

## Different patterns of aerenchyma formation in two hygrophytic species of *Paspalum* (Poaceae) as response to flooding

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Received 20 May 2004; accepted 27 November 2004

### Abstract

*Paspalum modestum* and *P. wrightii* are perennial grasses growing in permanent and seasonally flooded areas, respectively. The former produces short rhizomes and floating culms, the latter forms long rhizomes and erect culms. Variations in percentage aerenchymatous space (PAS) in different organs as a response to flooding was analysed using a clone of each species. Eighteen plantlets of each clone were cultivated during 7 months under flooded vs. unflooded conditions. After this period, roots, rhizomes, culms, and leaf sheaths were collected and prepared. PAS was measured using an image analysis device, and data were analysed using ANOVA.

Production of aerenchyma took place in both species within the cortical parenchyma of roots, rhizomes and culms, and the mesophyll of leaf sheaths, both in flooded and unflooded plants. Under flooding conditions PAS increased in both species, although the individual response of organs differed: whereas in *P. modestum* PAS increased primarily in substratum-fixed roots, in *P. wrightii* all organs produced additional aerenchyma uniformly. Contrasting responses are understood as adaptations to permanent and seasonal flooding, respectively.

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**Keywords:** *Paspalum modestum*; *Paspalum wrightii*; Phenotypic plasticity; Flooding; Aerenchyma; Growth form; Hygrophytic grasses; Wetland plants

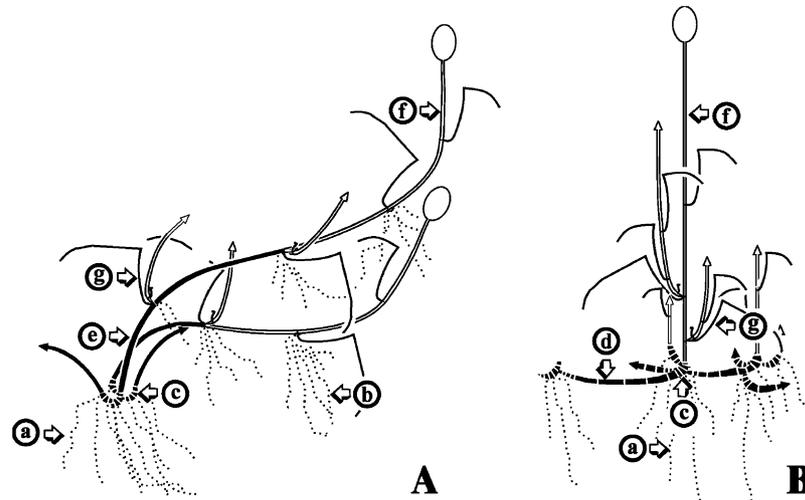
### Introduction

The genus *Paspalum* L. comprises about 330 (Zuloaga and Morrone, 2001) species distributed mainly in tropical to warm-temperate areas worldwide, with a primary diversity centre in tropical America (Chase, 1929; Clayton and Renvoize, 1986; Judziewicz, 1990).

*Paspalum* species occupy a wide range of habitats and show a remarkable diversity of growth forms (Rua and Gróttola, 1997). *Paspalum modestum* Mez and *P. wrightii* Hitchc. & Chase are two hygrophytic species promising as high-quality forage grasses for flooding areas (Pozzobon and Valls, 2003, *P. wrightii* mentioned under the synonym *P. hydrophilum* Henrard). They, respectively, grow in areas subject to permanent and periodical floods (Pozzobon and Valls, 2003), and differ because of their different growth habit. The tillers of *P. modestum* have a proximal portion comprising a few short internodes

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**Fig. 1.** Schematic representation of growth forms of: (A) *Paspalum modestum* and (B) *P. wrightii*. Ellipses represent inflorescences, perennial and annual axes depicted in black and white, respectively, leaves of lower nodes not depicted; letters in circles indicate regions at which transections were made: (A) fixed root, (B) floating root, (C) rhizome, short-internode region, (D) rhizome, long-internode region, (E) submerged culm, (F) emerged culm, and (G) leaf sheath.

rooted in the substrate, followed by a plagiotropic, floating portion which involves several long internodes and bears at each node a profuse tuft of caulogenous roots (Fig. 1A, cf. model PB4 in Rua and Gróttola, 1997). Such floating culms usually remain after flowering and originate a next generation of shoots. On the other hand, the tillers of *P. wrightii* bear a more developed proximal portion, which grows to some extent plagiotropically forming a rhizome composed of several relatively elongated internodes buried into the substrate (Fig. 1B, cf. models PU3 and PB3 in Rua and Gróttola, 1997). The distal portion of such tillers forms an orthotropic culm, so that the inflorescences are raised well above the water level. Renewal buds are located at the transitional zone between the rhizome and the culm, composed of some short internodes.

