mm and total leaf area of the most developed plantlet determined by the weight of leaf contours technique. The pooled shoot- and root-fresh and dry-weights (72 hours at 60 °C) of the remaining plantlets was also determined. All weights were determined to the nearest mg.

In the three additional containers two replications of three 8 mm diameter leaf disks per plant and container were taken, pooled, weighed (fresh weight), immersed in distilled water for three hours, weighed again (turgid weight) and dried for 72 hours at 60 °C and weighed (dry weight) to determine water content, relative water content and specific leaf area. Number of

stomata and number of non-stomata epidermal cells of the adaxial and abaxial surfaces were determined from transparent varnish prints of one leaf randomly selected from each of two randomly selected plantlets and four 0.181 mm² adjacent areas, devoid of veins, were observed at 400 × magnification using a light microscope Leica ATC 2000. Stomata conductance of adaxial and abaxial surfaces of three leaves randomly selected among all plantlets was determined with an automatic porometer Delta-T AP4. Relative chlorophyll content of eight leaves randomly selected among all plantlets was determined with a Chlorophyll Content Meter Minolta SPAD-502.

Statistical analyses

Recovery of germination completion was calculated in percentage as

$$RG = 100 (G_R / G_F) \quad [1]$$

in which G_R is the number of seeds that completed germination after being transferred from treatments to distilled water and G_F is the number of seeds that had failed to complete germination in treatments before transfer to distilled water (Khan and Gul 1998). The same equation was used to calculate continuance of germination completion (CG) applied to seeds that had failed to complete germination in the same treatment (NaCl, PEG or distilled water in controls). Because recovery of germination completion RG might result either from more time being allowed for seeds to complete germination even if kept on the same treatment, or from the withdrawal of the treatment (NaCl or PEG) we determined true recovery of germination completion as the difference between recovery RG and continuance of germination completion

CG.

Lag, rate and shape of germination completion were obtained from the three-term Weibull function (Weibull 1951):

$$G = 1 - \exp - \{ [(T - l) / k]^c \} \quad [2]$$

where G is the cumulative completion of germination at time T in proportion of total germination completion, l is a location parameter estimating here the latest time at which completion of germination is strictly zero which for all practical purposes means the time necessary for the first seed to complete germination, k is a scale parameter estimating the rate of the process with $l+k$ estimating here the time necessary for 63% of cumulative completion of germination and c a shape parameter estimating the symmetry of the distribution of germination completion over time with $3.25 \leq c \leq 3.61$ showing symmetry and representing a good approximation to the normal distribution, $c < 3.25$ positive asymmetry, $c > 3.61$ negative asymmetry (Dubey 1967; Bonner and Dell 1976). Weibull equations were fitted by least squares nonlinear regression without replication using the Marquardt method (Marquardt 1963). Fitted equations were only accepted after a consistency check of parameter estimates and germination predictions against the original data. The duration of germination completion, D_{100} , the number of days necessary to attain total germination completion minus the number of days necessary for the first seed to complete germination (l), was deduced from fitted equations.

In the salt and osmotic experiment, data of NaCl and PEG treatments were compared with controls and between them for each level of osmotic potential using Student's t tests.

However the response of plants to NaCl might involve osmotic potential effects and ionic effects of NaCl itself while the response to PEG would involve osmotic potential effects alone.

Therefore we set out to eliminate the osmotic component in the response of scorzonera to NaCl. Data of all experiments were expressed as a percentage of controls. Data of NaCl experiments were corrected to eliminate the osmotic component as NaCl–PEG, data of PEG experiments were normed so that controls are 0%. Thus, after corrections, negative values for PEG treatments mean reductions in relation to controls caused by osmotic potential alone; positive values for NaCl–PEG mean increases in relation to controls caused by ionic effects alone. Corrections were performed for total germination completion, lag of germination completion, D_{100} , root- and hypocotyl-length. Corrected data was regressed against osmotic potential (ψ_{π}) by fitting second order polynomials forced through the origin or by fitting modified Weibull equations:

$$Y = Y_{\max} \{ 1 - \exp - \{ [(-\psi_{\pi} - l) / k]^c \} \} \quad [3]$$

or

$$Y = -100 \{ 1 - \exp - \{ [(-\psi_{\pi} - l) / k]^c \} \} \quad [4]$$

where $Y_{\max}$ is the maximum value estimated for the dependent variable Y and l , k and c are location, scale and shape parameters as in equation 2.

From root (R) and hypocotyl (H) length we derived the partition of root and hypocotyl as

$$RHP_1 = 100 (R / R + H) \quad [5]$$

which may vary between 0% (only the hypocotyl grows, $R=0$ mm) and 100% (only the root grows, $H=0$ mm) the same for root and shoot length and for root and shoot dry mass, RSP_1 and RSP_w , respectively. From fresh, turgid and dry weights (W_F , W_T , W_D) we derived Water Content

as

$$WC = 100 (W_F - W_D / W_F) \quad [6]$$

and Relative Water Content as

$$RWC = 100 (W_F - W_D / W_T - W_D) \quad [7]$$

and from the area and dry weight of leaf disks (LA, LW_D) we derived Specific Leaf Area as

$$SLA = LA / LW_D \quad [8]$$

Because scorzonera leaves have stomata on both surfaces, leaf areas were calculated as the sum of the two surfaces. From the density of stomata (S) and non-stomata cells (NS_C) we derived Stomata Index as

$$SI = 100 (S / NS_C) \quad [9]$$

Whenever multiple comparisons of means were intended, homocedasticity was tested using the two-tailed *F* distribution and a comparison-wise type I error rate of 0.05 and if heterocedasticity was found, data was transformed using the Box-Cox family of transformations (Box and Cox 1964). Nested ANOVAs with unequal sample sizes were performed with the Satterthwaite approximation after checking for the adequacy of its use (Sokal and Rohlf 1995). Two sample comparisons were done with exact or approximate Student's *t* tests and a significant level of 0.05 after checking for homocedasticity as described above.

