Morpho-physiological responses of Alamo switchgrass during germination and early seedling stage under salinity or water stress conditions

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Running title: Germination of switchgrass under stress

Abstract

Switchgrass (***Panicum virgatum* L.)** is a warm perennial grass with valuable characteristics as a biofuel crop. To avoid competition with food crops, biofuel crops will be likely relegated to less productive soils such as marginal lands. Consequently, the salinity and water scarcity problems that commonly affect marginal lands, compromise biofuel crops germination, emergence and seedling establishment. The aims of this study were to study the germination and seedling growth of switchgrass under salinity and water stress, and to describe the morpho-anatomical responses of the roots and leaves in the seedlings to these stresses. The effect of salt and water stress was assessed using sodium chloride (NaCl) and polyethylene glycol 8000 (PEG) at the same water potentials of -0.8, -1.0 and -1.2 MPa. Seeds were moist prechilled for 7 days at 5 ºC and germinated at 30ºC/15 ºC (8 h light/16 h dark). NaCl treatments (-0.8 and -1.0 MPa) delayed germination rates but did not reduce the final germination percentage, whereas at a lower potential (-1.2 MPa) the final germination percentage was diminished. The effects of PEG (-1.0 and -1.2 MPa) on the germination rate and final percentage, were more detrimental than those induced by iso-osmotic concentrations of NaCl. PEG and NaCl reduced significantly the vigor index of -0.8 to -1.2 MPa. The morpho-anatomical changes such as the reduction in the root cross-section area and the thickening of the endodermis walls for both stress conditions and aerenchyma formation in the cortex under salinity, could significantly contribute in the survival and tolerance during the early seedling stages.

Keywords

Switchgrass; germination; seedling; anatomy; salinity; water stress.

Abbreviations

Ψ_w Water potential (MPa)

DAS Days After Sowing

FGP Final Germination Percentage

t_{50} Time to obtain 50 % germination

GR Germination Rate

RL Root Length

AL Aerial part Length

VI Vigor Index

Introduction

Switchgrass (*Panicum virgatum* L.) is a warm-season, short day C_4 perennial grass native to the North American prairie. It has a diverse geographic distribution being adapted to a wide range of climate and soil conditions [1]. There are two groups of switchgrass ecotypes classed by their habitat preference, ploidy level, and molecular markers. Upland ecotypes are hexaploid or octoploid, grow in drier environments, are shorter and thinner-stemmed, with rhizomes that are more vigorous. In contrast, lowland ecotypes are commonly tetraploid, generally grow in wetter environments, are taller, thicker and bunchy stemmed with less vigorous rhizomes.

Current and proposed uses for switchgrass are forage for grazing, haylage, erosion control, vegetative filter strips, reclamation/stabilization of sand dunes, fibre or pulp for paper, phytoremediation including smelter and mining sites, and biomaterials. In the last 20 years, switchgrass has begun to be studied for non-forage purposes such as bioenergy since it is a good candidate for direct combustion or conversion to liquid (bioethanol) or gaseous forms (biogas) energy feedstock [2]. Many reasons justify the use of switchgrass as a biomass crop for energy. Key advantages include high net energy production, low nutrient requirements, high water use efficiency and adaptation to marginal lands [2].

Switchgrass is a rhizomatous species that can be reproduced by seeds. However, often switchgrass seed germination and establishment are poor and slow, reducing its economic viability as a biomass feedstock [1]. Factors that contribute to seedling failures include seed dormancy, extreme environmental conditions, and inadequate seeding management.

Germination is a crucial phase in the plant life cycle, which determines the success of seedling establishment and subsequent growth [3]. Germination occurs after a series of metabolic events, only producing seedlings when the environmental conditions (temperature, humidity, light, oxygen, etc.) are favourable.

Salinity and drought stress are becoming particularly widespread in many regions and may affect more than 50 % of all arable land by 2050 [4]. While, both stresses repress plant growth and development due to the imposed osmotic stress, only salinity stress affects productivity of agricultural crops through ion toxicity [5–7]. Some plants have developed morphological, structural and functional adaptive strategies to cope with both stresses [8]. Aditionally, during salt stress, some plants develop various strategies to tolerate the saline conditions, i.e. ion homeostasis and compartmentalization, hormonal modulation, synthesis of antioxidants, and biosynthesis of osmoprotectants such as proline, glycine, betaine and sugars, among others [6]. Several studies have been conducted to study salinity and water stress tolerance in lowland and upland switchgrass genotypes in order to identify the morphological, physiological, biochemical and molecular adaptations [9–11].

Salt stress is one of the major limiting factors for the germination and growth of switchgrass cultivars such as Alamo [12], Blackwell, Cave-In-Rock and Kanlow [13, 14]. Based on the physiological parameters of 33 switchgrass populations, 2-months old plants subjected to salt stress (250 mM NaCl) in hydroponics system for 24 days demonstrated significant differences in salt tolerance between populations [20]. The same study concluded that in general lowland ecotypes were more tolerant to salt stress than the upland ones and suggested that this variation may be closely associated with their genetic background and origin. This work classified Alamo switchgrass cultivar as salt tolerant and Cave-in-Rock as salt sensitive [14].

Later, Kim *et al.* [15] demonstrated that proline levels were not positively correlated with salt tolerance in 46 switchgrass populations subjected to salt stress by irrigation for 30 days, revealing multiple response mechanism to salt stress. These mechanisms may not be delineated by ecotype designations.

In relation to the water stress, morphological and physiological measurements and metabolomics profiles revealed a wide variation in drought tolerance among 49 lowland and upland switchgrass populations [16, 17]. The most tolerant genotypes tended to have higher levels of abscisic acid, spermine, trehalose and fructose in comparison to most drought-sensitive ones [17]. A comparative study for differences in response to water deficit and nitrogen fertilizer in lowland (Alamo and Kanlow) and upland (Blackwell and Caddo) switchgrass cultivars suggested that Alamo is the best cultivar for forage and biomass production under drought and non-drought conditions [18]. This cultivar is also one of the most promising candidates for biofuel production, not only for its high biomass yields, but also because it has low fertilization requirements and is the most adapted, of the commercial lowland ecotypes, to unfavorable environments.

It is well established that plant responses to water or salt stress depend on the stage of development, stress intensity and duration. In our present study, we evaluated the effect of NaCl stress and water stress on the germination, emergence and the early growth of seedlings of Alamo switchgrass cultivar based not only on growth parameters, but also on and morpho-anatomical changes in roots and leaves. To the best of our knowledge this is the first report about the structural changes in roots and leaves induced by both types of stress for Alamo switchgrass cultivar during the seedling stage.

The aims of this study were (i) to study the germination and seedling growth of switchgrass under salinity and water stress and (ii) to describe the morpho-anatomical responses of the roots and leaves in the seedlings to these stresses.

Materials and Methods

Plant material

Switchgrass seeds cv. Alamo were hand harvested in November 2013, from adult plants grown at the Experimental Fields Service of the University of Barcelona (41°23′ N; 2°7′ E). Seeds were stored in paper bags at room temperature (22 ± 2 °C) and dry conditions (40 ± 5 % relative humidity (RH)).

Seed germination assays

Seeds were surface disinfected in 5 % sodium hypochlorite solution for 15 min and rinsed three times for 5 min using sterile distilled water. The germination assays were performed in sterilized 9-cm diameter Petri dishes. Seeds were sown on two layers of sterilized filter papers (Prat Dumas, Couze-St-Front, France) moistened with 2.5 mL of test solutions. Fresh test solutions were added weekly to ensure keeping constant experiment conditions. Dishes were sealed with Parafilm® (Bemis Company Inc., Neenah, Wisconsin, USA) to prevent evaporation and randomly placed in a light and temperature controlled incubator (ASL Ibercex, Madrid, Spain). Light was provided by cool white fluorescent lamps (OSRAM L36W/840, Munich, Germany). Light intensity was measured using a LI-COR quantum/meter (LI-188 B) giving a 76 ± 5.9 $\mu mol \cdot m^{-2} \cdot s^{-1}$ PAR. The germination assay was carried out following ISTA rules [19] for switchgrass. Seeds were moist prechilled for 7 days at 5 ºC and then were set to germinate at 30/15 ºC (light/dark) and 8/16 h photoperiod.

The number of germinated seeds were recorded daily for 28 days. Germinated seeds were scored and removed from the dish as soon as the roots protruded 2 mm through the pericarp. After each score, the dishes were re-sealed and randomly distributed in the germination chamber to avoid any positioning effects. Three germination assays were carried out yielding similar results; only data from third test is shown.

