BIOLOGIA PLANTARUM 51 (3): 436-442, 2007

Identification of two phenotypes of *Arabidopsis thaliana* under *in vitro* salt stress conditions

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Abstract

This study describes two phenotypes of *Arabidopsis thaliana* (ecotype Columbia) developed *in vitro* under salt stress (75 mM NaCl). The phenotypes 01 and 02 appeared visibly distinguishable by rosette morphology and competence to produce flowers. Phenotype 01, sensible to salt stress, accumulated high quantities of Na^+, showed a slight reduction in dry mass, and high protein and chlorophyll contents. Moreover, its anatomy exhibited some xeromorphic traits. Phenotype 02, clearly salt tolerant, showed a morphology similar to control plants, displaying typical phyllotactic rosette and flowering stalk production. Accumulation of Na^+, protein and chlorophyll contents were close to control plants. Reversion experiments on NaCl free MS medium, showed a partially recovered phenotype 01. A threshold salt stress concentration that permits the simultaneous development of two phenotypes, was found.

Additional key words: chlorophyll, leaf anatomy, morphology, proteins, sodium chloride.

Introduction

Salinity is an environmental challenge of worldwide importance, since one third of the world's irrigated lands are being deemed useless due to their salts excess (Owens 2001, Munns 2005). An early symptomatic feature of salt stress is the inhibition of plant growth (Zhu 2001). Delayed plant growth and development are interpretable as adaptive traits for plant survival, allowing them to use a significant part of photoassimilates to react to stress (Lazof and Bernstein 1999). Plants growing under stress can perform several strategies such as morphological modifications and/or changes in some physiological processes (Bohnert *et al.* 1995, Niu *et al.* 1995, Yeo 1998, Tester and Davenport 2003). Qualitative and quantitative changes in metabolite synthesis (Karimi *et al.* 2005), increased production and accumulation of reactive oxygen species (ROS) (Tsugane *et al.* 1999), occurrence of enhanced metabolic toxicity (Hasegawa *et al.* 2000), are some of the common indicators of stressed plants.

Furthermore, salt stress affects the expression of cell cycle progression genes (Burssens *et al.* 2000) and thus influences cell division. A perturbed pressure potential may also alter cell expansion and inhibit growth. All these cellular processes may be affected by an altered hormonal homeostasis under salt stress (Xiong and Zhu 2002). It is well documented that abscisic acid (ABA) content increases under salt stress (Bray 1997). Several mechanisms of protection and adaptation to hyper-ionic and hyper-osmotic stresses could take place to tolerate the injurious effects of salts, for example, vacuolar compartmentation of toxic ions (Binzel *et al.* 1988), regulation of ion transport from root into shoot by modifications of stomatal movements and accumulation of large quantities of ions in mature and old abscising leaves (Munns 1993).

The important plant model *Arabidopsis thaliana* L., is a glycophyte plant sensitive to growth inhibition and damage by salt stress in all developmental stages (Xiong and Zhu 2002), however, it possesses some degree of salt tolerance (Sanders 2000). At morphological level, *Arabidopsis thaliana* consists of annual rosette, with two phases of growth: vegetative and reproductive, classified by Bowman (1994). The vegetative rosette is characterized by a phyllotactic spiral leaf arrangement, with short internode elongation. The leaf number before the flowering depends on growth conditions and on

Received 9 December 2005, *accepted* 15 July 2006.
Abbreviations: EDTA - ethylenediaminetetraacetic acid; MS - Murashige and Skoog medium; ROS - reactive oxygen species.
Acknowledgements: This work was supported by *ex* 60 % funds from Ministry of University and Scientific and Technological Research (MURST) of Italy to Nello Bagni. Karina Ruiz Carrasco was supported by a grant from Ministry of Foreign Affairs of Italy. We are grateful to Dr. Gian Lorenzo Calzoni, University of Bologna for the macro photographs of treated and control plants.
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genotype. Later, the initials of leaf meristems in the vegetative phase switch to produce flowers, reaching the reproductive phase. Some leaves are formed along the flowering stalk, called cauline leaves, different from the rosette leaves.