An experiment-wise type I error-rate of 0.05 for coefficients calculated using the Dunn-

Šidák method (Ury 1976) was adopted for all linear regressions. Coefficients of determination (R^2) are always presented as proportion of the maximum R^2 possible (Draper and Smith 1998). Data are presented as mean $\pm$ SE. All statistical analyses were done with Statgraphics 4.2 (STCS, Inc., Rockville, MD, USA) except Box-Cox analyses and nested ANOVAs with unequal sample sizes done with BIOM (Rohlf 1992).

Results

Salt and osmotic effects on seed germination and seedling growth of scorzonera

Significant differences in total germination completion were found between control and NaCl and between control and PEG treatments at osmotic potentials of -0.49 MPa or lower. Also, at the same osmotic potential significant differences were found between NaCl and PEG treatments at of -0.49 MPa or lower (≥ 100 mM NaCl) with percentages of germination from NaCl treatments always higher than those from PEG treatments (Table 1A).

When seeds that failed to complete germination on NaCl and PEG treatments were transferred to distilled water no significant differences were found in total germination completion after eight days either between control and NaCl or PEG treatments or among replicates formerly assigned to NaCl or PEG treatments with the same osmotic potential (Table 1B). Recovery of germination completion (equation 1) of seeds previously treated with NaCl or PEG was almost always significantly different from the control except at -0.25 MPa but no significant differences were found among replicates formerly assigned to NaCl or PEG treatments with the same osmotic potential except at -1.21 MPa (Table 1D).

Conversely, total germination completion of seeds kept under the same NaCl or PEG

treatments eight more days showed significant differences in relation to the control, similar to those found previously in PEG treatments -0.97 MPa or lower while significant differences from the control completely disappeared in NaCl treatments except at -0.97 MPa (Table 1C). Continuance of germination completion (equation 1) was not significantly different from control in NaCl or PEG treatments but significant differences were found between treatments at osmotic potentials of -0.97 MPa or lower (Table 1E).

The effects of NaCl minus the effects of the iso-osmotic PEG solutions (NaCl-PEG) and the effects of the PEG solutions (PEG) on total germination completion after 16 days of incubation could be expressed by modified Weibull equations (equations 3 and 4) with $R^2=0.955$ and $R^2=0.996$, respectively. The decrease of osmotic potential alone was clearly inhibitory with complete inhibition estimated to occur at -1.10 MPa while the ionic effects of NaCl estimated by the removal of the osmotic component (NaCl-PEG) clearly countered the inhibition of osmotic effects alone (Fig. 1A). The increase of total germination completion by NaCl would mathematically asymptote at 150.6% which is biologically unrealistic. However, a 100% increase of total germination completion would be attained for an osmotic potential of -1.24 MPa which according to Lang (1967) corresponds to a NaCl concentration of ~ 255 mM.

Weibull equations (equation 2) could always be fitted to the relationship between germination after 16 days and NaCl or PEG treatments (mean $R^2=0.568\pm 0.072$).

Significant differences in lag of germination completion (l) were generally found between controls and NaCl or PEG treatments, in both cases with a direct relationship between l and the osmotic potential. A trend to non-significant differences between l values in NaCl and PEG treatments of the same osmotic potential was also found (Table 1F). Conversely, after the completion of germination had commenced no significant differences among treatments were found in the time required to total germination completion, D_{100} (Table 1G) which ranged

between 3.8 ± 2.0 day and 7.8 ± 0.3 day. Significant differences between the shape of germination completion over time (c) of controls and NaCl or PEG treatments were found at osmotic potentials of -0.73 MPa or lower in NaCl treatments (≥ 150 mM NaCl) and -0.49 MPa or lower in PEG treatments (Table 1H).

The relationship between osmotic potential and the effects of NaCl-PEG or the effects of PEG alone on l could be expressed by a second order polynomial ($P \leq 0.005$ for coefficients, lack of fit with $P = 0.348$, $R^2 = 0.987$ for NaCl-PEG; $P \leq 10^{-4}$ for coefficients, lack of fit with $P = 0.655$, $R^2 = 0.963$ for PEG) fitted by linear regression. Osmotic potential alone clearly explains the time needed for germination to end for the first seeds to do so. Ionic effects of NaCl estimated by the removal of the osmotic component (NaCl-PEG) clearly countered the delay associated with osmotic effects (Fig. 1B).