Germination was tested using sodium chloride (NaCl) and polyethylene glycol 8000 (PEG-8000) aqueous solutions prepared according to Sosa *et al.* [20] and Michel [21] in order to simulate salinity and water stresses. Three different water potentials (Ψ_w) were prepared for each NaCl and PEG solution (-0.8, -1.0 and -1.2 MPa). The saline solutions had equivalent concentrations of 200, 250 and 300 mM and an electrical conductivity (EC) of 18.5, 23.3 and 30.2 $dS \cdot m^{-1}$, respectively. The PEG solutions had a 24.46, 28.66 and 31.76 w/v concentrations (0.2546, 0.2866 and 0.3176 g PEG/g H_2O), respectively. Distilled water was used as control (0.0 MPa *C*).

Experiments were arranged in a completely randomized design. For each treatment, eight replicates of one–hundred seeds were set up. Four replicates were used to evaluate germination and four to evaluate seedling growth (see *Morphological seedling growth parameters and light microscopy* section below).

After the germination test (28 days), the non-germinated seeds were subjected to a 7 days recovery period to determine their viability and possible toxic effects of the solutions during germination. Thus, the seeds were transferred to Petri dishes with distilled water for 7 days under the same temperature and light regimes.

Germination data analysis

The Final Germination Percentage (FGP) was calculated using the following equation:

$$\text{FGP}\ (\%) = \frac{\text{Ng}}{\text{Nt}} \text{x}\ 100$$

where, Ng is the number of germinated seeds and Nt is the total number of seeds.

The time to obtain 50 % germination (t_{50}) was calculated according to the following formula modified by Farooq *et al.* [22]:

$$t50\ (days) = Ti\ \frac{\left(\frac{N}{2} - Ni\right)(Tj - Ti)}{Nj - Ni}$$

where, N is the final number of germinated seeds and Ni and Nj are the total number of seeds germinated in adjacent counts at time Ti and Tj respectively, when Ni < N2 < Nj .

The germination rate (GR) was estimated using a Modified Timson's Index:

$$\text{Germination rate} = \sum \frac{\text{G}}{\text{t}}$$

where G is the percentage of seed germination at 2-day intervals and t is the total germination period [23].

Morphological seedling growth parameters and light microscopy

Seeds were moist prechilled for 7 days at 5 ºC and germinated at 30/15 ºC (light/dark) with a 8/16 h photoperiod, according to ISTA guidelines [19]. Four Petri dishes containing 100 seeds were used for each treatment. Seedling growth was evaluated at 10, 20 and 28 days after sowing (DAS) seeds in 0.0 MPa control, NaCl and PEG 8000 at -0.8, -1.0 and -1.2 MPa solutions. For each treatment and time, four replicates of 20 randomly selected seedlings were taken from each Petri dish. Root length (RL) and aerial part length (AL) were measured using a calliper.

Vigor index (VI) was calculated using the following equation:

$$VI = (lroot + laerial)\ FGP$$

where lroot is the root length (mm) and laerial is the length of the aerial part (mm). This index was calculated using the length of the root and aerial part of each seedling (at 28 DAS) and the FGP of seeds subjected to the same treatment [24].

Root and first leaf samples of seedlings growing in control and -0.8 MPa NaCl and PEG solutions were collected at the end of the experiment (28 DAS). The samples were vacuum-infiltrated with 2.5 % (v/v) glutaraldehyde and 2 % (w/v) paraformaldehyde in 0.1 M sodium phosphate buffer (pH 7.4) and fixed for 24 - 48 h at 4 ºC. After four rinses in phosphate buffer (10 min, 4 ºC) samples were post-fixed in 1 % (w/v) osmium tetroxide (OsO_4) for 12 to 16 h at 4 ºC, dehydrated in an acetone-propylene oxide series and embedded in Spurr's epoxy resin [25]. Blocks were trimmed into pyramids using a pyramitome (TM60, Reichert). The root and leaf cross-sections (1 µm thickness) were obtained using a Leica Ultracut E ultramicrotome (Leica Microsystems AG, Germany). The root cross-sections were obtained 5 mm above the root tip, while the leaf cross-sections were obtained from the middle-portion of the leaf. The sections were stained with methylene blue 1 % (w/v) and examined using an Olympus BX50 light microscope (Olympus Corporation, Japan). Pictures were capture with an Olympus SC30 camera (Olympus Corporation, Japan). Images at magnifications of 20X and 40X were analysed using Image J (Image Processing and Analysis in Java). Measurements were conducted on three biological replicates (three cross-sections per sample). The total cross-section area, the central cylinder area, and the cross-section area of the protoxylem and metaxylem vessels plus the wall thicknesses of the protoxylem and metaxylem vessels and the inner wall of the endodermis thickness were measured in the root. The total leaf, mesophyll, epidermis and cuticle thickness, the number and area of bulliform cells per group, and the vascular bundle area were recorded in the leaf.

Statistical Analysis

The effects of NaCl and PEG on FGP, t_{50}, GR, RL, AL and VI were analysed using one-way ANOVA (with 7 treatment levels: 0.0 MPa control, -0.8 MPa NaCl, -1.0 MPa, -1.2 NaCl MPa, -0.8 MPa PEG, -1.0 MPa PEG and -1.2 MPa PEG). The morpho-anatomical parameters in the root and leaf were compared using

one-way ANOVA (with 3 treatment levels: 0.0 MPa control, -0.8 MPa NaCl and -0.8 MPa PEG). Differences between groups were evaluated using the Duncan test with a significance level of 5 %. The SPSS 20.0 software package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis of results.
The parameters measured were plotted using SigmaPlot (version 11.0, Systat Software Inc., Richmond, California, USA).

Results

Germination under salinity and water stress conditions

Seeds germinated in distilled water (0.0 MPa Control) showed a 96.5 FGP at 28 DAS (Fig. 1) and reached t_{50} in 3 DAS (Table 1). Salinity and water stress inhibited germination, decreased germination rates and delayed the onset of germination in a dose-dependent manner (Fig. 1, 2 and Table 1).

At 28 DAS, no significant differences were observed on the FGP at -0.8 and -1.0 MPa NaCl, when compared to the control (Fig. 1), although the GR decreased, being statistically different for each NaCl concentration (Fig. 2, Table 1). The t_{50} increased from 3 days for control to 6 and 11 DAS for -0.8 and -1.0 MPa NaCl, respectively (Table 1). Furthermore, a significant inhibition of FGP occurred under -1.2 MPa NaCl (Fig. 1) which also significantly decreased the GR and t_{50} was achieved 17 DAS (Fig. 2, Table 1).

The FGP at -0.8 MPa PEG showed no statistical differences when compared to the control and -0.8 or -1.0 MPa NaCl treated seeds (Fig. 1). However, the GR was significantly lower than the control and, in addition, t_{50} was reached on the 8th DAS, five days later than the control (Fig. 2, Table 1). In comparison, -1.0 and -1.2 MPa PEG significantly inhibited the FGP (70 % and 46 %, respectively) and decreased the GR (Fig. 1, Table 1). The t_{50} was reached at 20 DAS for -1.0 MPa PEG, while for the -1.2 MPa PEG treatment, the t_{50} could not be stated, because it was not reached the 50% germination at the end of the assay (Table 1). The FGP, t_{50} and GR did not vary significantly between -1.2 MPa NaCl and -1.0 MPa PEG treatments (Fig.1-Table 1).

Most of the un-germinated seeds from the NaCl and PEG treatments showed a recovery when transferring them to optimal conditions for one week. The FGP of -1.2 MPa NaCl treatment increased only from 75 % to 86 %, while the -1.0 and -1.2 MPa PEG treatments increased the FGP from 70 % and 46 %, respectively, to 90 % each in only 2 days (Fig. 2).

Seedling growth under salinity and water stress conditions

In the control condition (28 DAS), the root and aerial part length reached 14.46± 1.42 and 27.60 ± 2.10 mm, respectively (Fig. 3, Table 1). Both salinity and water stress treatments caused a significant length reduction, being even stronger in the aerial parts than in the roots (Fig. 3, Table 1). At -0.8 MPa NaCl, the RL was significantly reduced by 35 %, while at -1.0 and -1.2 MPa NaCl the reduction in RL was 77 % and 87 %, respectively (Fig. 3a, Table 1). A similar behaviour was observed in the aerial part growth, where AL was significantly reduced by 40 % at -0.8 MPa NaCl and by 85 % and 90 % at -1.0 and -1.2 MPa NaCl, respectively (Fig. 3b, Table 1).

In water stress conditions, -0.8 and -1.0 MPa PEG significantly reduced the RL by 51 % and 66 %, while -1.2 MPa PEG significantly reduced the RL by 96 % (Fig. 3a, Table 1). The aerial part growth was more affected by water stress since the -0.8 and -1.0 MPa PEG treatments resulted in significant reductions of 76

% and 96 %, respectively, when compared to the control. Furthermore, the -1.2 MPa PEG treatment totally inhibited AL growth (Fig. 3b, Table 1).

