

Flooding tolerance of *Paspalum dilatatum* (Poaceae: Paniceae) from upland and lowland positions in a natural grassland

Federico P.O. Mollard^{a,*}, Gustavo G. Striker^a, Edmundo L. Ploschuk^b,
Andrea S. Vega^c, Pedro. Insausti^a

^aIFEVA-CONICET, Av. San Martín 4453. CPA 1417 DSE Buenos Aires, Argentina

^bCátedra de Cultivos Industriales, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453. CPA 1417 DSE Buenos Aires, Argentina

^cCátedra de Botánica Agrícola, Facultad de Agronomía, Universidad de Buenos Aires, Av. San Martín 4453. CPA 1417 DSE Buenos Aires, Argentina

Received 12 July 2007; accepted 4 October 2007

Abstract

The grass *Paspalum dilatatum* Poir. subsp. *dilatatum* inhabits periodically flooded lowlands as well as non-flooded uplands of the flooding Pampa grasslands (Argentina), while *P. dilatatum* Poir. subsp. *flavescens* Roseng., B.R. Arrill. & Izag. inhabits only the upland sites. An experiment was designed to determine if there is local adaptation to flooding in physiological, anatomical and leaf morphological traits. To this end, plants of these populations were subjected to flooding (6 cm water depth) and control conditions (watered daily) for 60 days in an experimental garden. Flooded plants of the subsp. *dilatatum* from the lowland had 35% higher photosynthesis compared to controls without affecting their stomatal conductance, transpiration rate and leaf water potential. By contrast, both subsp. *dilatatum* and subsp. *flavescens* from the upland did not increase their photosynthesis, and had reduced their stomatal conductance and leaf transpiration rate by 35% and 45% when growing in flooded conditions. Upland populations had higher leaf water potential with respect to controls. All populations had high constitutive root aerenchyma (28–42%), and leaf sheath porosity increased by 75% in flooded conditions (from 22–28% to 35–48%). Leaf lengthening differed among populations according to their habitat: subsp. *dilatatum* from the lowland was the only one that had longer leaf sheaths and blade lengths when flooded. In contrast, flooded plants of subsp. *dilatatum* from the upland only increased leaf sheath length while subsp. *flavescens* neither increased leaf blade nor leaf sheath. In conclusion, both the physiological performance and the leaf length plasticity differed among populations. The results agree with those expected based on the species' habitat, and indicate the better adaptation to the flood-prone habitat of *P. dilatatum* subsp. *dilatatum* taken from a lowland area.

© 2008 Elsevier GmbH. All rights reserved.

Keywords: Aerenchyma; Carbon fixation; Flooding; Intraspecific variation; *Paspalum dilatatum*; Water relations

Introduction

Flooding is a strong natural selection factor that endangers the survival of individuals of a great number

*Corresponding author. Fax: +54 11 45148730.

E-mail address: fmollard@ifeva.edu.ar (F.P.O. Mollard).

of species (Jackson and Colmer, 2005; Justin and Armstrong, 1987; Kozłowski, 1984). Natural selection through floods can produce variations among locally adapted populations (Ashraf, 2003; Davy et al., 1990) and even intrapopulational specialization in response to flooding regimes (Lenssen et al., 2004). High outcrossing rates promote local adaptation because they favor the maintenance of enough genetic variation within a population (Parker et al., 2003); in contrast, clonal reproduction and polyploidy may predispose plants to show low levels of genetic variation but high physiological tolerance and plasticity to stress factors (Parker et al., 2003). Therefore, species with both sexual and asexual reproductive strategies might display a suite of adaptive traits that favor the maintenance of successful populations in stressful habitats like flooded grasslands.

In terrestrial ecosystems subjected to seasonal episodes of flooding, the tolerance to this stress factor varies among species. Plants of some species are very susceptible and can die during flooding but others actually benefit from the floods; in such plants, for example, physiological performance is enhanced (Jackson and Colmer, 2005; Naidoo and Mundree, 1993). Flooding tolerance depends on the development of physiological, anatomical and morphological responses related to survival under anaerobic soil conditions (Armstrong, 1979; Kozłowski and Pallardy, 1984; Pezeshki, 1994; Voisenek and Blom, 1989). Thus, plants may develop traits that ensure an efficient supply of oxygen to submerged tissues (Crawford, 2003; Jackson and Armstrong, 1999; Pezeshki, 1994;), to maintain basic physiological processes and survive waterlogging periods (Insausti et al., 2001; Naidoo and Mundree, 1993). The most common responses include aerenchyma formation (Justin and Armstrong, 1987), increased plant height and maintenance of stomatal conductance (Kozłowski and Pallardy, 1984; Naidoo and Naidoo, 1992). All these responses facilitate oxygen capture and its transport to submerged tissues (Colmer, 2003).