The relationship between osmotic potential and the effects of NaCl-PEG on D_{100} could be expressed by a first order polynomial ($P \leq 0.029$ for coefficients, lack of fit with $P = 0.450$, $R^2 = 0.690$) fitted by linear regression. No regression model could be fitted to PEG but ionic effects of NaCl (NaCl-PEG) clearly increased D_{100} (Fig. 1C).

With the sole exception of the NaCl treatment at -0.25 MPa, root and hypocotyl length of plants treated with NaCl or PEG were always significantly different from controls (Table 1I,J) but the partition of root and hypocotyl length (RHP_i) was generally unaffected by NaCl or by PEG treatments (Table 1K).

The relationship between osmotic potential and the effects of NaCl-PEG or the effects of PEG on root length could be expressed by modified Weibull equations (equations 3 and 4) with $R^2 = 0.740$ and $R^2 = 0.930$, respectively. The decrease of osmotic potential alone was clearly inhibitory with complete inhibition estimated to occur at -0.75 MPa while the ionic effects of NaCl (NaCl-PEG) clearly countered the inhibition by osmotic effects alone (Fig. 2A). The

increase of root length by NaCl alone would asymptote at 49.4% for an osmotic potential of – 3.51 MPa which according to Lang (1967) corresponds to a NaCl concentration of ~720 mM.

The relationship between osmotic potential and the ionic effects of NaCl (NaCl–PEG) or the effects of PEG on hypocotyl length could be expressed by modified Weibull equations (equations 3 and 4) with $R^2=0.809$ and $R^2=0.860$, respectively. The decrease of osmotic potential alone was clearly inhibitory with complete inhibition estimated to occur at –0.76 MPa while the ionic effects of NaCl (NaCl–PEG) clearly countered the inhibition by osmotic effects alone (Fig. 2B). The increase of hypocotyl length by NaCl alone would asymptote at 60.9% for an osmotic potential of –4.72 MPa which according to Lang (1967) corresponds to a NaCl concentration of ~960 mM.

Salt effects on early growth of scorzonera plantlets

Twelve days after sowing, coinciding with the beginning of NaCl treatments, five scorzonera plantlets were randomly selected and measured to assess scorzonera growth at the beginning of the experiment. Root length was 63.4 ± 8.4 mm, shoot length 108.6 ± 4.0 mm and the partition of root and shoot length 36.2 ± 2.3 %. Cotyledons were generally still present and leaves were mostly absent (mean number of leaves 0.4 ± 0.2). Cotyledon and leaf area (two surfaces) were 184.00 ± 9.56 mm² and 3.80 ± 2.73 mm² respectively. Root and shoot dry weight (cotyledons included) were 1.8 ± 0.2 mg and 9.2 ± 1.2 mg and the partition of root and shoot dry weight was 17.4 ± 2.9 %. Root and shoot water content were 92.1 ± 0.9 % and 91.8 ± 0.8 % respectively and relative root and shoot water content were 77.5 ± 2.8 % and 85.1 ± 0.9 % respectively.

In general, NaCl had no discernible effects in scorzonera growth after three weeks either in length, biomass, number and area of leaves, water content, stomata and non-stomata densities

or stomata conductance (Table 2). However, significant differences occurred, noteworthy in root length (Table 2A) and partition of root and shoot length (Table 2C), both significantly higher at 50 mM NaCl. Significant differences were also found in shoot length (Table 2B), shoot dry weight (Table 2H), total dry weight (Table 2I) and stomata conductance of abaxial surface of leaves (Table 2Y) all significantly lower at the highest or at the two higher concentrations of NaCl, while significant differences at intermediate NaCl concentrations were found in leaf water content (Table 2O), stomata index of adaxial surface (Table 2T) and stomata conductance of adaxial surface of leaves (Table 2X).

Conversely, the relative chlorophyll content (RCC) was always significantly increased by NaCl (Table 2Z), with a clear trend of increase of RCC with NaCl until a concentration of 150 mM followed by a decrease with a plateau at higher concentrations. Significant differences were also found between RCC at 150 mM and RCC at 200 mM or 250 mM ($P=0.027$ or $P=0.030$ respectively) but no significant differences were found between the latter ($P=0.754$).

Finally, a semi-formal sensory evaluation of scorzonera revealed a distinctive and increasingly salty taste in leaves of plantlets treated with NaCl, which was absent from the control.

Discussion

Salt and osmotic effects on seed germination and seedling growth of scorzonera

Effects of NaCl solutions on seed germination and on seedling or on plantlet growth might result from the osmotic potential of the solution, by not allowing the uptake of water, or from the ionic composition of the solute (Redmann 1974; Ryan et al. 1975; Bajji et al. 2002;

Zhou and Xiao 2010) or from both acting simultaneously. To evaluate the relative importance of the osmotic potential and of the ionic composition of NaCl solutions, experimental designs frequently include iso-osmotic NaCl and PEG treatments, and less frequently other osmotica like mannitol. In such designs the effects of PEG are assumed to represent the effects of osmotic potential alone and significant differences between the effects of NaCl and of PEG are implicitly taken as representing ionic effects.

In general, NaCl effects are much more intense than the effects of PEG either when halophyte (Tobe et al. 2000; Duan et al. 2004; Song et al. 2005; Hameed et al. 2013) or when non-halophyte species are tested (Bal and Chattopadhyay 1985; Tobe et al. 1999; Zhou and Xiao 2010), which has been usually interpreted as reflecting ionic rather than osmotic effects on target species.