Seedlings under control conditions had higher vigor index (VI) values. The VI decreased significantly at potentials of -0.8, -1.0 and -1.2 MPa (Fig. 4, Table 1). Finally, we observed that seedlings grown at -0.8 MPa NaCl had significantly higher VI when compared to all other treatments, although having significantly lowert VI than the control (Fig.4).

Root and leaf morpho-anatomical responses under salinity and water stress conditions

Seedlings showed a 49629 ± 2955 μm^2 root cross-sectional area in the control (0.0MPa) (Table 2). Furthermore, the unistratified rhizodermis showed isodiametric thin-walled cells, lacked cuticle, and possessed trichoblasts. At 5 mm above the root tip (cutting height), the cortex, comprising five cell layers, did not show an exodermis although it had developed four layers of polyhedral parenchymal thin-walled cells, with intercellular spaces. Furthermore, the innermost layer, the endodermis, showed slight thickenings of 0.85 ± 0.07 µm in the inner tangential and radial walls (Table 2), which masked the Casparian strips. In addition, an unistratified pericycle with periclinal divisions opposite the five poles of protoxylem alternating with the phloem strands was identified in the vascular cylinder. Finally, the centripetal xylem differentiation showed protoxylem vessels with a cross-section area of 57.6 ± 2.3 μm^2 and a wall thickness of 0.8 ± 0.1 µm (Table 2), and a metaxylem vessel at the root centre with a cross-section area of 781.5 ± 72.9 μm^2 and a wall thickness of 0.47 ± 0.09 µm (Fig. 5a, Table 2).

Anatomical changes were observed under stress conditions, mainly in the cortex and the central cylinder (Fig. 5c and e). Under salinity stress (-0.8 MPa NaCl), the root cross-section area decreased significantly (36 %) in comparison with values found in control seedlings (Table 2). This treatment had no effect on the cross-section area of the central cylinder but affected the cortex dramatically because it induced the formation of aerenchyma due to a severe collapse of cortical cells (Fig. 5c). Furthermore, the endodermis showed a significant increase in U-shaped cell wall thickenings compared to the control (1.29 ±0.06 µm *versus* 0.85 ±0.07 µm) (Fig. 5a and c, Table 2). The salinity treatments also significantly increased both the cross-section area (37 %) and the wall thickness (90 %) of protoxylem vessels, however, it significantly decreased (by about 50 %) the cross-section area of the metaxylem vessels and the wall thickness increased about 148 % relative to control seedlings (Table 2). In turn, the -0.8 MPa PEG treatment dramatically reduced both the root and the central cylinder cross-section areas by approximately 45 % relative to the values found in control seedlings. A significant increase in the thickness of the endodermis was also observed in PEG treatment that resembled the observations made under salinity stress (Fig. 5e, Table 2). The cross-section areas of the protoxylem (0.86 ± 0.14 μm^2) and the metaxylem (298.1 ± 42.5 μm^2) vessels were significantly reduced under water stress in comparison to the values of control under salinity stress treatments (Table 2). In addition, PEG significantly reduced the protoxylem and metaxylem wall thicknesses in comparison to salinity stress, although no perceptible changes were observed in this parameter when compared to the control seedlings (Table 2).

The anatomical analysis of the first leaf indicated a typical Kranz mesophyll of C_4 NAD-ME species. Control leaves showed a total thickness of 68.9 ± 8.0 µm (Table 2). The epidermal layers (covered by the cuticle) of the adaxial and abaxial surfaces were characterized by elliptical fundamental cells; and the

stomatal complexes in both surfaces indicated an amphistomatic condition (Fig. 5b, d and f). The adaxial epidermis was thicker than the abaxial epidermis (7.3 ±3.1 versus 5.0 ±1.9 µm), whereas the cuticle thickness in both surfaces was similar (0.35 ± 0.15 and 0.48 ± 0.21 µm) (Table 2). Sets of colourless epidermal cells known as motor or bulliform cells were evident at the intercostal regions of the adaxial epidermis (Fig. 5b). They were arranged in groups of 4-5 cells (fan-shaped) occupying an area of 1348.5 ± 210.0 μm^2 (Fig. 5b). First order-vascular bundles, elliptical in outline, were observed in the centre of the blade, with 3-4 mesophyll cells between the adjacent secondary and tertiary bundles. Between the vascular system and the dermal system, the fundamental system (mesophyll) showed a thickness of 55.7 ± 4.1 µm (Table 2). It was characterized by the presence of an outer layer of radial chlorenchyma that overlapped the inner layer, or Kranz sheath, and the innermost "mestome sheath" surrounding the vascular bundle, which occupied an area of 4375.5 ± 119.8 μm^2 (Table 2). Besides that, islets formed by 15-20 sclerenchyma cells at the ends of the limbs, and girders in the costal zones associated with the first and second-order vascular bundles (opposite the Kranz bundle sheath) were observed (Fig. 5b, d and f). Under salinity stress, the total thicknesses of the leaf and the mesophyll were increased by 36 % and a 40 % respectively, and the cuticle thicknesses of both the adaxial and abaxial epidermis were increased approximately two-fold in comparison to control seedlings (Table 2). Similar changes were induced in the PEG treatment where the total thickness of the leaf and the mesophyll thickness increased by 50 % and 40 %, respectively, in comparison to the values observed in control seedlings (Table 2). The cuticle thickness in the adaxial epidermis was increased by about 70 % and the cuticle thickness in the abaxial epidermis was increased about two-fold with respect to the control (Table 2). No significant differences between the thickness of either the adaxial or the abaxial epidermises, the number and area of bulliform cells or the vascular bundle area were found in leaves of control seedlings control and those submitted to salinity or water stress (Table 2).

Discussion

Our results demonstrated that Alamo switchgrass was more tolerant to salinity than water stress, both at the germination stage and at the seedling developmental phase. The specific morpho-anatomical changes observed in seedlings under salinity and water stress might be considered an adaptive strategy in this environmental conditions [39, 62].

Effects of stress conditions on germination

In our experimental conditions, salinity stress induced by -0.8 MPa NaCl (200 mM) and -1.0 MPa NaCl (250 mM) did not reduce the FPG significantly, but decreased the GR. In contrast, the reduction in FPG and GR was evident at -1.2 MPa NaCl (300 mM (Fig. 1, Table 1). The progressive delay in germination associated to the lower GR induced by NaCl (Table 1) might be due to an osmotic effect that provokes a decrease in water uptake, which in turn makes it difficult to reach the minimal hydration level for successful seed germination [4]. A previous study has reported that 1 % NaCl (170 mM) significantly decreased Alamo cultivar seed germination from 82 % in the control to 36 % [12]. Recent studies have found a strong salt-tolerance variation among switchgrass cultivars [26]. Nevertheless, these differences in the threshold of salinity for a significant reduction in germination could not onlybe dep3endent on genotype, but also on the experimental conditions [27]. As an example, seed germination of *Panicum turgidum* has been reported

as significantly reduced by salinity levels above 50 mM and this effect was more pronounced at 20-30 ºC than at 15-25 ºC [28].

Liu *et al.* [17] classified Alamo switchgrass as salt tolerant based on physiological parameters of 20-month-old plants after 24 days of salt stress treatment (250 mM NaCl). Our results agree with this classification because in our experimental conditions this cultivar could even germinate at -1.2 MPa NaCl. The electrical conductivity (30.2 $dS \cdot m^{-1}$) of a -1.2 MPa NaCl solution represents a high concentration of ions (Na^+ and Cl^-) only tolerated by halophyte species such as quinoa (*Chenopodium quinoa)* [29], common name (*Limonium stocksii)* and common name (*Suaeda fruticose)* [30]. Interestingly, not all of the non-germinated seeds treated with -1.2 MPa NaCl germinated when transferred to optimal conditions (Fig. 2). This suggests that this level of salinity inhibits germination due to both osmotic and ion-toxicity effects [6].