The perennial grass *P. dilatatum* Poir. encompasses a group of closely related taxa that display a number of different cytotypes (Souza-Chies and Cavalli-Molina, 1995) widely distributed in temperate grasslands of South America (Soriano, 1991). *P. dilatatum* subsp. *flavescens* Roseng., B.R. Arrill. & Izag. is an allotetraploid cytotype with sexual reproduction, while *P. dilatatum* subsp. *dilatatum* is an apomictic allohexaploid or allohexaploid taxon (Espinoza and Quarín, 2000; Souza-Chies and Cavalli-Molina, 1995). *P. dilatatum* subsp. *dilatatum* is very common in the Flooding Pampa Grasslands (Argentina) where floods occur mainly in winter or spring but also in summer (Insausti et al., 1999). It is also prevalent in upland communities where flooding does not take place (Burkart et al., 1990) and there is some preliminary evidence of intraspecific differentiation between popula-

tions of *P. dilatatum* subsp. *dilatatum* situated in both communities (Loreti and Oesterheld, 1996). Otherwise, subsp. *flavescens* is a less frequent taxon that, contrary to what should be expected based on its sexual reproduction and potential for micro-evolution, its distribution is more restricted than that of subsp. *dilatatum* and restricted to non-flooded upland grasslands. Therefore, in *P. dilatatum*, apomixis seems to be the reproductive strategy that is associated with the existence of established populations in lowland habitats.

Because the mentioned populations will certainly share a high number of characters due to common ancestry, *P. dilatatum* becomes an invaluable tool for the study of adaptive trait divergence associated with flooding. The aim of this work was to evaluate which trait or combination of traits is associated with the occurrence of subsp. *dilatatum* in flooding habitats. Particularly, we addressed the following question: Are there any differences among populations in physiological, anatomical or leaf morphological traits in response to flooding? Answering this question will help identify critical features that discriminate between flooding tolerant and intolerant populations of a grass like *P. dilatatum*.

Materials and methods

Plant material and experimental design

Plants of *P. dilatatum* subsp. *dilatatum* and *P. dilatatum* subsp. *flavescens* were removed from two different plant communities located in upland and lowland sites along a topographic gradient in the Flooding Pampa Grasslands, Argentina. The subsp. *flavescens* and subsp. *dilatatum* plants from upland (hereafter “*dilatatum* Upland”) were taken from a plant community characterized by *Melica brasiliensis* Ard., *Borreria dasycephala* (Cham. & Schltdl.) Bacigalupo & E. L. Cabral, and *Echium plantagineum* L. Plants of subsp. *dilatatum* from lowland stands (“*dilatatum* Lowland”) were taken from a community characterized by *Piptochaetium montevidense* (Spreng.) Parodi, *Ambrosia tenuifolia* Spreng., *Eclipta bellidioides* (Spreng.) Sch. Bip. ex S.F. Blake and *Mentha pulegium* L., one of the most widespread plant associations of these grasslands (Burkart et al., 1990). Both sites have contrasting water regimes due to their relative topographical positions and soil characteristics: the uplands are associated with soils of moderate drainage, never-flooded, positioned 50–100 cm higher than the lowlands, which have clayey soils of poor drainage that experience annual floods (Soriano, 1991). At each site, seven distant adult plants were carefully collected in small soil blocks and transported to the Faculty of Agronomy, University of

Buenos Aires. Plants were immediately transplanted inside a greenhouse to pots (3l) with a mixture of sand and topsoil from the grassland as a substrate (1:1) and vegetatively propagated for 6 months. This period was assumed to erase original environmental effects as demonstrated previously for this species (Loreti and Oosterheld, 1996). After this, three similarly sized tillers per plant were removed, transplanted to individually bigger pots (7l), and placed interspersed in an experimental garden in the same faculty. Two months later, two treatments were applied for 60 days following a completely randomized design with seven replicates: (1) *flooding*: pots were flooded and maintained with a water level of 6 cm above the soil surface (2) *control*: pots were watered daily and allowed to drain freely. Treatments ran from spring to early summer.

Physiological measurements

Stomatal conductance and transpiration rate were measured in the youngest fully expanded leaf blade of each plant in a similar position using a LI-1600M steady-state porometer (LI-Cor Inc., Lincoln, NE, USA). Leaf water potential was recorded immediately on the same leaves with a Scholander-type pressure chamber (Bio-Control, Buenos Aires, Argentina). The net CO₂ exchange was measured on the same type of leaves with a LI-6200 portable photosynthesis system (LI-Cor Inc., Lincoln, NE, USA). Measurements were taken 1 day before the end of the experiment, at noon (PPFD = $1673 \pm 68 \mu\text{mol m}^{-2} \text{s}^{-1}$).

Tissue porosity and anatomical observations

At the end of the experiment, gas-filled porosity was quantified in fresh samples of young roots and sheaths using the pycnometer method (Sojka, 1988), based on the increase in weight that occurs when air spaces of plant tissues are replaced by water after maceration. Quantification of porosity in aerial and submerged tissues, such as leaves and roots, allows us to infer differences in the capacity of internal aeration from shoot to root among *Paspalum* populations (Justin and Armstrong, 1987). In addition, root and leaf sheath samples were cut, carefully washed and preserved in 70% alcohol until needed. Root segments comprising the apical 3 cm of the tip and leaf sheath segments 1 cm below the ligule were dehydrated in an ethanol series and embedded in paraffin wax. Cross-sections of 8–10 μm thickness of root and leaf sheath samples (respectively) were cut with a rotatory microtome, double stained with Safranin—Fast Green and mounted in Canada balsam. For each population, light microscope studies on randomly selected root and sheath cross-sections from each plant were made using an

optical microscope (Zeiss Axioplan, Zeiss, Oberkochen, Germany).