However, effects of NaCl are not always larger than those of PEG are reported. Similar effects by NaCl and PEG have been reported to occur (Tobe et al. 2006) implying that all effects are osmotic, and in other cases lower effects by NaCl than by PEG were reported (Tobe et al. 1999; Tobe et al. 2000; Song et al. 2005) and so specific ionic effects of NaCl somehow favours target species in comparison with iso-osmotic PEG treatments.

Our data show that both germination and seedling growth of scorzonera are affected by PEG, and thus that osmotic potential alone can affect scorzonera. Effects of PEG on total germination completion were found eight days after sowing and on root growth five days after sowing for osmotic potentials of -0.49 MPa or lower and for all osmotic levels in hypocotyl length. However, osmotic effects on total germination completion were fully reversed when seeds were transferred from PEG solutions to distilled water and high recovery of germination completion was observed.

Globally, NaCl acts on germination and early growth of scorzonera in much the same way

that PEG does. Both PEG and NaCl effects on total germination completion were completely reversed when seeds that fail to complete germination were transferred to distilled water. However, NaCl consistently affects scorzonera germination and early growth less intensely than PEG or affects scorzonera only at lower osmotic potentials than PEG does.

Experimentally disentangling osmotic and ionic effects when testing NaCl or any other salts is hardly possible and that is why iso-osmotic treatments by PEG or other osmotica are performed followed by comparisons between NaCl and PEG at iso-osmotic levels. However, the two sets of data can be used post-experiment to estimate the ionic effects of NaCl by explicitly subtracting from NaCl effects the osmotic effects of PEG alone.

By doing so, it is easily seen both graphically (Fig. 1 and 2) and otherwise (Table 1), that the ionic effects of NaCl are highly beneficial to germination and early growth of scorzonera when compared with osmotic effects alone. These beneficial effects of the ionic component of NaCl may be explained because, contrary to the much larger molecules of PEG 6000 plus their rigid behaviour in water solutions (Michel and Kaufmann 1973), NaCl can be taken up by seeds and seedlings. Then NaCl may be used in the build-up of a negative cell water potential that enables seeds and seedlings to take water from the saline solution and thus to germinate, to complete germination and grow. Thus, scorzonera has the ability to sequester NaCl even when the salt is in relatively high concentrations in the surrounding medium which, according to Khan (2002) and Khan and Gul (2008), makes this species an ideal or, at least, a very attractive candidate for saline agriculture.

Salt tolerance is a complex trait both genetically and physiologically involving many different processes (Flowers 2004; Kronzucker and Britto 2011). However the use of NaCl as osmoticum is well established (Kronzucker and Britto 2011) and the accumulation of Na^+ and Cl^- in vacuoles is known to allow plants to maintain osmotic potentials low enough to drive water

into cells (Blumwald 2000).

Germination of scorzonera also shows a high ability to recover after being subjected to a high level of saline stress and compares very favourably with values assembled by Khan and Gul (2008) for halophytes of temperate or sub-tropical regions.

High values of recovery of total germination completion have been interpreted as the result of osmotic effects rather than ion toxicity (Khan 2002; Duan et al. 2004). This would imply that NaCl effects at low osmotic potentials were independent from the ionic composition of the solutions and were essentially the same as those of PEG treatments. However, it has also been stated that ionic effects are in place when the germination completion recovery is significantly different between treatments and distilled water controls, otherwise these effects would be purely osmotic (Pujol et al. 2000). Thus NaCl and especially PEG effects on scorzonera germination at low osmotic potentials would result from the ionic composition of solutions which is exactly the opposite of the conclusion reached above. It is possible to conceive that osmotic and ionic effects are present and responsible for seed germination responses. This is probably the case in germination and early growth responses of scorzonera to NaCl, but the contradiction between the two conflicting conclusions strongly suggests that recovery data can only provide unequivocal information on the transient- or permanent-nature of effects.

Comparing recovery and continuance percentages also suggests that recovery of germination completion when seeds were treated with NaCl and then transferred to distilled water was essentially the result of more time available for germination which accounts for a high fraction of recovery, an aspect rarely if ever considered in recovery studies. Conversely more time for germination on PEG treatments at lower osmotic potentials did not result in increased germination percentages with germination inhibition being transient and depending upon the maintenance of osmotic stress. This also implies that the response of scorzonera seeds to NaCl is

fundamentally different to their response to PEG.

Salt effects on early growth of scorzonera plantlets

Contrary to what occurred in the germination and seedling growth experiment, scorzonera plantlets were essentially insensitive to NaCl treatments started twelve days after seed sowing to seedlings having grown in the absence of salt stress for approximately eight days and data of differences between air and leaf temperature suggest that plantlets were not facing important difficulties to dissipate heat by transpiration.

The only clear exception to plantlet insensitivity to NaCl treatments was relative chlorophyll content which significantly increased with concentration until 150 mM NaCl and stabilized at lower values of chlorophyll content at 200 mM and 250 mM NaCl. Relative chlorophyll content is known to strongly correlate with leaf chlorophyll concentration (Markwell et al. 1995) and to be a good indicator of leaf nitrogen status (Hoel and Solhaug 1998). Thus our data suggest that the high tolerance to NaCl of scorzonera plantlets might involve a very strong mobilization of nitrogen to increase chlorophyll concentration in leaves in order to sustain the increasing requirements of energy needed to maintain tolerance as NaCl concentration increases.