Genetic engineering of *Arabidopsis* salt tolerance has been attempted using mutational and transgenic approaches, one of which involves the transfer of transgenes designed to constitutively express genes involved in salt tolerance in other plants or organisms (Denby and Gehring 2005). In particular in the overexpression of the vacuolar (Apse *et al.* 1999) and plasma membrane *SOS1* (Shi *et al.* 2003), Na^+/H^+ antiporter gene, improved significatively the salt tolerance by preventing the build-up of toxic levels of cytosolic Na^+. Another strategy in studying and manipulating *Arabidopsis* salt tolerance, could also be represented by the analysis of natural variability under salt stress conditions. Quesada *et al.* (2002) analysed 102 wild-type races of *Arabidopsis* germinating on 250 mM NaCl finding a wide range of variability among them and suggesting that the natural variation of *Arabidopsis* in NaCl tolerance is under polygenic control. Confirming this hypothesis a detailed transcriptome analysis conducted with a GeneChip microarray technique (Kreps *et al.* 2002) identified a set of mRNAs stress-specific in response to 100 mM NaCl exposure.

The aim of this study was to provide more information on the behaviour of *Arabidopsis thaliana* L. (ecotype Columbia) grown *in vitro* under salt stress conditions, with particular attention to variations in morphometric, morphological and some biochemical parameters.

Materials and methods

Plant culture and growth conditions: *Arabidopsis thaliana* L. (ecotype Columbia) seeds carrying the homozygous recessive glabra marker (*gl1)* (*Lehle Seeds*, Round Rock, Texas, USA), were imbibed in sterile water for 30 min and then incubated for 4 min in 70 % (v/v) ethanol and 4 min 10 % (v/v) commercial bleach. The seeds were washed 5 times in sterile water and then resuspended in 0.15 % (m/v) *Bactoagar* and kept at 4 °C for 24 h. Seeds were horizontally sowed in glass jars with ¼ strength Murashige and Skoog (1962; MS) medium (*Gibco, Invitrogen,* Milano, Italy) with 0.6 % (m/v) *Phytagel* (*Sigma*, Milano, Italy) without or with NaCl. At first, the experiments were performed at different NaCl concentrations, *i.e.*, 3, 10, 25, 50, 75, 100, and 200 mM, in order to determine the sublethal concentration. The selected concentration was 75 mM NaCl, since higher concentrations lead to the death of plants. Glass jars with 13 seeds were kept at 16-h photoperiod (25 W m^{-2} irradiance) at 22 °C.

The sampling was performed in the reproductive growth phase according to Bowman (1994) taking into account the ontogenetic time. Control plants were collected at 31 d after sowing while the 75 mM NaCl treated plants were harvested 46 d after sowing and divided in two groups phenotypically different. In order to determine if the morphological features of the two group of plants were due just to salt stress exposure or also to genetic characteristics, phenotype 01 and 02 salt-treated plants were transferred (46 d after sowing) to NaCl free MS media to verify a possible phenotype reversion.

When collected all the plants were separated into roots, leaf rosettes and, if present, flowering stalks, immediately frozen with liquid N_2 and stored at -80 °C or used for microscopy experiments.

Microscopy: Small fragments of leaves were fixed in 0.1 M Na-phosphate buffer (pH 7) with 3 % (v/v) glutaraldehyde, for 3 h at 4 °C, post fixed in aqueous 1 % (v/v) osmium tetroxide, dehydrated in buffer added ethanol series and finally, embedded in glycolmethacrylate resin (*Technovit® 7100*, *Bio-Optica*, Milan, Italy), as reported by Gutmann and Feucht (1991). Semithin sections, obtained using an ultramicrotome (*Reichert-Jung Ultracut E Microtome*, *Leica Microsystems*, Nussloch, Germany), were stained with 1 % toluidine blue and/or neutral red and observed under a *Leitz Orthoplan* light microscope (*Leitz-Service*, Como, Italy). To be able to compare control and treated plants, the cross sections for the different measurements and counting (cells or cell layers) were done in the same leaf position.

Morphometric parameters (leaf blade area and thickness, number of mesophyll cell layers, leaf cell and intercellular spaces area) were calculated dividing each photographic cross-section into four transepts and all the cells and intracellular spaces inside these lines were evaluated by the *AutoCAD2000* program (*Autodesk*, San Francisco, CA, USA). At least twenty different measurements were done for each parameter with a minimum of five photos per sample. The morphometric analyses were repeated two times with two independent sets of plants obtaining similar results. The results of Table 1 and Fig. 1 refer to a single set of plants.