Leaf morphology

At the end of the experiment, blade and sheath lengths were measured separately on the youngest fully expanded leaves of three tillers per plant. This allowed us to investigate the de-submergence capacity of plants in each population, a well-known trait closely associated with plants better adapted to flooding (Naidoo and Mundree, 1993).

Statistical analyses

Physiological, anatomical and morphological data were analyzed within populations by Student's *t*-test. Leaf sheath and root porosity data were transformed prior to analyses by $\arcsin \sqrt{x}$ to satisfy the assumption of normality and homogeneity of variance. Results are presented as untransformed mean \pm standard error. All tests were performed using GraphPad Prism 4.0 for Windows (GraphPad Software, San Diego California, USA).

Results

Physiological responses

The *P. dilatatum* population from the lowland maintained their stomatal conductance unaltered under flooded conditions ($P > 0.05$) (Fig. 1A). According to such stomatal behavior, the *dilatatum* Lowland plants did not display differences in the leaf transpiration rate between treatments ($P > 0.05$) (Fig. 1B). In contrast, populations from the upland site reacted in a physiologically similar way to flooding: both *dilatatum* and subsp. *flavescens* decreased their stomatal conductances by 34% and 46%, respectively ($P < 0.01$ for *dilatatum* Upland; $P < 0.05$ for subsp. *flavescens*) (Fig. 1A). These decreases in stomatal conductance of plants in both populations from the upland were correlated with lower transpiration rates. The decrease was 36% for *dilatatum* Upland and 45% for subsp. *flavescens* ($P < 0.01$ for *dilatatum* Upland; $P < 0.05$ for subsp. *flavescens*; Fig. 1B). Remarkably, *dilatatum* Lowland had a net photosynthesis rate 35% higher in flooded conditions than in control ones ($P < 0.05$) (Fig. 1C). Neither *dilatatum* Upland nor subsp. *flavescens* showed any differences in net photosynthetic rate between treatments ($P > 0.05$) (Fig. 1C). In line with the transpiration behavior of both upland populations, similar physiological responses between *dilatatum* Upland and subsp. *flavescens* were also observed in leaf water potential: this