The plateau found in relative chlorophyll content at the two higher NaCl concentrations also suggests that in the conditions of the experiment, nitrogen requirements of plantlets to maintain growth and to tolerate those salt concentrations equalled or exceeded nitrogen availability. The significant inhibition of shoot length, and shoot and total dry weight at 250 mM NaCl clearly supports this reasoning.

Conclusions

Scorzonera, or at least the cultivar used in this study, acted as a naturally salt tolerant species, able to complete germination and successfully produce seedlings and to recover from NaCl stress at concentrations as high as 250 mM, at which a number of halophytes show much lower germination or recovery percentages (Khan and Gul 2008; Gul et al. 2013). NaCl effects were essentially due to the osmotic component while the ionic component counteracted the former. Germination recovery from NaCl effects is partly true recovery but the preponderance of the recovery results from more time allowed to complete germination, an issue that has been rarely if ever examined in studies of this type.

When NaCl was applied to twelve days old seedlings, the growth of scorzonera after three weeks was almost insensitive to it except in the concentration-dependent increase of relative chlorophyll content. Further research is needed before definitive conclusions can be reached but strong evidence exists that the high tolerance of scorzonera plantlets to NaCl might partly depend upon the availability of nitrogen.

More studies are also required to ascertain whether the capacity to tolerate NaCl shown in germination and seedling phases and in early plantlet growth would be maintained during the whole life cycle but the fact that tolerance to NaCl appears to increase from seed to plantlet makes scorzonera a promising crop for salinized soils.

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Fig. 1. Effects of polyethylene glycol 6000 (PEG) and of sodium chloride (NaCl) after the removal of osmotic component (NaCl-PEG) on germination of *Scorzonera hispanica* 16 days after sowing, as a percentage of controls normed to be 0%.

(A) Observed means ($\pm$ SE) of total germination completion (G_T) on polyethylene glycol (PEG, solid squares) and sodium chloride (NaCl-PEG, open squares) and predicted responses. $G_T = 100 \{1 - \exp\{-[(-\psi_\pi - 0.048)/0.798]^{6.102}\}\}$ for PEG (thick line); $G_T = 150.642 \{1 - \exp\{-[(-\psi_\pi - 0.398)/0.797]^{1.795}\}\}$ for NaCl-PEG (thin line). All treatments with $n=2$ except controls ($\psi_\pi=0$ MPa) with $n=4$.

(B) Observed means ($\pm$ SE) of lag of germination completion (l) on polyethylene glycol (PEG, solid squares) and sodium chloride (NaCl-PEG, open squares) and predicted responses. $l = -1478.823\psi_\pi^2$ for PEG (thick line); $l = -889.050\psi_\pi - 2408.129\psi_\pi^2$ for NaCl-PEG (thin line). All treatments with $n=2$ except controls ($\psi_\pi=0$ MPa) with $n=4$.

(C) Observed means ($\pm$ SE) of duration of germination completion on polyethylene glycol (PEG, solid squares) and sodium chloride (NaCl-PEG, open squares) and predicted responses. $D_{100} = -83.461\psi_\pi$ for NaCl-PEG (thin line). All treatments with $n=2$ except controls ($\psi_\pi=0$ MPa) with $n=4$.

Fig. 2. Effects of polyethylene glycol 6000 (PEG) and of sodium chloride (NaCl) after removal of the osmotic component (NaCl-PEG) on growth of *Scorzonera hispanica* seedlings five days after sowing as a percentage of controls normed to be 0%.

(A) Observed means ($\pm$ SE) of root length (R) on polyethylene glycol (PEG, solid squares) and sodium chloride (NaCl-PEG, open squares) and predicted responses. $R = 100 \{1 - \exp\{-[(-\psi_\pi - 0.098)/0.398]^{3.439}\}\}$ for PEG (thick line); $R = 49.386 \{1 - \exp\{-[(-\psi_\pi - 0.096)/0.806]^{1.819}\}\}$ for NaCl-PEG (thin line). All treatments with $n=15$.

(B) Observed means ($\pm$ SE) of hypocotyl length (H) on polyethylene glycol (PEG, solid squares) and sodium chloride (NaCl-PEG, open squares) and predicted responses $H = 100 \{1 - \exp\{-[(-\psi_{\pi} - 0.099)/0.399]^{3.309}\}\}$ for PEG (thick line); $H = 60.859 \{1 - \exp\{-[(-\psi_{\pi} - 0.086)/0.784]^{1.424}\}\}$ for NaCl-PEG (thin line). PEG treatments at $\psi_{\pi} = -0.487$ MPa and NaCl-PEG at $\psi_{\pi} = -1.21$ MPa with $n=13$, NaCl-PEG at $\psi_{\pi} = -0.73$ MPa with $n=10$, at $\psi_{\pi} = -0.97$ MPa with $n=8$; otherwise $n=15$.

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Table 1. Means±standard errors and samples size of seed germination percentage and growth of *Scorzonera hispanica* seedlings treated with sodium chloride (NaCl) or polyethylene glycol 6000 (PEG) at various osmotic potentials (ψ_{π}).