Aerenchyma occurs regularly in hygrophytic plants (Sculthorpe, 1967). The relative amount of this tissue can however vary plastically in response to the anaerobic conditions caused by flooding (Sultan, 1995). Increase of aerenchyma production is stimulated by ethylene accumulation, and enhances the efficiency of oxygen transfer from aerial to submerged organs, making the survival in flooding habitats possible (Jackson and Drew, 1984; Justin and Armstrong, 1987; Evans, 2003). Nevertheless, the location and distribution of aerenchymatous tissue can be characteristic of a given species, and the plastic response of this feature can vary from one organ to another (Sultan, 1995).

Some investigation on the foliar anatomy of *Paspalum* species has been carried out (Türpe, 1967; Aliscioni and Arriaga, 1998; Aliscioni, 2000), but the variation of anatomical features under different environmental conditions only has been studied in *P. dilatatum* Poir. (Vasellati et al., 2001; Insausti et al., 2001).

The aim of the present paper is to analyse the relative amount of aerenchyma in different organs of *P. modestum* and *P. wrightii*, and to explore the variation of this trait as a response to different hydric soil conditions.

## Materials and methods

### Plant material and experimental design

An assay was carried out using plants of *P. modestum* and *P. wrightii* obtained from cloning of one individual of each species, using as source of rootlings two plants maintained under cultivation in the “Lucien Hauman” Botanical Garden of the Buenos Aires University (Buenos Aires, Argentina). The voucher specimens are the following:

*P. modestum*: Argentina, prov. Corrientes, dpto. Gral. San Martín, ruta nac. 14, ca. río Aguapey, G. H. Rua et al. 146, 14-01-1994 (BAA).

*P. wrightii*: Brasil, estado Mato Grosso, ruta Transpantaneira, km 11, frente a la escuela munic. Silverio N. Rondon, frente faz. Ipiranga, G. H. Rua et al. 95, 17-03-1996 (UFMT, BAA).

Eighteen rootlings of each species were placed in plastic pots containing a substrate composed of volcanic gravel and garden soil (2:1 v/v). The plants were cultivated outdoors through a period of 7 months (October–May), under two different water conditions of the substrate: field capacity and flooding. In order to get this, the pots with the plants were randomly distributed within 36 plastic containers, a half of which were previously drilled at the bottom to allow draining. All

plants were watered regularly, in such a way that in intact containers the water level was continuously kept at least 1 cm above the substrate. After this period, material from all plants was collected and preserved in FAA (formalin-acetic acid-70 per cent ethanol, 10:5:85 v/v) for later microscopical analysis, including substratum-fixed and floating/aerial roots, rhizomes, submerged and emerged culms and leaf sheaths of *P. modestum*, and fixed roots, compressed and elongated portions of rhizomes, emerged culms and leaf sheaths of *P. wrightii* (Fig. 1).

### Anatomical preparation and aerenchymatous space measurement

All materials were dehydrated in an ethanol series, and embedded in paraffin. Transverse sections 15 µm thick were cut using a rotary microtome. Root sections were obtained from young roots at ca. 5 cm away from the apex, whereas the remaining organs were cut at the middle portion. Sections were double-stained with safranin-fast green (D'Ambrogio de Argüeso, 1986). Percentage aerenchymatous space (PAS, i.e. the ratio between the area occupied by aerenchyma and the total cross-sectional area) in each organ was measured using an optical microscope (Zeiss Axioplan, Zeiss, Oberkochen, Germany) connected to an image analyser (Imagination Px, Imagination Corp., Beaverton, OR, USA). Microphotographs were taken through the Zeiss Axioplan microscope.

### Statistical analyses

PAS was analysed for each species separately using two-way ANOVA ( $n = 8$ ), with flooding treatment and plant organ as main factors. Before analysis, data were arcsine square-root transformed (Steel and Torrie, 1988). A Kolmogorov-Smirnov  $D$ -test indicated that the data were well fitted to normality ( $D = 0.08$ ). Post-hoc multiple comparisons between means were performed using Tukey test.

### Results

Production of aerenchymatous spaces took place in both species within the cortical parenchyma of roots, rhizomes and culms, and the mesophyll of leaf sheaths. Such spaces were observed both in flooded and unflooded plants. Even though aerenchyma was more abundant in roots and leaf sheaths, it occurred in all examined organs of both species. Although not quantified, it was also observed in leaf blades of *P. modestum* (previously documented by Aliscioni, 2000), but not in those of *P. wrightii*.

In roots, air spaces appear as large radiating cavities, which are separated by longitudinal radiating plates composed of either intact cells or remnants of collapsed cells, connecting the internal and external rings of living tissues (Fig. 2A). Thus, the observed pattern do not differ from the common one occurring among grass roots (Metcalf, 1960; Justin and Armstrong, 1987). In all examined roots a hypodermis was noticeable, composed of 2 or 3 layers of cells with thickened, lignified walls (Fig. 2A). No difference among treatments was detected regarding this trait.