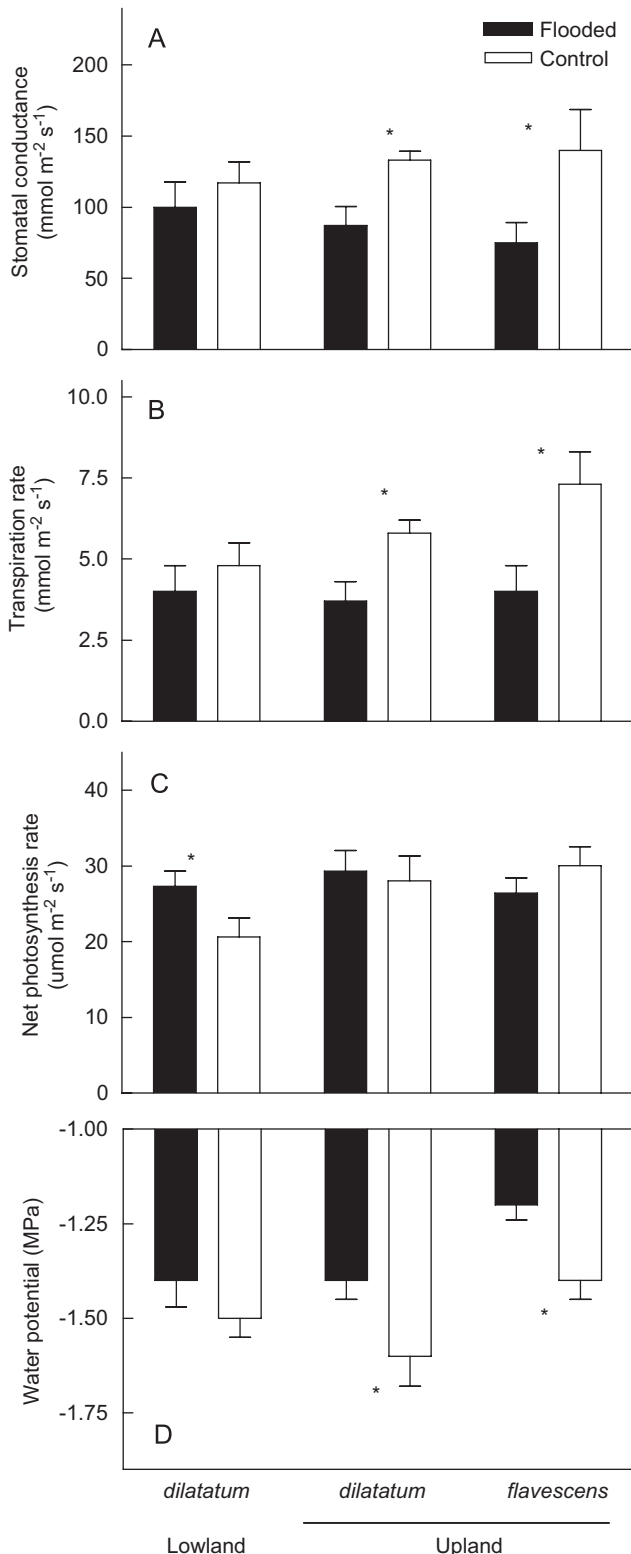


Fig. 1. Physiological measurements on leaves of *P. dilatatum* populations subjected to flooding or control treatments. (A) stomatal conductance, (B) transpiration rate, (C) net photosynthetic rate, (D) water potential. Values are mean \pm S.E. of seven replicates. Asterisks indicate significant differences between treatments based on student's *t*-tests ($P < 0.05$).

was higher in flooded conditions than in control ones ($P < 0.05$ for *dilatatum* Upland; $P < 0.01$ for subsp. *flavescens*) (Fig. 1D). By contrast, flooding did not affect the leaf water potential of *dilatatum* Lowland plants ($P > 0.05$) (Fig. 1D).

Leaf sheath and root anatomy

Flooding increased leaf sheath porosity in all populations, ranging from 62% to 74% ($P < 0.05$ for *dilatatum* Lowland; $P < 0.01$ for *dilatatum* Upland and subsp. *flavescens*) (Table 1). This response resulted from the development of larger-sized lysigenous lacunae in the parenchyma (Fig. 2A, C, E), which was not observed in leaf sheath cross-sections of control plants (Fig. 2B, D, F). Otherwise, control roots of all *P. dilatatum* populations had high constitutive porosity (Table 1), which corresponded to an extensive system of lysigenous aerenchyma tissue arranged radially in the root cortex (Fig. 3). Longitudinal lacunae were separated by rows of parenchymatic cells and surrounded by a ring of sclerenchymatic cells in the exodermis (Fig. 3). This root structure, common to both subspecies of *P. dilatatum*, resembled a bicycle wheel and corresponds to the *graminaceous* root structural type defined by Justin and Armstrong (1987). Flooding did not increase root porosity in any population ($P > 0.05$ for all populations) (Table 1).

Leaf morphology responses

Flooding affected leaf lengthening differentially in the three populations. In the *dilatatum* Lowland-flooded plants, both leaf sheaths and leaf blades were longer than in plants growing in drained soil ($P < 0.05$ in both cases; Table 2). By contrast, upland plants did not respond to flooding like *dilatatum* Lowland: flooded plants of *dilatatum* Upland had a larger leaf sheath ($P < 0.05$) but a leaf blade length similar to controls ($P < 0.05$). Meanwhile, plants of subsp. *flavescens* had similar leaf sheath and leaf blade lengths irrespective of whether they had grown under flooding or drained conditions ($P > 0.05$; Table 2).

Discussion

Flooding had a positive effect on the physiological performance of *P. dilatatum* subsp. *dilatatum* Lowland that did not occur in either of the Upland populations. Flooded plants of *dilatatum* Lowland increased their net photosynthetic rate relative to controls (Fig. 1), fully agreeing with values obtained by Insausti et al. (2001) for a lowland population. Moreover, *dilatatum* Lowland plants did not decrease either stomatal conductance or

Table 1. Leaf sheath and root porosity (%) of *P. dilatatum* populations grown for 60 days under flooding and control treatments

	Leaf sheath porosity (%)		Root porosity (%)	
	Flooding	Control	Flooding	Control
<i>dilatatum</i> Lowland	35.4±45	21.8±3.2*	38.3±2.7	42.7±3.1 n.s
<i>dilatatum</i> Upland	48.3±2.2	28.0±3.7*	32.2±1.3	32.2±1.3 n.s
<i>flavescens</i>	45.3±1.5	25.9±4.0*	30.3±3.4	28.6±0.9 n.s

Values are mean ± S.E. of seven replicates. * $P < 0.05$; n.s., not significant. Comparisons between treatments were based on student's *t*-tests.

transpiration rate upon flooding. Stomatal closure during flooding is a behavior that regulates the water balance of susceptible plants and is a critical response in preventing leaf dehydration (Ashraf, 2003; Baruch, 1994; Bradford and Hsiao, 1982). Remarkably, physiological behavior of *dilatatum* Lowland contrasted with that observed in *dilatatum* Upland and *flavescens* plants: both reduced stomatal conductance and transpiration rate in flooding conditions. This indicates that flooding was a stress factor that affected water relations of upland populations. In this way, the decrease in transpiration rate along with the higher leaf water potential in flooded conditions suggest that stomatal conductance was effective in regulating the water status of upland plants (Fig. 1D). Contrary to the situation in *dilatatum* Lowland, upland plants did not increase their rate of photosynthesis in flooded conditions. In spite of their high constitutive photosynthesis rate, they could not take advantage of a situation that was beneficial to *dilatatum* Lowland: a flooded soil with high solar irradiance and high temperature (Fig. 1; Insausti et al., 2001). In consequence, the differential physiological performance of *dilatatum* Lowland with respect to the other populations suggests that they are locally adapted populations with a different response to flooding.