ψ_{π} (MPa)	A) Total germination completion (%)			B) Total germination completion (%)			C) Total germination completion (%)		
	NaCl (8 days)	<i>P</i>	PEG (8 days)	NaCl+Water (8+8 days)	<i>P</i>	PEG+Water (8+8 days)	NaCl (16 days)	<i>P</i>	PEG (16 days)
0	93.0±2.5;4		93.0±2.5;4	95.0±1.9;4		95.0±1.9;4	95.0±1.9;4		95.0±1.9;4
– 0.25	87.0±1.9;4 (0.107)	[0.537]	90.0±4.2;4 (0.560)	92.0±4.0;2 (0.471)	[0.553]	96.0±4.0;2 (0.804)	94.0±2.0;2 (0.765)	[1.000]	94.0±2.0;2 (0.765)
– 0.49	80.3±4.4;4 (0.046)	[0.011]	40.0±10.3;4 (0.012)	98.0±2.0;2 (0.391)	[0.500]	100.0±0.0;2 (0.080)	96.2±0.0;2 (0.589)	[0.413]	90.0±6.0;2 (0.344)
– 0.73	33.0±5.7;4 (7.45×10 ^{–5})	[0.011]	2.0±1.2;4 (5.28×10 ^{–8})	100.0±0.0;2 (0.080)	[0.205]	94.0±2.0;2 (0.765)	84.0±8.0;2 (0.125)	[0.457]	66.0±18.0;2 (0.351)
– 0.97	35.4±8.9;4 (7.92×10 ^{–4})	[0.028]	0.0±0.0;4 (4.36×10 ^{–5})	94.1±2.1;2 (0.784)	[0.307]	98.0±2.0;2 (0.391)	76.0±0.0;2 (0.002)	[0.035]	4.0±4.0;2 (1.76×10 ^{–5})
– 1.21	49.5±9.4;4 (0.004)	[0.014]	0.0±0.0;4 (4.36×10 ^{–5})	88.0±4.0;2 (0.137)	[0.500]	92.0±0.0;2 (0.215)	90.2±1.8;2 (0.195)	[0.012]	0.0±0.0;2 (1.80×10 ^{–5})
ψ_{π} (MPa)	D) Recovery of germination completion (%)			E) Continuance of germination completion (%)			F) Lag of germination completion (days)		
	NaCl	<i>P</i>	PEG	NaCl	<i>P</i>	PEG	NaCl	<i>P</i>	PEG
0	27.8±14.7;3		27.8±14.7;3	27.8±14.7;3		27.8±14.7;3	0.7±0.2;4		0.7±0.2;4
– 0.25	37.5±12.5;2 (0.677)	[0.488]	60.0;1 (0.387)	58.3±8.3;2 (0.223)	[0.293]	41.7±8.3;2 (0.537)	1.4±0.01;2 (0.037)	[0.465]	1.3±0.2;2 (0.146)
– 0.49	93.8±6.2;2 (0.043)	[0.423]	100.0±0.0;2 (0.032)	77.5±2.5;2 (0.080)	[0.494]	84.4±7.9;2 (0.065)	1.5±0.05;2 (0.057)	[0.006]	2.1±0.01;2 (0.009)
– 0.73	100.0±0.0;2 (0.032)	[0.087]	93.9±1.9;2 (0.040)	73.8±13.8;2 (0.123)	[0.753]	65.7±17.7;2 (0.199)	2.1±0.7;2 (0.044)	[0.182]	6.7±2.2;2 (0.218)
– 0.97	90.7±0.2;2 (0.050)	[0.068]	98.0±2.0;2 (0.035)	63.3±3.3;2 (0.160)	[0.008]	4.0±4.0;2 (0.304)	3.5±0.03;2 (0.001)		–
– 1.21	70.8±4.2;2 (0.111)	[0.037]	92.0±0.0;2 (0.043)	82.2±5.3;2 (0.067)	[0.004]	0.0±0.0;2 (0.239)	2.6±0.2;2 (0.004)		–