Sun *et al.* [12] reported that water stress simulated by the addition of PEG (1 – 7.5 % (w/v)), did not have a great effect on *in vitro* germination of Alamo cultivar in comparison to the inhibitory effect of NaCl (0.1 – 1.0 %). Our results demonstrated that the inhibitory effects of PEG (-1.0 and -1.2 MPa) on both the onset, the FPG, and the GR were more detrimental than those induced by isosmotic concentrations of NaCl. This discrepancy between the results of this study and previous works could be due to differences in experimental conditions such as: i) a different plant culture system (filter paper germination assay versus *in vitro* culture); ii) the method of PEG application (in aqueous solution *versus* in nutrient media); iii) the concentrations of the osmotic solutions tested (25-31 % w/v in our experiments *versus* 1-7.5 % w/v); iv) the frequency of changes of the osmotic solutions and v) the temperature regimes (15/30ºC *versus* room temperature), among others. The strongest germination inhibition effectiveness of PEG in comparison to NaCl isosmotic solutions has been found in other species like durum wheat (*Triticum durum)* [3], sunflower (*Helianthus annuus)* [31] and rice *(Oryza sativa)* [32]. The inhibitory effect of PEG, which is a non-ionic, water-soluble and non-penetrating solute, could be attributed to an osmotic stress that decreases seed hydration and results in lower water absorption, as stated by Murillo and Amador in common name (*Vigna unguiculate)* [33]. In our experimental conditions, the transfer of non-germinated seeds from PEG solutions to distilled water resulted in 100 % germination recovery at all osmotic potential levels (Fig. 2). This suggests that PEG only has an osmotic effect and does not produce toxicity or deleterious effects, which is in accordance with Murillo-Amador [33].

Effects of stress conditions on seedling growth and anatomical features

Our results showed that NaCl and PEG in the range of -0.8 to -1.2 MPa significantly reduced both the RL and AL. Although, Alamo seedlings looked healthy with no signs of injury, its emergency capacity or vigor was diminished in unfavourable environmental conditions. Thus, early seedling growth was more sensitive than germination to both stress treatments. Early seedling growth was affected by a water potential of -0.8 MPa that also reduced the root diameter near the root tip. Similar differences in sensitivity have been documented in other species like quino [34] and common name (*Echinochloa crusgalli)* [35]. The reduction in plant growth detected in Alamo seedlings under saline conditions and water stress (Fig 3a and b) is a general phenomenon in plants under stress [5], although it manifests differently in different organs. In general, roots are the first organs that respond under salt stress and RL is commonly affected than AL. This

has been reported in *Trailblazer*, *Cave-in-Rock*, *Blackwell* and *Kanlow* switchgrass cultivars under salt – alkaline stress [11]. However, in our study, the inhibition of length was more prominent in aerial parts than in roots, as reported in sunflower [36], common name (*Carthamus tinctorius)* [37] and canola (*Brassica juncea)* [3]. For both RL and AL, PEG functioned as a stronger growth inhibitor than NaCl, at isosmotic concentrations as reported in common name [37], durum wheat [33] and common name (*Vigna unguiculate)* [38]. These results suggest that osmotic dehydration is the main factor affecting Alamo seedling growth. The relatively minor effects of NaCl compared to PEG at isosmotic concentrations could be due to differences in the membrane reflection coefficient between the osmotic species, which is lower for NaCl. As a result, the osmotic adjustment of seedlings subjected to salt stress should be faster and more efficient than in PEG isosmotic solutions. Thus, the salt stressed seedlings should experience lower water stress than those growing in PEG solutions as reported by Ayala-Cordero *et al.* [39]. An alternative observation was documented by Ji *et al.* [40] in wheat who demonstrated that low levels of PEG-mediated osmotic stress not only inhibited the roots growth, but also the premature differentiation of the root apical meristem (RAM) and stimulated the development of long and extensive root hair growth and the outgrowth of lateral roots.

Our results reveal that in addition to inhibiting RL and AL, -0.8 MPa NaCl and PEG induced other morpho-anatomical changes in roots and the first leaf. These effects are more apparent at the root anatomy, perhaps because they are the first organs in contact with the NaCl and PEG solutions, and this would be an adaptive advantage under stress conditions.

At the root level, the reduction in cross-section area has been proposed as a trait for increasing root hydraulic conductivity and plant acquisition of water and productivity [41]. Our results agree with those observed in others species such as common name (*Chloris gayana)* [39], common name (*Prosopis strombulifera)* [41], common name (*Cynodon dactylon)* [42] and cotton [43] under salinity and wheat (*Triticum aestivum)* [3] and rice [38] under water deficit. In our study, the salinity decreased the root cross-section area mainly due to cortex width reduction (Table 2). The cortex is associated with the development of aerenchyma that may facilitate gas exchange and the radial transport of salts, nutrients and water through the root as reported for grass species like common name (*Imperata cylindrica)* [44], common name (*Sporobolus arabicus)* [45] and common name *(Aeluropus lagopoides)* [39]. Additionally, the formation of aerenchyma tends to prevent oxygen deficiency and thus supports uninterrupted xylem transport [46]. Under water stress, the reduction in root cross-section area was due to a change in both cortex width and the vascular cylinder. A decrease in the vascular cylinder has been observed in other systems under salinity and could be associated with a reduction in root hydraulic conductivity and the aerial part development [47]. Thus, the differential inhibition of the aerial part length induced by PEG with respect to isosmotic concentrations of NaCl (Fig. 3) could be explained to a greater inhibition of root water transport by PEG treated seeds in comparison to NaCl, which has also been documented in the leaf development of hydroponically grown maize seedlings [39]. The results presented in the current work may not reflect the salt- or water stress-induced disturbances at the organ level as described by Ceccoli *et al.* [39]. It is worth noting that our anatomical observations were performed in a zone near the root tip, which is just a small part of the entire root system. Further work will be required to obtain information at different distances from the root tip.

The remarkable reduction in cross-section area of metaxylem vessels observed in roots of switchgrass seedlings grown under NaCl and PEG stress (Table 2) matched the findings of Akram *et al.* [48] in wheat under salinity stress and in common name [49] under drought. This response could constitute an efficient strategy to transport water by lowering the risk of embolisms (protecting the xylem against cavitation: a critical adaptation because narrow vessels are less prone to damage caused by embolism) and increasing water –flow resistance [50].

Several lines of evidence have demonstrated that salt stress and drought affect the width of Casparian strips and induce suberification of the endodermis in many plants species, suggesting a strategy to block the entry of toxic elements into the root vasculature and to prevent water and oxygen loss [51, 52]. Indeed, our results indicate an increase in the thickness of the cell wall endodermis that match those reported in *Gossypium hirsutum* [53], *Oriza sativa* [54] and the facultative halophyte *Aeluropus littoralis* [55] under salinity conditions. Also, they match the findings of Vasellati *et al.* [50] who reported the formation of a conspicuous endodermis in *Paspalum dilatatum* under drought stress. Contrary to our expectations, neither the NaCl nor the PEG treatments induced the formation of exodermis closer the root tip, despite its being a common response to water stress conditions [56]. However, we cannot exclude its formation at several centimetres from the tip, as mentioned by Enstone *et al.* [56]. Thus, our results revealed that near the root tip the endodermis might limit the free apoplastic diffusion of sodium ions into the vascular stream. This could lead to the accumulation of sodium ions in tissues peripheral to the endodermis.

Structural changes in the first leaf were similar under both stress situations and they mainly affected the thickness of the mesophyll and the cuticles. The increase in mesophyll thickness match the findings in *Mentha x piperita* plants treated with PEG 100 $g.L^{-1}$ [57] and in most plants [58] under salinity stress but is opposite to observations made in *Cynodon dactylon* [42]. At the epidermal level, both stresses increased the thickness of the cuticle, which is an effective mechanism against water loss under stress [59]. However, no changes were induced in either the number or the area of bulliform cells, unlike reports from other species such as *Imperata cylindrica* [60], *Sporobolus arabicus* [61] and *Aeluropus lagopoides* [38] under salinity conditions. Under our NaCl salinity conditions, epidermal salt glands were not observed, which is contrary to the findings in the upland switchgrass Cave-in-Rock cultivar in green house experiments [26]. This anatomical feature is one among the diverse mechanisms that may be used to handle salt stress, and our results suggest that the Alamo cultivar follows different strategies to cope with salinity. Kim *et al.* [15] reported the existence of multiple response modes to salt stress among switchgrass populations. To our knowledge, there is no information about the mechanisms involved in drought and salt tolerance in Alamo. For this reason, our study could be used as the basis for future investigations to clarify the salinity and drought tolerance mechanisms in this cultivar.

Conclusions

Alamo switchgrass cultivar is more tolerant to salinity than drought stress. In general, this study revealed that Ψ_w of -0.8 MPa affected only the germination rate, whereas a Ψ_w of -1.0 and -1.2 MPa affected the FGP and germination rate under both salinity (NaCl) and water stress (PEG) treatments. Nevertheless, the PEG treatments had a greater effect on the FGP, GR and seedling growth when compared to the NaCl

treatments at a given Ψ_w. This behaviour would be related to the water uptake, which is reduced in response to PEG while salt stress effects are probably linked to ion accumulation.