Determination of dry mass and sodium content: For dry mass measurement, 100 mg of fresh plant material from control and treated plants were oven-dried in paper sacks at 80 °C until no differences in dry mass were detected.

Control, phenotype 01 and phenotype 02 entire plants were used for sodium quantification. The samples were weighed and divided in 100 mg lots, rinsed 2 times with deionised water, dried in an 80 °C oven, and digested in 10 cm^3 deionised water, 3 cm^3 65 % perchloric acid and 1 cm^3 65 % nitric acid followed by warming twice for

10 min in a microwave oven (3.1 kW). Then, 2 cm^3 of 12 M HCl was added. The homogenate was boiled until complete clearing, the solution was filtered and brought to 50 cm^3 final volume with deionised water. Na^+ content was determined by atomic absorption spectrophotometer (*PerkinElmer 5000*, *PerkinElmer Life and Analytical Sciences Inc.*, Boston, MA, USA) (David 1962). en different measurements were performed for each parameter. The analyses were performed on seven independent sets of plants obtaining similar results. The results of Table 2 refer to a single set of plants.

Determination of protein and chlorophyll contents: Four independent samples (200 mg each) of control, phenotype 01 and 02 leaf tissues were homogenized in 5 volumes of 100 mM Tris-HCl buffer, pH 7.6, containing EDTA 50 μM and 25 μM pyridoxal phosphate and centrifuged at 20 000 *g* for 30 min at 4 °C. The supernatant and pellet were separated and the latter was washed, centrifuged twice and finally resuspended in five buffer volumes. Protein determinations of both fractions were performed according to Lowry *et al.* (1951) using bovine serum albumin as standard. Chlorophylls were determined according to Smith and Benitez (1955) in control, phenotype 01 and 02 plants (four each). Each sample was homogenised with five volumes of 80 % (v/v) acetone and centrifuged 10 min at 1 100 *g*. The absorbance of the clear supernatant was measured at 663 nm and 645 nm with a spectrophotometer (*Jasco 7800*, *Jasco Europe*, Carpi, Italy). All the measurements were performed in triplicate samples.

Results

Phenotypes description and leaf anatomy: Regular development of basal leaf clusters in control plants, forming the typical *Arabidopsis* rosette, indicated that the *in vitro* culture conditions were adequate to prevent any stress damage. Seeds sowed in MS plus 75 mM NaCl, displayed delayed radicle formation and cotyledons emergence begun to show the first alteration at seed germination stage. All germinated seeds produced basal rosette leaves (data not shown).

The plants grown on MS with 75 mM NaCl displayed, during the reproductive phase, the occurrence of two different phenotypes, phenotype 01 and 02, showing different reproductive behaviour, while the plants grown on MS plus 50 mM reached the flowering stage, even if with a delay in flowering stalk production (data not shown). No more than 31 % of the individual plants grown in MS plus 75 mM NaCl produced flowers, originating the phenotype 02. On the other hand, phenotype 01 (69 % of the plant population), remained in the vegetative phase producing only leaves. A similar pattern was described in *Arabidopsis* by Bowman (1994), who closely correlated the increase in leaf number with a delay in the transition to flowering.