Ψ_{π} (MPa)	G) D_{100} (days)			H) Shape of germination completion			I) Root length (mm)		
	NaCl	P	PEG	NaCl	P	PEG	NaCl	P	PEG
0	4.9±1.4;4		4.9±1.4;4	2.4±0.8;4		2.4±0.8;4	19.5±0.5;15		19.5±0.5;15
− 0.25	9.9±0.3;2 (0.075)	[0.017]	7.1±0.2;2 (0.350)	1.6±0.05;2 (0.533)	[0.023]	2.0±0.05;2 (0.768)	16.9±1.2;15 (0.069)	[0.500]	15.9±1.0;15 (0.003)
− 0.49	7.8±0.3;2 (0.236)	[0.461]	7.5±0.2;2 (0.285)	2.4±0.2;2 (0.992)	[1.27×10 ^{−4}]	23.7±0.1;2 (5.87×10 ^{−5})	10.7±0.5;15 (1.13×10 ^{−12})	[2.86×10 ^{−5}]	7.2±0.4;15 (1.70×10 ^{−17})
− 0.73	7.3±0.7;2 (0.324)	[0.240]	3.8±2.0;2 (0.677)	24.6±0.4;2 (5.48×10 ^{−5})	[0.195]	11.9±6.6;2 (0.382)	5.3±0.4;15 (3.98×10 ^{−20})		—
− 0.97	5.9±0.03;2 (0.490)		—	17.7±2.1;2 (0.001)		—	4.6±0.3;15 (1.75×10 ^{−18})		—
− 1.21	6.7±0.02;2 (0.287)		—	21.8±1.3;2 (1.81×10 ^{−4})		—	7.9±0.7;15 (6.63×10 ^{−14})		—
Ψ_{π} (MPa)	J) Hypocotyl length (mm)			K) RHP ₁ (%)					
	NaCl	P	PEG	NaCl	P	PEG			
0	13.4±0.7;15		13.4±0.7;15	59.5±1.3;15					
− 0.25	11.1±0.4;15 (0.006)	[0.204]	10.2±0.5;15 (0.001)	59.8±1.4;15 (0.865)	[0.724]	60.6±1.6;15 (0.599)			
− 0.49	8.9±0.4;15 (2.61×10 ^{−6})	[7.55×10 ^{−6}]	5.4±0.5;13 (8.18×10 ^{−10})	54.6±1.0;15 (0.005)	[0.070]	63.1±4.4;15 (0.443)			
− 0.73	5.5±0.5;10 (1.59×10 ^{−8})		—	64.1±6.9;15 (0.519)		—			
− 0.97	4.4±0.5;8 (8.20×10 ^{−9})		—	73.2±6.9;15 (0.071)		—			
− 1.21	7.6±0.7;14 (1.39×10 ^{−6})		—	53.8±3.8;15 (0.181)		—			

Note: Numbers inside round brackets are the significance levels of exact or approximate Student’s t test for differences between treatments and control (Ψ_{π} =0 MPa); numbers inside square brackets (P) are the significance levels for differences between NaCl and

PEG treatments at the same ψ_{π} level. **A** is the total germination completion of seeds on NaCl or PEG eight days after sowing, **B** is the total germination completion of seeds on NaCl or PEG eight days after sowing plus eight days in distilled water, **C** is the same but continuously on NaCl or PEG. Recovery of germination completion refers to the total germination completion of **B**, continuance of germination completion refers to the total germination completion of **B**. Lag of germination completion is the time necessary for the first seed to complete germination; D_{100} is the duration of germination completion, the time necessary to attain total germination completion minus the time necessary to the first seed to complete germination; shape of germination completion is a parameter estimating the symmetry of the distribution of germination completion over time; RHP_1 is partition of root (R) and hypocotyl (H) length calculated as $100 (R / R + H)$.

Table 2. Means±standard errors and samples size of early growth of *Scorzonera hispanica* plantlets treated with sodium chloride (NaCl)

NaCl (mM)	A) Root length (mm)	B) Shoot length (mm)	C) RSP ₁ (%)	D) Root diameter (mm)	E) Shoot diameter (mm)	F) Number of leaves	G) Root dry weight (mg)	H) Shoot dry weight (mg)
0	127.0±6.2;32	170.4±8.7;32	43.0±1.5;32	1.8±0.2;31	1.6±0.1;32	2.4±0.2;32	35.0±5.7;4	56.4±4.3;4
50	149.0±7.6;30 (0.024)	170.6±8.6;30 (0.983)	46.8±1.6;30 (0.034)	1.5±0.2;29 (0.671)	1.4±0.1;29 (0.115)	2.3±0.2;30 (0.815)	59.8±31.6;4 (0.493)	43.7±8.1;4 (0.215)
100	136.7±8.0;28 (0.611)	164.7±6.5;28 (0.615)	45.7±1.7;28 (0.652)	1.5±0.1;27 (0.599)	1.7±0.1;28 (0.760)	2.4±0.2;28 (0.989)	48.4±24.5;4 (0.627)	50.7±4.0;4 (0.372)
150	109.4±7.7;33 (0.393)	168.0±7.1;33 (0.759)	38.7±1.9;33 (0.324)	1.7±0.1;33 (0.886)	1.9±0.1;33 (0.151)	2.3±0.2;33 (0.835)	25.2±3.7;4 (0.202)	56.5±1.5;4 (0.991)
200	129.9±8.1;32 (0.763)	144.3±6.3;32 (0.131)	46.4±1.6;32 (0.273)	1.6±0.1;32 (0.798)	1.7±0.1;32 (0.544)	2.3±0.2;32 (0.844)	30.9±6.6;4 (0.656)	63.1±11.8;4 (0.618)
250	120.1±8.7;34 (0.639)	132.9±7.3;34 (0.041)	46.5±1.5;34 (0.300)	1.4±0.1;33 (0.587)	1.5±0.1;34 (0.904)	2.3±0.2;34 (0.866)	21.3±3.4;4 (0.085)	42.0±3.4;4 (0.039)
NaCl (mM)	I) Total dry weight (mg)	J) RSP _w (%)	K) RWC (%)	L) SWC (%)	M) TWC (%)	N) Leaf area (cm ²)	O) LWC (%)	P) LRWC (%)
0	91.4±7.2;4	37.8±4.0;4	86.9±1.5;4	80.4±4.9;4	84.1±2.3;4	26.62±5.50;4	88.3±0.5;2	83.5±0.2;2
50	103.6±27.8;4 (0.688)	49.7±12.4;4 (0.398)	82.1±6.8;4 (0.542)	88.7±1.4;4 (0.152)	84.7±4.1;4 (0.911)	32.29±7.39;4 (0.561)	87.8±1.1;2 (0.690)	82.4±2.4;2 (0.683)
100	99.1±21.9;4 (0.750)	41.8±10.9;4 (0.743)	82.4±6.4;4 (0.540)	87.3±0.7;4 (0.688)	84.8±3.0;4 (0.864)	24.51±5.60;4 (0.797)	83.7±0.4;2 (0.020)	82.7±1.3;2 (0.623)
150	81.7±4.5;4 (0.296)	30.4±3.1;4 (0.200)	84.5±2.5;3 (0.427)	85.2±4.0;3 (0.498)	85.4±3.5;3 (0.756)	21.01±1.83;3 (0.440)	85.1±1.1;2 (0.126)	81.1±1.9;2 (0.334)
200	94.0±18.1;4 (0.901)	32.1±2.6;4 (0.278)	83.0±3.1;3 (0.277)	79.7±4.0;3 (0.928)	81.0±3.7;3 (0.482)	25.97±2.44;3 (0.928)	87.1±1.1;2 (0.427)	83.8±0.5;2 (0.631)
250	63.3±6.8;4 (0.029)	32.9±2.3;4 (0.338)	83.8±2.8;3 (0.336)	83.4±2.8;3 (0.647)	83.5±2.8;3 (0.878)	26.88±10.50;3 (0.982)	89.1±0.9;2 (0.534)	84.1±0.8;2 (0.541)