The most important anatomical changes due to salinity or drought stress occurred in the roots, which is the first organ to develop and experience salt excess or water scarcity. Furthermore, the reduction in the root cross-section area, aerenchyma formation in the cortex under salinity and the thickening of the endodermis walls could help avoid solute uptake and dehydration during early growth stages and thus could increase survival and tolerance of this species under salinity stress conditions.

Acknowledgements

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References

1. Duclos D, Ray D, Johnson D, Taylor A (2013) Investigating seed dormancy in switchgrass (*Panicum virgatum* L.): Understanding the physiology and mechanisms of coat-imposed seed dormancy. Ind Crops Prod 45:377–387. doi: 10.1016/j.indcrop.2013.01.005
2. Hashemi M, Sadeghpour A (2013) Establishment and Production of Switchgrass Grown for Combustion : A Review. Int J Plant Biol Res 1:1002.
3. Almansouri M, Kiner J, Lutts S (2001) Effect of salt and osmotic stresses on germination in durum wheat (*Triticum durum* Desf.). Plant Soil 231:243–254. doi: 10.1023/A:1010378409663
4. Wang W, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. Planta 218:1–14. doi: 10.1007/s00425-003-1105-5
5. Munns R (2002) Comparative physiology of salt and water stress. Plant Cell Environ 25:239–250. doi: 10.1046/j.0016-8025.2001.00808.x
6. Munns R, Tester M (2008) Mechanisms of salinity tolerance. Annu Rev Plant Biol 59:651–81. doi: 10.1146/annurev.arplant.59.032607.092911
7. Llanes A, Andrade A, Masciarelli O, et al (2016) Drought and salinity alter endogenous hormonal profiles at the seed germination phase. Seed Sci Res 26:1–13. doi: 10.1017/S0960258515000331
8. Kramer P, Boyer J (1995) Water relations of plants and soils. Elsevier Academic Press, San Diego, California
9. Quinn LD, Straker KC, Guo J, et al (2015) Stress-Tolerant Feedstocks for Sustainable Bioenergy Production on Marginal Land. Bioenergy Res 8:1081–1100. doi: 10.1007/s12155-014-9557-y
10. Aimar D, Calafat M, Andrade a M (2011) Drought Tolerance and Stress Hormones : From Model Organisms to Forage Crops. Plants Environ 272. doi: 10.5772/1958
11. Liu Y, Zhang X, Miao J, et al (2014) Evaluation of Salinity Tolerance and Genetic Diversity of Thirty-Three Switchgrass (Panicum virgatum) Populations. Bioenergy Res 7:1329–1342. doi: 10.1007/s12155-014-9466-0
12. Sun G, Stewart CN, Xiao P, Zhang B (2012) MicroRNA expression analysis in the cellulosic biofuel crop switchgrass (Panicum virgatum) under abiotic stress. PLoS One. doi: 10.1371/journal.pone.0032017
13. Liu Y, Wang Q, Zhang Y, et al (2014) Synergistic and Antagonistic Effects of Salinity and pH on Germination in Switchgrass (*Panicum virgatum* L.). PLoS One. doi: 10.1371/journal.pone.0085282
14. Liu Y, Zhang X, Miao J, et al (2014) Evaluation of Salinity Tolerance and Genetic Diversity of Thirty-Three Switchgrass (*Panicum virgatum*) Populations. Bioenergy Res 7:1329–1342. doi: 10.1007/s12155-014-9466-0
15. Kim J, Liu Y, Zhang X, et al (2016) Analysis of salt-induced physiological and proline changes in 46 switchgrass (*Panicum virgatum*) lines indicates multiple response modes. Plant Physiol Biochem 105:203–212. doi: 10.1016/j.plaphy.2016.04.020
16. Barney JN, Mann JJ, Kyser GB, et al (2009) Tolerance of switchgrass to extreme soil moisture stress: Ecological implications. Plant Sci 177:724–732. doi: 10.1016/j.plantsci.2009.09.003

17. Liu Y, Zhang X, Tran H, et al (2015) Assessment of drought tolerance of 49 switchgrass (Panicum virgatum) genotypes using physiological and morphological parameters. Biotechnol Biofuels 8:1–18. doi: 10.1186/s13068-015-0342-8
18. Stroup JA, Sanderson MA, Muir JP, et al (2003) Comparison of growth and performance in upland and lowland switchgrass types to water and nitrogen stress. Bioresour Technol 86:65–72. doi: 10.1016/S0960-8524(02)00102-5
19. International Seed Testing Association ISTA (2012) International rules for seed testing. Seed Sci Technol 21:288.
20. Sosa L, Llanes A, Reinoso H, et al (2005) Osmotic and Specific Ion Effects on the Germination of Prosopis strombulifera. Ann Bot 96:261–267. doi: 10.1093/aob/mci173
21. Michel B (1983) Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. Plant Physiol 72:66–70. doi: 10.1104/pp.72.1.66
22. Farooq M, Basra SMA, Ahmad N, Hafeez K (2005) Thermal Hardening: A New Seed Vigor Enhancement Tool in Rice. J Integr Plant Biol 47:187–193. doi: 10.1111/j.1744-7909.2005.00031.x
23. Khan MA, Ungar IA (1984) The Effect of Salinity and Temperature on the Germination of Polymorphic Seeds and Growth of *Atriplex triangularis* Willd. Am J Bot 71:481. doi: 10.2307/2443323
24. Fina BL, Lupo M, Dri N, et al (2016) Comparison of fluoride effects on germination and growth of Zea mays, Glycine max and *Sorghum vulgare*. J Sci Food Agric 96:3679–3687. doi: 10.1002/jsfa.7551
25. Spurr AR (1969) A low-viscosity epoxy resin embedding medium for electron microscopy. J Ultrastruct Res 26:31–43.
26. Kim SB, Rayburn AL, Voigt T, et al (2012) Salinity Effects on Germination and Plant Growth of Prairie Cordgrass and Switchgrass. Bioenergy Res 5:225–235. doi: 10.1007/s12155-011-9145-3
27. Carson MA, Bachle S, Morris AN (2016) Germination and Growth of Panicum virgatum Cultivars in a NaCl Gradient. In: Khan MA, Boër B, Ozturk M, et al (eds) Sabkha Ecosyst. Vol. V Am. Tasks Veg. Sci. Springer International Publishing, Switzerland, pp 287–297
28. Al-Khateeb SA (2006) Effect of salinity and temperature on germination, growth and ion relations of *Panicum turgidum* Forssk. Bioresour Technol 97:292–298. doi: 10.1016/j.biortech.2005.02.041
29. Panuccio M, Jacobsen S, Akhtar S, Muscolo A (2014) Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. AoB Plants. doi: 10.1093/aobpla/plu047
30. Hameed A, Rasheed A, Gul B, Khan MA (2014) Salinity inhibits seed germination of perennial halophytes *Limonium stocksii* and *Suaeda fruticosa* by reducing water uptake and ascorbate dependent antioxidant system. Environ Exp Bot 107:32–38. doi: 10.1016/j.envexpbot.2014.04.005
31. Luan Z, Xiao M, Zhou D, et al (2014) Effects of Salinity, Temperature, and Polyethylene Glycol on the Seed Germination of Sunflower (*Helianthus annuus* L.). Sci World J. doi: 10.1155/2014/170418