The number of rosette leaves was of 10 ± 2 in control, 105 ± 3 in phenotype 01 and 7 ± 2 in phenotype 2 plants, with generally one flowering stalk per plant in control and phenotype 02 and none in phenotype 01. Phenotype 01 (Fig. 1*E*) showed peculiar morphological features, very different from the control (Fig. 1*A*), with extremely small sized plants. Each plant produced numerous little leaves which wrap each other up, showing very reduced internodal segments. Long, thin roots were present. The phenotype 01 plants never reached the reproductive phase. Phenotype 02 (Fig. 1*G*) displayed a macro-morphology similar to the control: however, the plant was slightly smaller in size, with a cluster of basal leaves reduced in number and size. This plant had a long flowering stalk, that reached the reproductive phase later in comparison with control plants (the flower became visible 31 d after sowing in control plants, whereas 46 d after sowing in phenotype 02 plants). In control plants, upper epidermis cells were small in apical leaf, while were much more developed in basal one (Fig. 1*B,C*). Between the irregularly shaped lower epidermis cells, some little stomata were discernable, both in apical and in basal leaf sections. The mesophyll consisted of one-layered palisade tissue, better distinguishable in the basal leaf. Spongy tissue cells were differently shaped, adjoining to very extended, communicating intercellular space system. The leaf texture appeared loose in both leaves (Fig. 1*B,C*). The most important and substantial feature in the cross section of the phenotype 01 apical leaf (Fig. 1*D*) was the presence of a very compact mesophyll, with a compressed photosynthetic parenchyma, showing few and small or absent intercellular space systems. Upper and lower epidermis cells were turgescent and larger than the mesophyll cells. Palisade and spongy tissues were hardly distinguishable, the mesophyll being formed by small, narrow, closely neighbouring cells. The same features were displayed in the fully expanded phenotype 01 basal leaf (Fig. 1*F*). Phenotype 02 very young apical leaf (Fig. 1*H*) appeared very reduced in thickness, with large-celled upper and lower epidermis. The four-layered mesophyll presented small almost round shaped cells, very close to each other, so its texture was very compact, with an absent inter-cellular space system. Phenotype 02 basal leaf (Fig. 1*I*) appeared thicker than the apical one, due to the larger mesophyll cells, especially below the upper epidermis. The mesophyll, consisting of one-layered palisade tissue, presented very large cells and some intercellular spaces, although reduced in size and number. Small cells, not quite interpretable as a typical spongy tissue, were in contact with the lower epidermis.

Leaf morphometry: The phenotype 01 leaf area resulted dramatically reduced in both apical and basal leaves. Leaf blade was much thicker, coherent with increased cell

layer number in phenotype 01 (Table 1). The intercellular space system area appeared to be extremely reduced (a 4-6 fold reduction) in both apical and basal leaves (Table 1). In phenotype 02 plants, leaf area, thickness and cell area values were, according to phenotype 02 morphology, similar to control plants. In basal leaves of phenotype 02 plants, the intercellular spaces area showed lower values than phenotype 01 plants and extremely reduced values when compared with control plants. The leaf cell layer number was the same as in control plants (Table 1).