All *P. dilatatum* populations contained high amounts of constitutive aerenchyma in roots, and increased leaf sheath porosity under flooding conditions. The formation of aerenchyma in roots and leaf sheaths suggests some degree of flooding tolerance in all populations because this improves oxygenation of submerged tissues by permitting the flow of oxygen from shoots by diffusion (Jackson and Armstrong, 1999; Laan et al., 1990). The occurrence of this adaptation in all populations might be due to the origin of both *P. dilatatum* subspecies. Both have genomes closely related to *P. juergensii* Hack. and *P. intermedium* Munro ex Morong & Britton (Espinoza and Quarín, 2000; Pitman et al., 1987). The last diploid and other diploid *Paspalum* species with slightly different genome forms inhabit the wetlands of South America and have a high content of constitutive aerenchyma in their organs (Burkart, 1969; Molina and Rúgolo de Agrasar, 2006; Rosengurtt et al., 1970) so it is possible that *P. dilatatum* populations

share their traits. Also, the fact that populations that inhabit never-flooded sites conserve high constitutive root porosity suggests that this trait may not represent an important cost in that environment (Oosterheld and McNaughton, 1991). Alternatively, high constitutive root porosity of upland populations may not be an adaptation to flooding *per se*; another factor may favor this trait in such sites. For example, it has been reported that soils of these grasslands have a low content of available phosphorus (Lavado and Taboada, 1987) and that low phosphorus availability favors the formation of cortical aerenchyma in roots. This is thought to decrease respiratory requirements and, thereby, the metabolic burden of soil exploration (Fan et al., 2003).

Morphological responses were completely in accordance with the physiological behavior of each population. Both leaf sheath and leaf blade length along with an unaltered stomatal conductance under flooding of subsp. *dilatatum* Lowland reveals its high capability to emerge from water and to capture atmospheric oxygen (Grimoldi et al., 1999; Laan et al., 1990; Voisenek et al., 2006). In contrast, the lack of (or a limited) leaf length along with the stomatal closing in flooded plants of *dilatatum* Upland and subsp. *flavescens* suggest a less plastic response to flooding compared to *dilatatum* lowland plants. Consequently, if flooding is intensified (e.g., in duration, water depth) such morphophysiological limitations of the upland populations could constrain their performance to a greater extent due to the difficulty of recovering adequate contact with air (Grimoldi et al., 1999).

In conclusion, the improved physiological and leaf morphological responses of *P. dilatatum* subsp. *dilatatum* from lowland areas in respect of the population of the same subspecies from upland suggest the existence of locally flood-adapted populations. Additionally, the finding that the morpho-physiological behavior of *dilatatum* Upland resembled that of subsp. *flavescens* that also inhabits uplands reinforces these notions. Our findings reinforce the point that flooding can exert a strong selective pressure on plant species populations. Also, the appearance of flooding tolerant populations, within one apomictic lineage such as subsp. *dilatatum*, demonstrates the importance of limited gene exchange