32. Alam M, Stuchbury T, Naylor R (2002) Effect of NaCl and PEG Induced Osmotic Potentials on Germination and Early Seedling Growth of Rice Cultivars Differing in Salt Tolerance. Pakistan J Biol Sci 5:1207–1210.
33. Murillo-Amador B, López-Aguilar R, Kaya C, et al (2002) Comparative Effects of NaCl and Polyethylene Glycol on Germination, Emergence and Seedling Growth of Cowpea. J Agron Crop Sci 188:235–247. doi: 10.1046/j.1439-037X.2002.00563.x
34. Panuccio MR, Jacobsen SE, Akhtar SS, Muscolo A (2014) Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. AoB Plants 6:1–18. doi: 10.1093/aobpla/plu047
35. Rahman M, Ungar I (1990) The effect of salinity on seed germination and seedling growth of *Echinochloa crusgalli*. Ohio J Sci 90:13–15.
36. Kaya MD, Okçu G, Atak M, et al (2006) Seed treatments to overcome salt and drought stress during germination in sunflower (*Helianthus annuus* L.). Eur J Agron 24:291–295. doi: 10.1016/j.eja.2005.08.001
37. Pandey M, Penna S (2017) Time course of physiological, biochemical, and gene expression changes under short-term salt stress in *Brassica juncea* L. Crop J 5:219–230. doi: 10.1016/j.cj.2016.08.002
38. Mokhberdoran F, Kalat Nabavi S, Haghighi Sadrabadi R (2009) Effect of Temperature, Iso-Osmotic concentration of NaCl and PEG agents on germination and some seedling growth yield in rice (*Oryza sativa* L.). Asian J Plant Sci 8:409–416.
39. Céccoli G, Ramos JC, Ortega LI, et al (2011) Salinity induced anatomical and morphological changes in *Chloris gayana* Kunth roots. Biocell 35:9–17.
40. Wasson AP, Richards RA, Chatrath R, et al (2012) Traits and selection strategies to improve root systems and water uptake in water-limited wheat crops. J Exp Bot 63:3485–3498. doi: 10.1093/jxb/ers111
41. Reinoso H, Sosa L, Ramírez L, Luna V (2004) Salt-induced changes in the vegetative anatomy of Prosopis strombulifera (Leguminosae). Can J Bot 82:618–628. doi: 10.1139/b04-040
42. Hameed M, Ashraf M, Naz N, Al-Qurany F (2010) Anatomical adaptations of *Cynodon dactylon* (L.) Pers. from the salt range Pakistan, to salinity stress. I. Root and stem anatomy. Pakistan J Bot 42:279–289.
43. Zhang L, Ma H, Chen T, et al (2014) Morphological and physiological responses of cotton (*Gossypium hirsutum* L.) plants to salinity. PLoS One. doi: 10.1371/journal.pone.0112807
44. van der Weele CM, Canny MJ, McCully ME (1996) Water in aerenchyma spaces in roots. A fast diffusion path for solutes. Plant Soil 184:131–141. doi: 10.1007/BF00029283
45. Naz N, Hameed M, Nawaz T, et al (2013) Structural adaptations in the desert halophyte *Aeluropus lagopoides* (Linn.) Trin. ex Thw. under high salinity. J Biol Res 19:150–164.
46. Gibbs J, Turner D, Armstrong W, et al (1998) Response to oxygen deficiency in primary maize roots. I. Development of oxygen deficiency in the stele reduces radial solute transport to the xylem. Aust J Plant Physiol 25:745–758.
47. Chazen O, Hartung W, Neumann PM (1995) The different effects of PEG 6000 and NaCI on leaf

development are associated with differential inhibition of root water transport. Plant Cell Environ 18:727–735. doi: 10.1111/j.1365-3040.1995.tb00575.x

48. Akram M, Akhtar S, Javed I-U-H, et al (2002) Anatomical Attributes of Different Wheat (Triticum aestivum) Accessions/Varieties to NaCl Salinity. Int J Agric Biol Int J Agri Biol 4:166–168.
49. Momayezi M, Zaharah R, Hanafi M (2012) The effects of cation ratios on root lamella suberization in rice (*Oryza sativa* L.) with contrasting salt tolerance. Int J Agron 2012:1–8. doi: 10.1155/2012/769196
50. Vasellati V, Oesterheld M, Medan D, Loreti J (2001) Effects of flooding and drought on the anatomy of *Paspalum dilatatum*. Ann Bot 88:355–360. doi: 10.1006/anbo.2001.1469
51. Doblas VG, Geldner N, Barberon M (2017) The endodermis, a tightly controlled barrier for nutrients. Curr Opin Plant Biol 39:136–143. doi: 10.1016/j.pbi.2017.06.010
52. Líška D, Martinka M, Kohanová J, Lux A (2016) Asymmetrical development of root endodermis and exodermis in reaction to abiotic stresses. Ann Bot 118:667–674. doi: 10.1093/aob/mcw047
53. Reinhardt DH, Rost TL (1995) On the correlation of primary root growth and tracheary element size and distance from the tip in cotton seedlings grown under salinity. Environ Exp Bot 35:575–588. doi: http://dx.doi.org/10.1016/0098-8472(95)00018-6
54. Fahn A, Cutler D (1992) Xerophytes. In: Borntraeger G (ed). Berlin, Stuttgart,Germany, p 176
55. Barzegargolchini B, Movafeghi A, Dehestani A, Mehrabanjoubani P (2017) Increased cell wall thickness of endodermis and protoxylem in *Aeluropus littoralis* roots under salinity: The role of LAC4 and PER64 genes. J Plant Physiol 218:127–134. doi: 10.1016/j.jplph.2017.08.002
56. Enstone DE, Peterson CA, Ma F (2002) Root Endodermis and Exodermis: Structure, Function, and Responses to the Environment. J Plant Growth Regul 21:335–351. doi: 10.1007/s00344-003-0002-2
57. Búfalo J, Rodrigues TM, de Almeida LFR, et al (2016) PEG-induced osmotic stress in *Mentha x piperita* L.: Structural features and metabolic responses. Plant Physiol Biochem 105:174–184. doi: 10.1016/j.plaphy.2016.04.009
58. Kozlowski TT (1997) Responses of woody plants to flooding and salinity. Tree Physiol 17:490. doi: https://doi.org/10.1093/treephys/17.7.490
59. Hameed M, Nawaz T, Ashraf M, et al (2013) Physioanatomical adaptations in response to salt stress in *Sporobolus arabicus* (Poaceae) from the Salt Range, Pakistan. Turk J Botany 37:715–724. doi: 10.3906/bot-1208-1
60. Hameed M, Ashraf M, Naz N, et al (2014) Physiological adaptative characteristics of *Imperata cylindrica* for salinity tolerance. Biologia (Bratisl) 69:1148–1156. doi: 10.2478/s11756-014-0417-1
61. Zhang H, Irving LJ, McGill C, et al (2010) The effects of salinity and osmotic stress on barley germination rate: sodium as an osmotic regulator. Ann Bot 106:1027–1035. doi: 10.1093/aob/mcq204
62. Ayala-Cordero G, Terrazas T, López-Mata L, Trejo C (2006) Morpho-anatomical changes and photosynthetic metabolism of *Stenocereus beneckei* seedlings under soil water deficit. J Exp Bot

57:3165–3174. doi: 10.1093/jxb/erl078

Figure 1

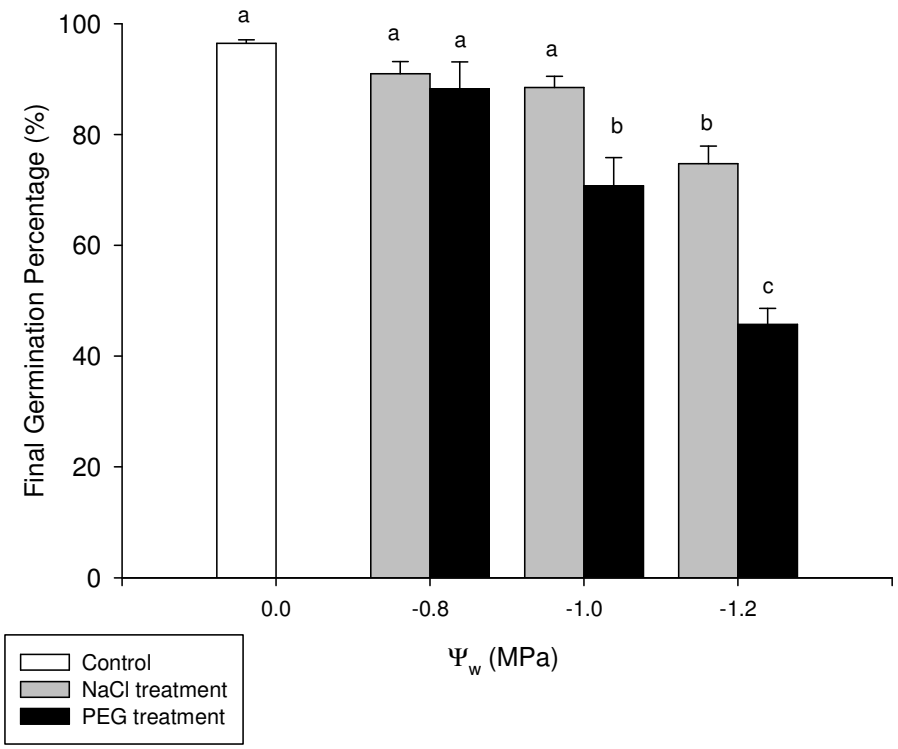

Fig. 1 Final germination percentage of Alamo switchgrass. The seeds germinated under different water potentials, salinity (NaCl) or water stress (PEG). Values represent the mean ± SE of four replicates. Different letters indicate significant differences ($P < 0.05$) between treatments according to Duncan test