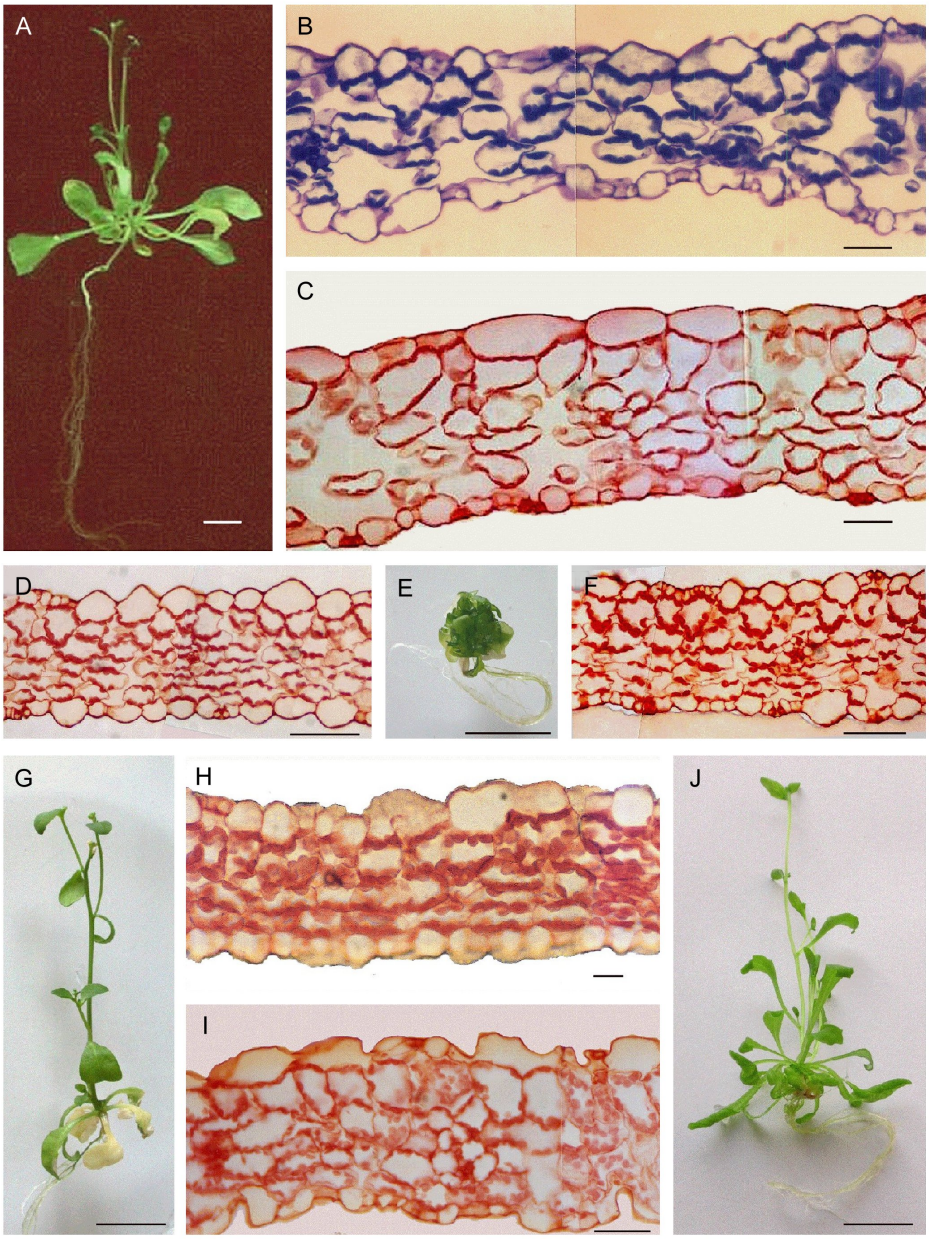

Fig. 1. *Arabidopsis thaliana* L. ecotype Columbia at reproductive growth phase. *A* - Control plant at 31 d after sowing shows a well developed rosette, with two emerged inflorescence shoots. *B* and *C* - Cross sections of apical (*B*) and basal (*C*) leaves of control plants. They display large stomatal cavities and loose mesophyll. *D* - Cross section of salt stress phenotype 01 apical leaf shows a very compact mesophyll, with small intercellular space system and more mesophyll cell layers. *E* - Phenotype 01 plants, 46 d after sowing on MS medium plus 75 mM NaCl, show a very little rosette, with high leaf numbers and drastically reduced leaf area. *F* - Cross section of phenotype 01 basal leaf, displaying the same features as apical ones. *G* - Phenotype 02 plants, 46 d after sowing in MS media plus 75 mM NaCl, phenotypically similar to control plants. *H* and *I* - Cross sections of phenotype 02 apical (*H*) and basal (*I*) leaves. Mesophyll shows anatomical features, similar to control plant mesophyll regarding cell layer numbers, even if highly compacted. *J* - Revertant plant of the phenotype 01, after 18 d from transfer to NaCl free MS medium. It shows morphological features similar to control plant, with fully expanded leaf rosette and two emerged flowering stalks. *A*, *E*, *G* and *J bar* = 1 cm; *B*, *C*, *D*, *F*, *H* and *I bar* = 100 μm.

Table 1. Morphological parameters measured in control (31 d after sowing) and 75 mM NaCl treated (46 d after sowing) apical and basal leaves of *Arabidopsis thaliana* L. (ecotype Columbia). Each value is the mean of 20 measurements ± SE; * - values were significantly different from the control at $P < 0.05$.

Parameters	Apical leaf control	phenotype 01	phenotype 02	Basal leaf control	phenotype 01	phenotype 02
Leaf area [mm^2]	70 ± 9	5 ± 3*	64 ± 7	88 ± 12	16 ± 6*	86 ± 20
Leaf thickness [μm]	120 ± 20	180 ± 10	130 ± 10	180 ± 10	220 ± 10	190 ± 10
Mesophyll cell layers	4	6	4	4	6	4
Mesophyll cell area [μm^2]	791 ± 124	935 ± 90*	755 ± 66	1964 ± 234	1105 ± 58*	1513 ± 90*
Intercellular spaces area [μm^2]	2061 ± 252	316 ± 67*	629 ± 130*	2514 ± 235	411 ± 60*	484 ± 44*

Sodium, protein and chlorophyll contents: Dry mass measurements (Table 2) were performed on control, phenotype 01 and 02 plants collected at reproductive stage (31 d for control and 46 d after sowing for phenotype 01 and 02 plants). According to the plant morphology, phenotype 01 plants had the lowest dry mass, while control and phenotype 02 plants displayed comparable values.