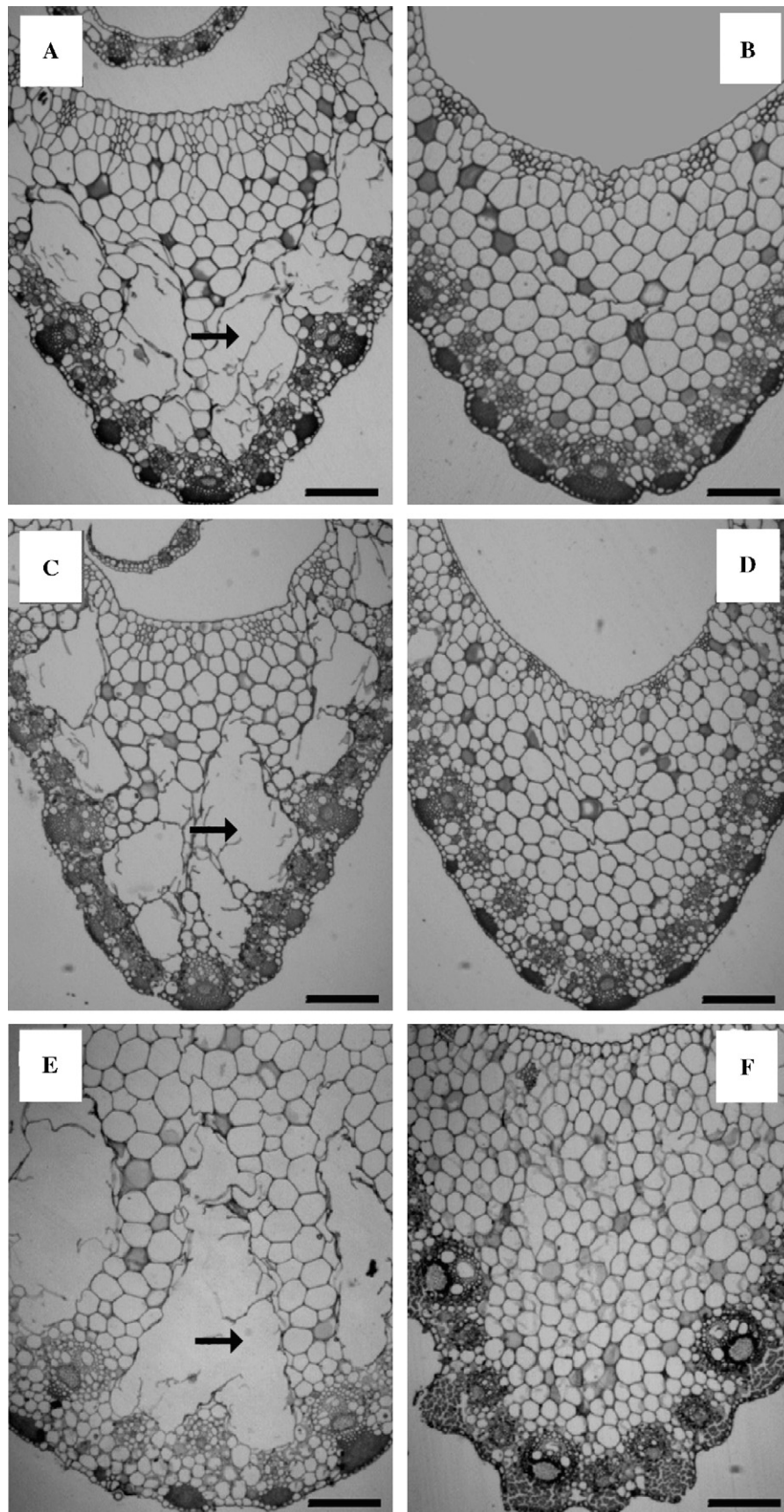


Fig. 2. Transverse sections of leaf sheaths (keel in detail) of *P. dilatatum* subsp. *dilatatum* from lowlands (A, B), *P. dilatatum* subsp. *dilatatum* from uplands (C, D) and *P. dilatatum* subsp. *flavescens* (E, F) grown for 60 days under flooding (A, C, E) and control (B, D, F) treatments. Arrows indicate lysigenous aerenchyma. The bar represents 180 μm.