NaCl (mM)	Q) SLA (mm ² mg ⁻¹)	R) S adaxial (No. mm ⁻²)	S) NS _C adaxial (No. mm ⁻²)	T) SI adaxial (%)	U) S abaxial (No. mm ⁻²)	V) NS _C abaxial (No. mm ⁻²)	W) SI abaxial (%)	X) g _s adaxial (mmol m ⁻² s ⁻¹)
0	60.3±0.0;2	52.5±2.8;8	302.6±10.6;8	17.4±0.7;8	42.1±2.1;8	304.6±10.1;8	13.8±0.4;8	251.7±52.0;3
50	55.3±5.0;2 (0.500)	60.8±3.4;8 (0.493)	355.1±12.1;8 (0.312)	17.1±0.6;8 (0.781)	48.4±1.4;8 (0.342)	344.0±6.7;8 (0.306)	14.1±0.5;8 (0.746)	273.0±61.2;3 (0.804)
100	43.1±0.0;2 (0.194)	70.5±6.1;8 (0.382)	381.3±24.6;8 (0.344)	18.4±0.7;8 (0.462)	71.1±7.9;8 (0.241)	420.0±32.2;8 (0.266)	16.6±0.7;8 (0.224)	92.3±18.7;3 (0.045)
150	40.4±2.7;2 (0.086)	52.5±1.8;8 (0.915)	397.9±10.4;8 (0.119)	13.2±0.5;8 (0.021)	44.9±2.8;8 (0.706)	373.0±5.8;8 (0.147)	12.1±0.8;8 (0.744)	105.0±41.2;3 (0.092)
200	46.7±3.6;2 (0.164)	58.7±4.2;8 (0.649)	357.8±6.2;8 (0.195)	16.3±1.0;8 (0.697)	69.8±6.7;8 (0.263)	407.6±26.0;8 (0.242)	17.0±0.9;8 (0.204)	104.7±9.0;3 (0.050)
250	55.3±5.0;2 (0.500)	71.8±4.0;8 (0.153)	468.3±11.0;8 (0.057)	15.4±0.9;8 (0.259)	67.0±2.4;8 (0.072)	415.8±16.2;8 (0.136)	16.3±0.9;8 (0.260)	152.2±89.8;3 (0.382)
NaCl (mM)	Y) g _s abaxial (mmol m ⁻² s ⁻¹)	Z) RCC						
0	214.0±47.4;3	35.5±1.4;8						
50	285.0±50.2;3 (0.362)	39.1±0.9;8 (0.045)						
100	17.1±6.2;3 (0.051)	40.4±1.0;8 (0.011)						
150	25.1±8.8;3 (0.053)	47.7±1.4;8 (2.34×10 ⁻⁵)						
200	5.3±2.6;3 (0.048)	43.3±1.1;8 (5.11×10 ⁻⁴)						
250	6.2±1.3;3 (0.048)	42.7±1.5;8 (0.003)						

Note: Numbers inside round brackets are the significance levels of differences between treatments and control (NaCl=0 mM) tested by nested ANOVA (**A–F** and **R–W**) or by exact or approximate Student's t test (**G–Q** and **X–Z**). RSP_I and RSP_w are partitions of root

(R) and shoot (S) length or dry weight calculated as $100(R/R+S)$. RWC, SWC, TWC and LWC are water contents of roots, shoots, roots plus shoots and leaves; LRWC is relative water content of leaves; SLA is specific leaf area; S and NS_c are densities of stomata and non-stomata epidermal cells of adaxial- or abaxial-surfaces; SI is stomata index calculated as $SI = 100 (S / NS_c)$; g_s is stomata conductance of adaxial- or abaxial-surfaces; RCC is relative chlorophyll content.

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