Plants grown on 75 mM NaCl supplemented MS medium showed a relevant Na^+ accumulation in their roots and aerial parts (Table 2). In particular, phenotype 01 showed 1.8 and 20-fold higher Na^+ content when compared to phenotype 02 and control plants, respectively.

Significant differences were shown in protein content (expressed per unit of leaf area). Both control and phenotype 02 plants showed almost equal amount of protein about 4 times lower than phenotype 01. Both chlorophyll *a* and *b* contents in phenotype 01 plants were higher than in phenotype 02 and control plants (Table 2), in agreement with direct observation of a dark green colour of phenotype 01 salt-treated plants.

Table 2. Effects of salinity on *Arabidopsis thaliana* L. (ecotype Columbia) control (31 d after sowing) and 75 mM NaCl treated phenotype 01 and 02 plants (46 d after sowing). Dry mass and Na^+ content were measured in whole plants, protein and chlorophyll contents in rosette leaves. Each value is the mean of 10 measurements ± SE. (*) values were significantly different from the relative control at least for $P < 0.05$ (Student *t*-test).

Parameters	Control	Phenotype 01	Phenotype 02
Dry mass [mg]	7.0 ± 0.2	6.5 ± 0.1	7.5 ± 0.3
Na^+ content [mg g^{-1}]	3.5 ± 0.3	69.9 ± 0.8*	38.4 ± 0.7*
Protein [μg mm^{-2}]	1.1 ± 0.3	9.4 ± 0.6*	2.7 ± 0.9*
Chl *a* [μg mm^{-2}]	4.3 ± 0.8	6.0 ± 1.1	2.9 ± 0.4
Chl *b* [μg mm^{-2}]	2.0 ± 0.4	3.1 ± 0.5	1.7 ± 0.2

Reverting experiment: In order to determine if the morphological features of the two different phenotypes were due just to salt stress exposure or also to genetic characteristics, the salt-treated plants were transferred (46 d after sowing) to NaCl free MS media. After 10 d from the transfer, phenotype 01 had developed more vigorous rosettes, with numerous and miscellaneous leaves (Fig. 1*J*) (about 34 ± 2 per rosette). The leaves close to the stem were very small, resembling the rosette leaves developed in MS medium with NaCl. Leaves with elongating petiole and leaf area similar to the control ones, were instead present along the stem.

The reproductive growth phase was achieved by reverted phenotype 01 plants 18 d after transfer, with the production of two or more flowering stalks, like control plants. Moreover, some biochemical data such as protein and chlorophyll *a+b* contents (12.39 ± 0.21 mg g^{-1}(FM) and 4.51 ± 0.59 mg g^{-1}(FM), respectively) were close to values of control plants (14.86 ± 0.75 mg g^{-1}(FM) and 6.66 ± 1.27 mg g^{-1}(FM), respectively). It was not possible to express these data as μg mm^{-2}, due to the fact that the plants presented heterogeneous leaves: the apical leaves reverted to a normal shape, while the basal ones did not change after the experiment.

Phenotype 02 plants growing in NaCl free MS medium displayed features decidedly similar to the control ones.

Discussion

In our experimental system, we studied *Arabidopsis thaliana* (ecotype Columbia) through its life cycle from seed germination to flower production, grown *in vitro* without or with NaCl. *Arabidopsis thaliana* is considered a salt sensitive species, in which the most vulnerable phases are seed germination and seedling stages (Xiong and Zhu 2002). Our results reveal that vegetative rosette phase is affected by salt stress at 75 mM NaCl 21 d after sowing (data not shown) while only phenotype 02, but not phenotype 01, reached the reproductive stage (46 d after sowing).

Plants growing in MS medium with 75 mM NaCl displayed turgescent, large upper and lower epidermis cells, interpretable as water storing cells, frequently

reported as a structural response, which enables the plant to survive in adverse conditions. However, several xeromorphic features like thick cuticle, sclerenchymatous bundle sheath, sunken stomata, sclerenchymatic hypodermis were not present. This can be explained as a result of optimal culture conditions in controlled environment. Moreover, neither phenotype 01 nor 02 showed cells with plasmolysis symptoms; in fact, the plasmalemma was closely adherent to the cell wall and the central cell portions were presumably occupied by the vacuolar system. Mimura *et al.* (2003) considered the increasing vacuolar volume as one of important mechanisms of salt tolerance of plant cells. In fact, the decrease of cytoplasmic volume can generate the necessary increase in osmolarity to counterpart the increasing vacuolar osmolarity, required to maintain pressure potential. This can lead to produce less compatible solutes in the cytoplasm and keep high cytoplasmic potassium concentration (Mimura *et al.* 2003). This mechanism needs additional investigations.