Figure 2

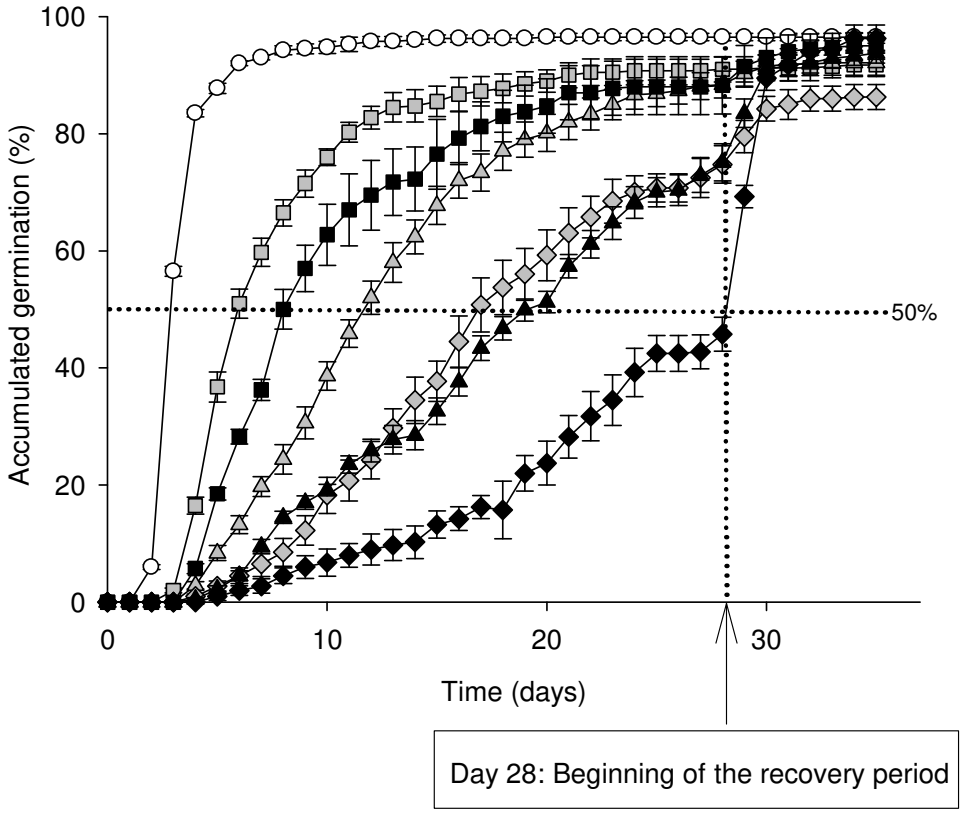

Fig. 2 Time courses of percentage germination of Alamo switchgrass in response to salinity (NaCl) and water stress (PEG). White, grey and black symbols correspond to control, NaCl and PEG treatment, respectively. Water potentials (Ψ_w): 0.0 MPa (○), -0.8 MPa (□), -1.0 MPa (Δ) and -1.2 MPa (◊). Horizontal dotted line indicates the 50 % germination. After 28 days, un-germinated seeds were transferred to distilled water to study the recovery of germination during 7 days under the same temperature and light regimes. Vertical dotted line (day 28) indicates the start recovery period. Values represent the mean ± SE (error bars) of four replicates

Figure 3

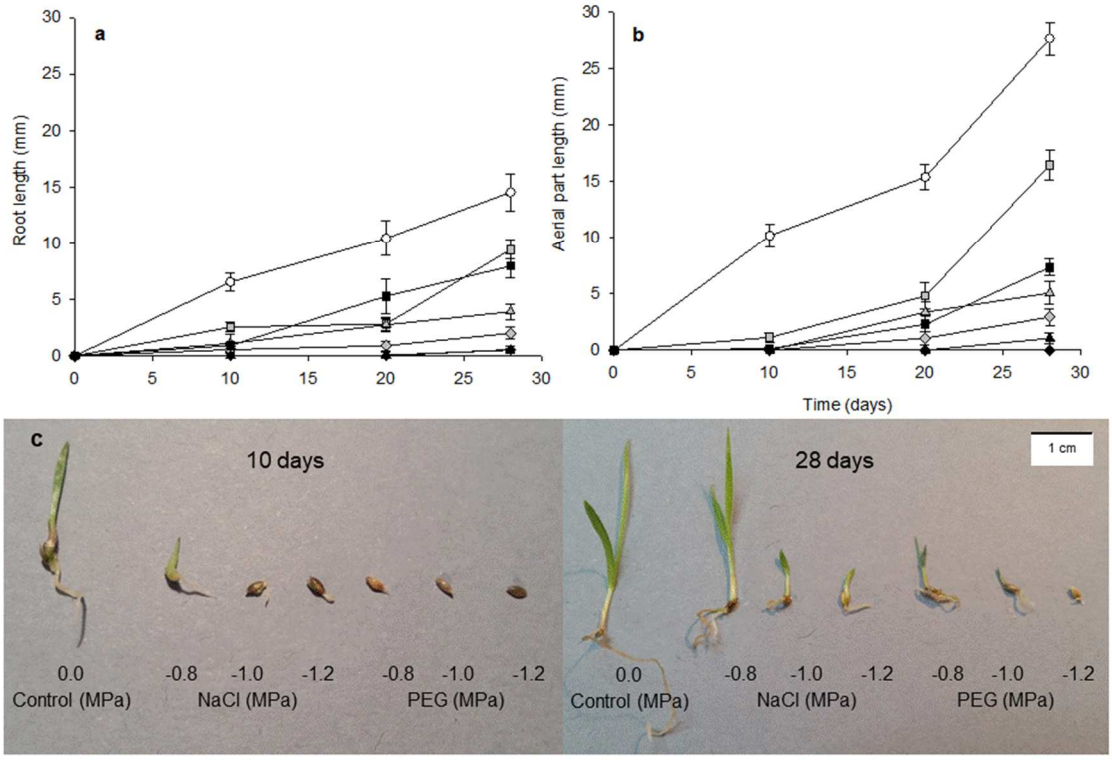

Fig. 3 Growth responses of Alamo switchgrass germinated under salinity (NaCl) or water stress (PEG). (a) Root length, (b) aerial part length and (c) seedlings at 10, 20 and 28 days after sowing (DAS). White, grey and black symbols correspond to control, NaCl and PEG treatment, respectively. Water potentials (Ψ_w): 0.0 MPa (○), -0.8 MPa (□), -1.0 MPa (Δ) and -1.2 MPa (◊). Values represent the mean ± SE of twenty replicates

Figure 4

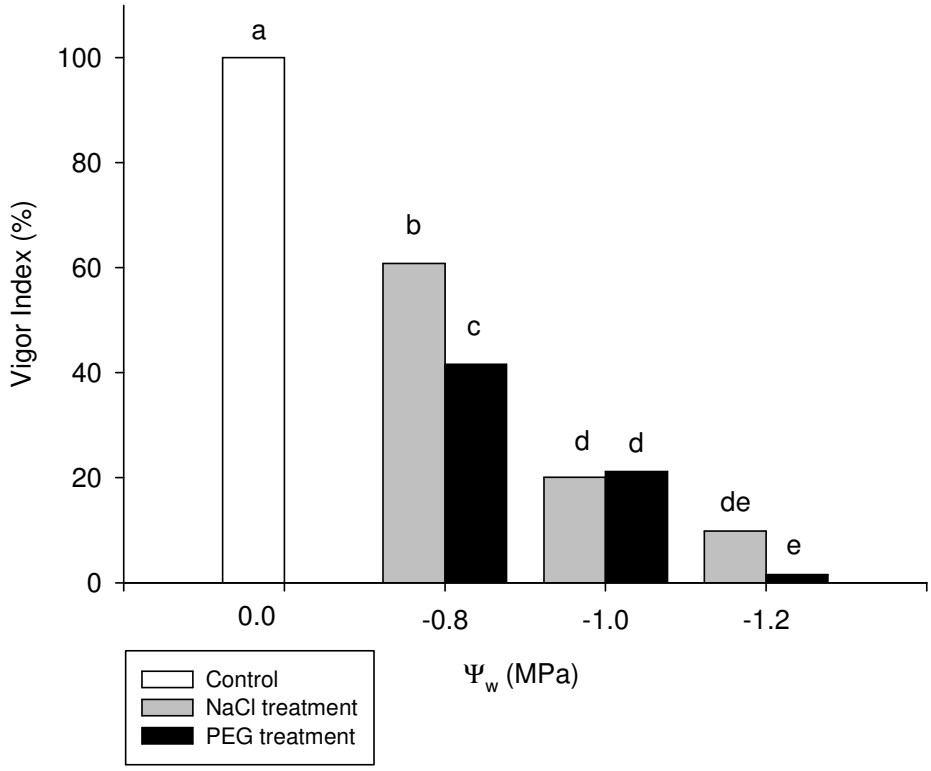

Fig. 4 Vigor index of Alamo switchgrass as a response of the germination and growth seedling under salinity (NaCl) or water stress (PEG), expressed as % of the control taken as 100 %. Different letters indicate significant differences ($P < 0.05$) between treatments according to Duncan test