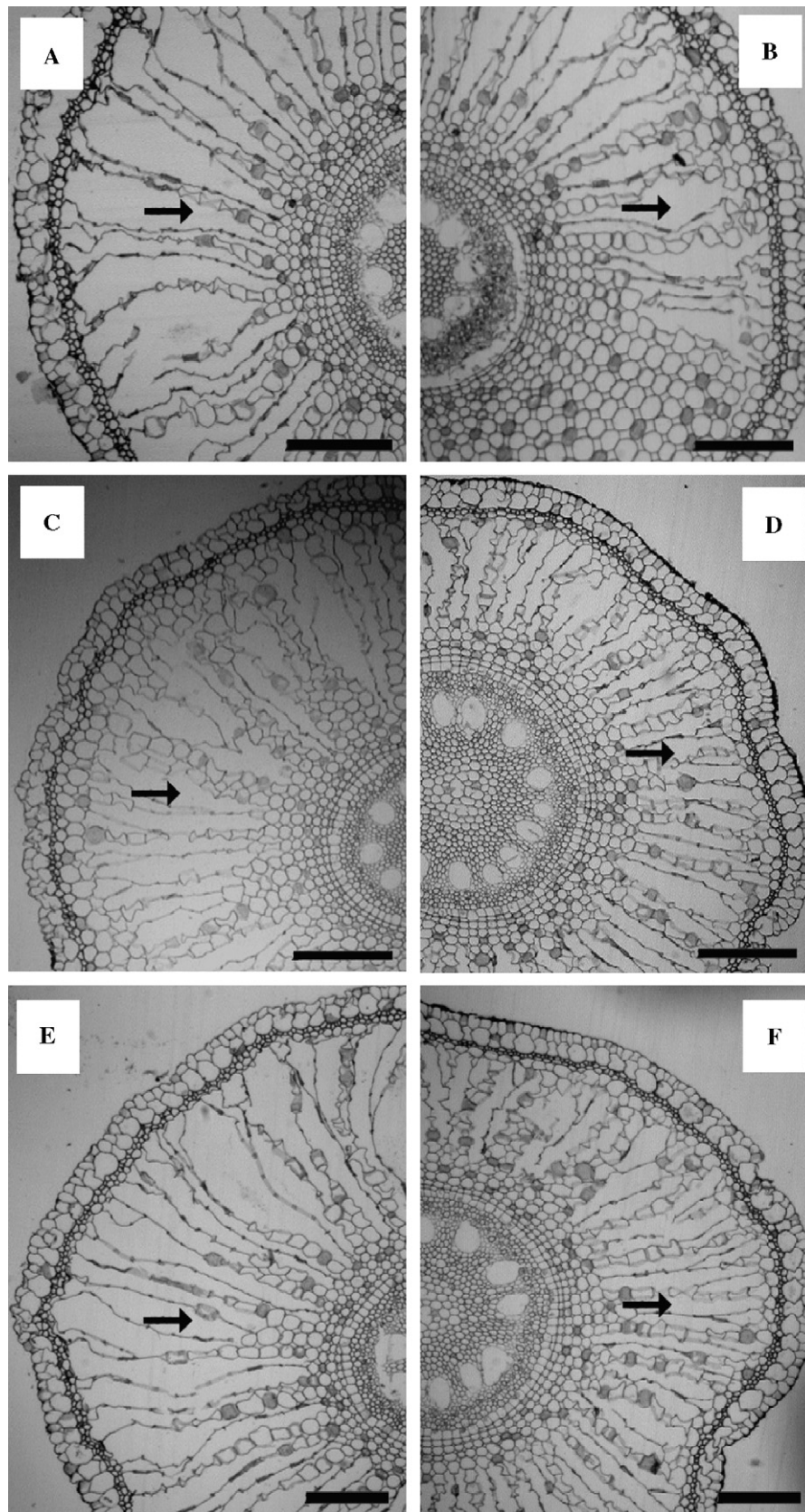


Fig. 3. Transverse sections of roots of *P. dilatatum* subsp. *dilatatum* from lowlands (A, B), *P. dilatatum* subsp. *dilatatum* from uplands (C, D) and *P. dilatatum* subsp. *flavescens* (E, F) grown for 60 days under flooding (A, C, E) and control (B, D, F) treatments. Arrows indicate lysigenous aerenchyma. The bar represents 180 μm.

Table 2. Leaf sheath and blade lengths of *P. dilatatum* populations grown for 60 days under flooding and control treatments

	Leaf sheath length (cm) (\pm S.E.)		Leaf blade length (cm) (\pm S.E.)	
	Flooding	Control	Flooding	Control
<i>dilatatum</i> Lowland	6.4 \pm 0.5	4.5 \pm 0.4*	10.8 \pm 0.6	8.7 \pm 0.5*
<i>dilatatum</i> Upland	7.0 \pm 0.5	5.0 \pm 0.5*	11.3 \pm 0.8	9.9 \pm 1.1 n.s
<i>flavescens</i>	6.3 \pm 0.5	5.3 \pm 0.5 n.s	14.5 \pm 0.9	16.1 \pm 1.2 n.s

Values are mean \pm S.E. of seven replicates. * P < 0.05; n.s., not significant. Comparisons between treatments were based on student's *t*-tests.

on the generation of differentiated populations (Davy et al., 1990). Further research on the effects of flooding on other life-history traits (seed production, seedling survival) might reveal the importance of this stress factor in determining population fitness.

Acknowledgments

We specially thank A. Grimoldi for his interesting comments on early versions of this manuscript, R. León for his invaluable support throughout the study and G. Zarlavsky for her technical assistance with anatomical work. This study was supported by a grant from ANPCyT Foncyt–PICT 08-09934.