In the phenotype 01, photosynthetic parenchyma was highly stratified with very little intercellular spaces giving a high leaf thickness (Fig. 1*D*,*F*). The very compact texture of mesophyll layers, where palisade and spongy tissues were not distinguishable and where leaf size was small, were referred to as xeromorphic leaf traits, as reported for plant populations living in arid conditions (Roth 1984, Ofir and Kigel 2003).

In terms of resistance, phenotype 01 was considered less tolerant to 75 mM NaCl concentration than phenotype 02, due to the fact that it accumulated the highest amount of Na^+ and never produced flowers. However, some degrees of tolerance were present in the phenotype 01. This phenotype responded to saline stress changing growth pattern (Zhu 2001), generating numerous, thick and little leaves and not producing flowers (Fig. 1*E*). Inhibition of leaf elongation has been described in several species under conditions of hyper-osmolarity (Romero-Aranda *et al.* 2001, Ghoulam *et al.* 2002). In addition, the reduced petiole length observed in phenotype 01 could be interpreted as a consequence of cell elongation inhibition rather than cell division arrest (Burssens *et al.* 2000).

The phenotype 02 was more tolerant to salt stress than phenotype 01, because it accumulated a reduced amount of Na^+ (Table 2) and it produced a flowering stalk, even if flowering phase was retarded. The leaf number and leaf area were reduced when compared with controls. Phenotype 02 leaf tissues were organized similarly to the control mesophyll tissues, showing an identical cell layer number. Values of cellular area, area and number of intercellular spaces were close to control (Table 1).

Dry mass accumulation was only slightly reduced in phenotype 01, whereas in phenotype 02 the value was similar to control plants. The most plausible explanation is that treated plants invest in structural compounds to counteract negative effects caused by stress (Zhu 2001), since water lost is not significantly different in control and treated individuals. This hypothesis was supported by the increase of protein and chlorophyll contents (expressed as $\mu g\ mm^{-2}$), taking into account the relevant leaf thickness observed in treated plants (Table 2).

Particularly, the chlorophyll content was also coherent with a dark green colour displayed by phenotype 01 leaf rosette. Romero-Aranda *et al.* (2001) observed a similarly high chlorophyll contents in tomato plants, when exposed to salt stress, but it was attributed to the reduced leaf area expansion, being more affected than the structural component of the leaf. Conversely, in our system, besides a reduced leaf area, leaf anatomy shows clearly increased leaf thickness in treated plants, implying structural feature modifications.

When phenotype 01 plants were removed from salt stress and transferred to a medium without NaCl, they developed after 18 d morphological traits with intermediate features between control and phenotype 02. At a metabolic level, the patterns of dry mass gain and protein accumulation were very similar to control plants. Probably, reverted plants were able to recover quickly from saline stress to some extent, even if the chlorophyll content remained low, with respect to controls. De Herralde *et al.* (1998) described a similar behaviour in *Argyranthemum coronopifolium*, a non-halophyte plant, suggesting injuries in the photosynthetic apparatus by toxic effect of salt concentration.

Some points of interest of this screening about the behavior in *Arabidopsis* growing under saline stress are the following: *1*) as regard the less Na^+ content in the phenotype 02, we could suppose that a difference exists at root level, like the involvement of the exclusion sodium root proteins (SOS1 and AtHKT1), as reviewed by Xiong and Zhu (2002) and Chinnusamy *et al.* (2004). Further studies are in progress in order to determine the presence of these proteins and to measure their activity. *2*) Moreover, information about the hereditariness of the phenotype 02 could help us to understand if the phenotype 02 behaviour is due to an adaptative trait or to acclimatization to salt stress. 3) Regarding the ability to produce flowers of the phenotype 02, it could be interesting to study the saline stress effects on genes that regulate the transition from the vegetative phase to the reproductive phase. Several gene sequences involved in shoot apical meristem (SAM) differentiation have been reported for *Arabidopsis* (Hempel *et al.* 1997, Fletcher 2002, Simpson 2004).

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