Figure 5

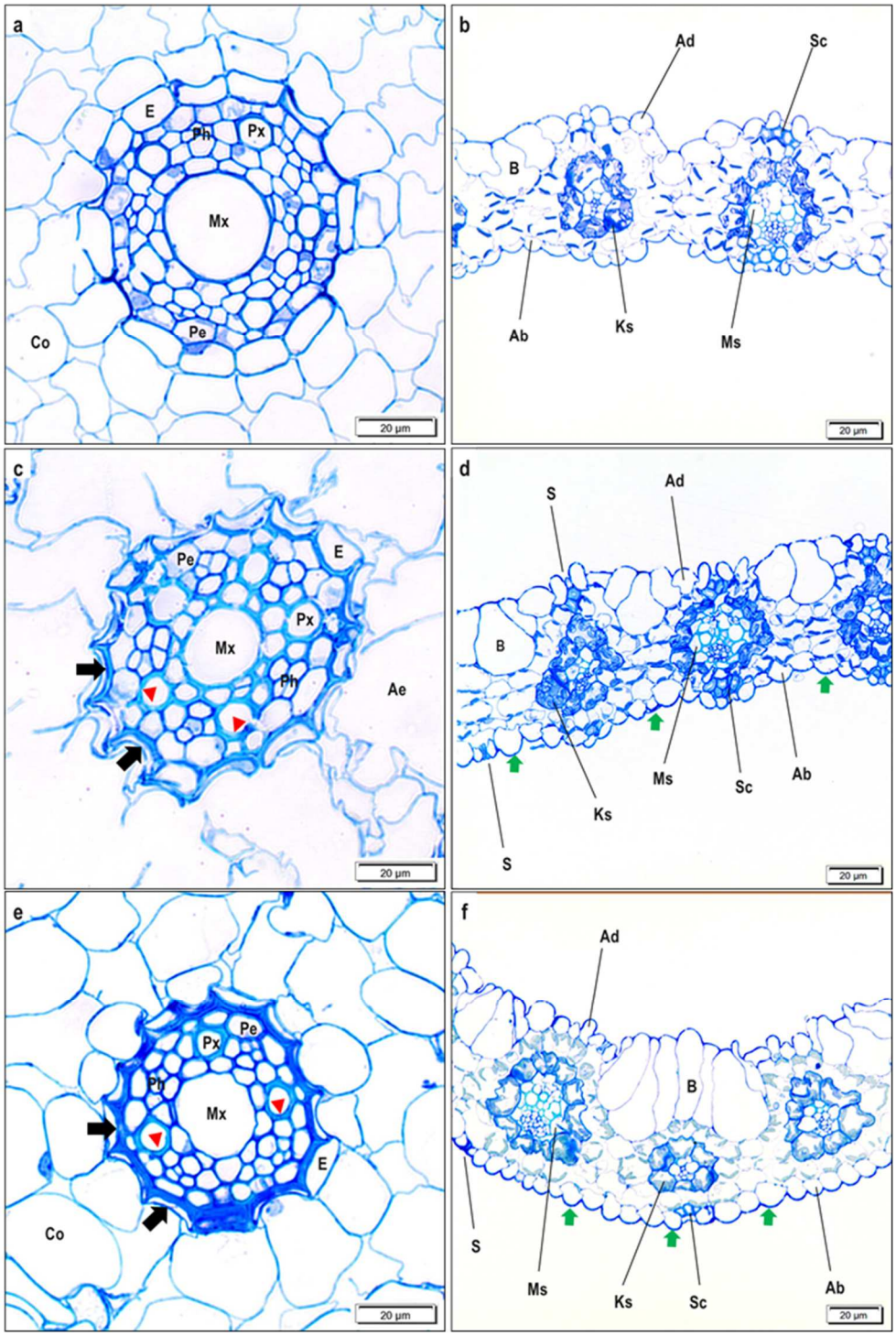

Fig. 5 Anatomical responses of Alamo switchgrass during early stage under salinity (NaCl) or water stress (PEG). 0.0 MPa control (a, b), -0.8 MPa NaCl (c, d) and -0.8 MPa PEG (e, f) treatments. Cross-sections of roots (a, c and e) showing the central cylinder and the inner cortex, u-shaped endodermis cell wall (black arrows), the protxylem walls (red arrows) under salinity (c) and water stress (e). Cross-sections of the first leaf (b, d and f) showing thickening of abaxial cuticle under salinity (d) and water stresses (f) (green

arrows). Ab, Abaxial epidermis; Ad, Adaxial epidermis; Ae, Aerenchyma; B, Bulliform cell; Co, Cortex; E, Endodermis; K, Kranz bundle sheath; Pe, Pericycle; Ph, Phloem; Ms, Mestome sheath; Mx, Metaxylem; Px, Protoxylem; S, Stomata complex; Sc, Sclerenchyma cells. Scale = 20 µm

Table 1 Response of t_{50}, GR, RL, AL and VI in Alamo switchgrass under salinity (NaCl) or water stress (PEG). Values represent the mean ± SE of four replicates to t_{50}, GR and of twenty replicates to RL, AL and VI. Different letters indicate significant differences ($P < 0.05$) between treatments according to Duncan test

Seed treatment	Ψ_w (MPa)	t_{50} (days)	GR	RL (mm)	AL (mm)	VI
Control	0.0	3±0.1e	87±0.5a	14.5±1.5a	27.6±2.1a	1658.0±150.0a
NaCl	-0.8	6±0.3d	70±1.6b	9.4±0.9b	16.4±1.3b	1008.6±77.9b
	-1.0	11±0.7b	53±1.3d	3.3±0.7de	4.3±1.0cd	332.8±63.1d
	-1.2	17±0.4a	37±2.5e	1.9±0.5e	2.8±0.7de	162.9±42.5de
PEG	-0.8	8±0.3c	62±3.7c	7.2±0.1bc	6.1±0.9c	690.2±102.9c
	-1.0	20±0.4a	33±1.7e	4.9±1.3cd	1.0±0.5e	351.3±90.2d
	-1.2	-	17±1.4f	0.6±0.3e	0.0±0.0e	25.4±14.8e

t_{50}: the time to obtain 50 % germination; **GR**: germination rate index; **RL**: Root Length, **AL**: Aerial part Length; **VI**: Vigor Index.

Table 2 Morpho-anatomical response of Alamo switchgrass during early stage under salinity (NaCl) or water stress (PEG). Values represent the mean ± SE of three replicates. Different letters indicate significant differences (P <0.05) between

	Seed treatments		
cal parameters	***C* (0.0 MPa)**	**NaCl (-0.8 MPa)**	**PEG (-0.8 M**
ss section			
ection area (μm^2)	49621.1 ±2954.9a	31629.8 ±2541.3b	21106.5 ±14
cylinder area (μm^2)	5673.6 ±530.7a	5130.2 ±476.7a	2693.5 ±19
all of the endodermis thickness (µm)	0.9 ±0.1b	1.3±0.1a	1.4 ±0.1
ection area of Protoxylem vessel (μm^2)	57.6 ±2.3b	79.0 ±9.5a	25.5 ±0.4
ylem vessel wall thickness (µm)	0.8 ±0.1b	1.5 ±0.3a	0.9 ±0.2
ection area of Metaxylem vessel (μm^2)	781.5 ±73.0a	408.0 ±16.2b	298.2 ±42
ylem vessel wall thickness (µm)	0.5 ±0.1b	1.2 ±0.2a	0.6 ±0.1
ss section			
hickness (µm)	68.9±8.0b	93.8±3.1ab	103.4±13.9
nyll thickness (µm)	55.7±4.2b	76.9±3.7ab	79.6±9.8
l epidermis thickness (µm)	7.4±3.1a	7.5±1.2a	10.9±1.8
l epidermis thickness (µm)	5.7±2.0a	7.7±1.4a	11.3±3.0
thickness of adaxial epidermis (µm)	0.4±0.2b	0.8±0.1a	0.6±0.1a
thickness of abaxial epidermis (µm)	0.5±0.2b	0.8±0.2a	0.9±0.1a
rm number of cells per group	4.0±0.6a	4.7±0.4a	4.7±0.4a
rm cell area per group (μm^2)	1348.5±210.0a	1954.1±520.9a	1294.8±45
ar bundle area* (μm^2)	4375.5±119.8a	3428.3±317.2a	3623.0±80

treatments according to Duncan test. * The vascular bundle area including: radiate chlorenquima + Kranz sheath