References

- Armstrong, W., 1979. Aeration in higher plants. In: Woolhouse, H.W. (Ed.), *Advances in Botanical Research*, vol. 7. Acad. Pr., London, pp. 225–332.
- Ashraf, M., 2003. Relationships between leaf gas exchange characteristics and growth of differently adapted populations of Blue panicgrass (*Panicum antidotale* Retz) under salinity or waterlogging. *Plant Sci.* 165, 69–75.
- Baruch, Z., 1994. Responses to drought and flooding in tropical forage grasses. II. Leaf water potential, photosynthetic rate and dehydrogenase activity. *Plant Soil* 164, 97–105.
- Bradford, K.J., Hsiao, T.C., 1982. Stomatal behaviour and water relations of waterlogged tomato plants. *Plant Physiol.* 70, 1508–1513.
- Burkart, A., 1969. *Flora Ilustrada de Entre Ríos* (Argentina). Instituto Nacional de Tecnología Agropecuaria, Buenos Aires.
- Burkart, S.E., León, R.J.C., Movia, C.P., 1990. Inventario fitosociológico de un pastizal de la depresión del Salado (Prov. de Bs. As) en un área representativa de sus principales ambientes. *Darwiniana* 30, 27–69.
- Colmer, T.D., 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant Cell Environ.* 26, 17–36.
- Crawford, R.M.M., 2003. Seasonal differences in plant responses to flooding and anoxia. *Can. J. Bot.* 81, 1224–1246.
- Davy, A.J., Noble, S.M., Oliver, R.P., 1990. Genetic variation and adaptation to flooding in plants. *Aquat. Bot.* 38, 91–108.
- Espinoza, F., Quarín, C.L., 2000. $2n+n$ Hybridization of Apomictic *Paspalum dilatatum* with diploid *Paspalum* species. *Int. J. Plant Sci.* 161, 221–225.
- Fan, M., Zhu, J., Richards, C., Brown, K.M., Lynch, J.P., 2003. Physiological roles for aerenchyma in phosphorus-stressed roots. *Funct. Plant Biol.* 30, 493–506.
- Grimoldi, A.A., Insausti, P., Roitman, G.G., Soriano, A., 1999. Responses to flooding intensity in leontodon taraxacoides. *New Phytol.* 141, 119–128.
- Insausti, P., Chaneton, E.J., Soriano, A., 1999. Flooding reverted grazing effects on plant community structure in mesocosms of lowland grassland. *Oikos* 84, 266–276.
- Insausti, P., Grimoldi, A.A., Chaneton, E.J., Vasellati, V., 2001. Flooding induces a suite of adaptive plastic responses in the grass *Paspalum dilatatum*. *New Phytol.* 152, 291–299.
- Jackson, M.B., Armstrong, W., 1999. Formation of aerenchyma and the process of plant ventilation in relation to soil flooding and submergence. *Plant Biol.* 1, 274–287.
- Jackson, M.B., Colmer, T.D., 2005. Response and adaptation by plants to flooding stress. *Ann. Bot.* 96, 501–505.
- Justin, S.H.F.W., Armstrong, W., 1987. The anatomical characteristics of roots and plant response to soil flooding. *New Phytol.* 106, 465–495.
- Kozłowski, T.T., 1984. Extent, cause, and impact of flooding. In: Kozłowski, T.T. (Ed.), *Flooding and Plant Growth*. Acad. Pr., Orlando, pp. 165–193.
- Kozłowski, T.T., Pallardy, S.G., 1984. Effects of flooding on water, carbohydrate and mineral relations. In: Kozłowski, T.T. (Ed.), *Flooding and Plant Growth*. Academic Press, Orlando, pp. 165–193.
- Laan, P., Tosserams, M., Blom, C.W.P.M., Veen, B.W., 1990. Internal oxygen transport in rumex species and its significance for respiration under hypoxic conditions. *Plant Soil* 122, 39–46.
- Lavado, R.S., Taboada, M.T., 1987. Soil salinization as an effect of grazing in native grassland soil in the flooding pampa of Argentina. *Soil Use Manag.* 3, 143–148.
- Lenssen, J.P.M., Van Kleunen, M., Fischer, M., De Kroon, H., 2004. Local adaptation of the clonal plant *Ranunculus reptans* to flooding along a small-scale gradient. *J. Ecol.* 92, 696–706.
- Loreti, J., Oosterheld, M., 1996. Intraspecific variation in the resistance to flooding and drought in populations of

- Paspalum dilatatum* from different topographic positions. *Oecologia* 108, 279–284.
- Molina, A.M., Rúgolo de Agrasar, Z.E., 2006. Flora Chaqueña: Gramíneas. Instituto Nacional de Tecnología Agropecuaria, Buenos Aires.
- Naidoo, G., Mundree, S.G., 1993. Relationship between morphological and physiological responses to waterlogging and salinity in *Sporobolus virginicus* (L) Kunth. *Oecologia* 93, 360–366.
- Naidoo, G., Naidoo, S., 1992. Waterlogging responses of *Sporobolus virginicus* (L) Kunth. *Oecologia* 90, 445–450.
- Oosterheld, M., McNaughton, S.G., 1991. Interactive effect of flooding and grazing on the growth of Serengeti grasses. *Oecologia* 88, 153–156.
- Parker, I.M., Rodriguez, J., Loik, M.E., 2003. An evolutionary approach to understanding the biology of invasions: Local adaptation and general-purpose genotypes in the weed *Verbascum thapsus*. *Conserv. Biol.* 17, 59–72.
- Pezeshki, S.R., 1994. Plant response to flooding. In: Wilkinson, R.E. (Ed.), *Plant–Environment Interactions*. Marcel Dekker, New York, pp. 289–321.
- Pitman, M.W., Burson, B.L., Bashaw, E.C., 1987. Phylogenetic relationships among *Paspalum* species with different base chromosome numbers. *Bot. Gaz.* 148, 130–135.
- Rosengurtt, B., Arrillaga de Maffei, B.R., Izaguirre de Artucio, P., 1970. Gramíneas Uruguayas. Universidad de la República, Montevideo.
- Sojka, R.E., 1988. Measurement of root porosity (volume of root in space). *Environ. Exp. Bot.* 28, 275–280.
- Soriano, A., 1991. Río de la plata grasslands. In: Coupland, R.T. (Ed.), *Ecosystems of the World 8A. Natural Grasslands. Introduction and Western Hemisphere*. Elsevier, Amsterdam, pp. 367–407.
- Souza-Chies, T.T., Cavalli-Molina, S., 1995. Variability in seed production and germination in *Paspalum dilatatum* group (Gramineae). *Rev. Bras. Biol.* 55, 127–139.
- Voesenek, L.A.C.J., Blom, C.W.P.M., 1989. Growth responses of *Rumex* species in relation to submergence and ethylene. *Plant Cell Environ.* 12, 433–439.
- Voesenek, L.A.C.J., Colmer, T.D., Pierik, R., Millenaar, F.F., Peeters, A.J.M., 2006. How plants cope with complete submergence. *New Phytol.* 170, 213–226.