Aerenchymatous spacing in roots, culms, and leaf sheaths (Figs. 2A–D) results principally from death and posterior collapsing of cells, thus it corresponds to the lysigenous type (Smirnoff and Crawford, 1983; Jackson and Drew, 1984; Justin and Armstrong, 1987), although schizogeny can occur as well. When living cells remain, they are cubic and leave intercellular spaces which appear rhomboid in cross section.

#### *P. modestum*

ANOVA revealed significant effects of flooding treatment ( $P < 0.00007$ ) and plant organ ( $P < 0.000001$ ) on the PAS. A significant treatment  $\times$  organ interaction ( $P < 0.008$ ) was also detected, so that differences between control and flooded plants varied from one organ to another (Fig. 3A). In fact, multiple comparison of means indicates that the difference in PAS between control and flooded plants was only significantly explained by the enhanced formation of air spaces in substratum-fixed roots ( $P < 0.0002$ ).

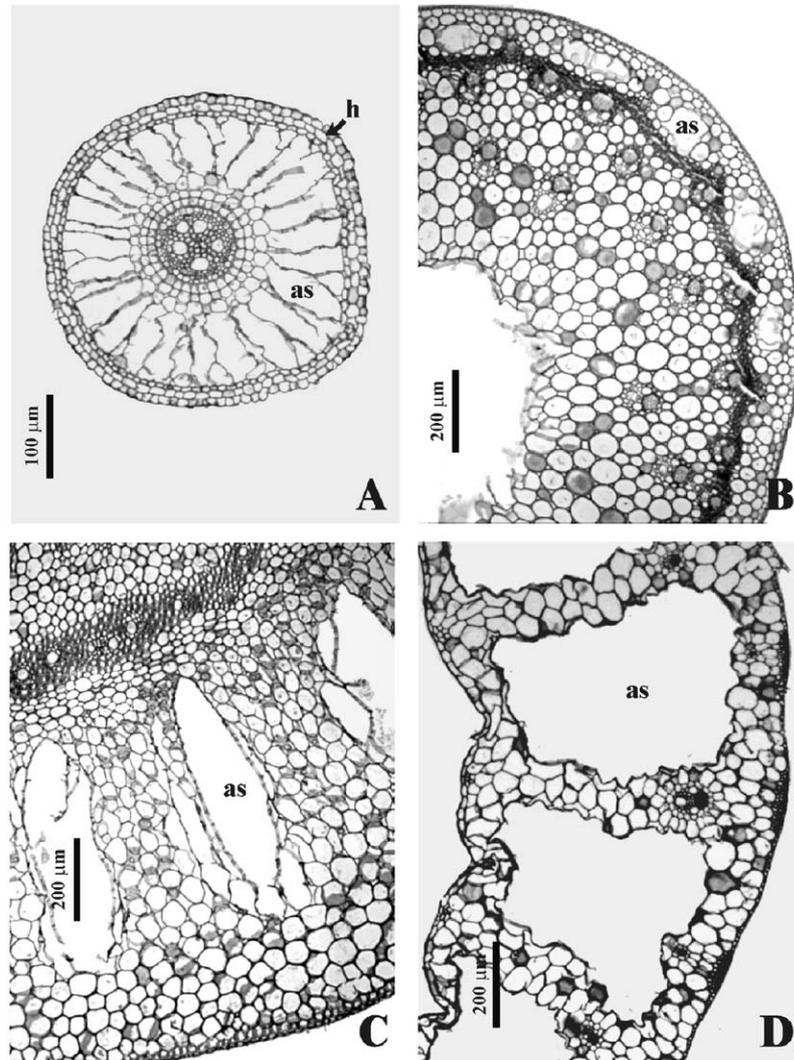
#### *P. wrightii*

Two-way ANOVA revealed significant effects of flooding treatment ( $P < 0.0003$ ) and plant organ ( $P < 0.000001$ ) on PAS. No significant treatment  $\times$  organ interaction was detected, thus no individual organ made a substantial contribution to the global variation (Fig. 3B).

Although not quantified, some morphological traits were additionally observed. All plants flowered towards the end of the assay period. All flooded plants of *P. wrightii* develop longer culms than control plants. Flooded plants of both species develop copious adventitious roots, arising from floating culms in *P. modestum* and from rhizomes in *P. wrightii*.

### Discussion

Both *P. modestum* and *P. wrightii* increased the percentage of aerenchymatous space in response to flooding. This behaviour, as well as the occurrence of a



**Fig. 2.** Location of air spaces in different organs of *P. modestum* and *P. wrightii*: (A) fixed root of *P. modestum*, (B) emerged culm of *P. wrightii*, (C) rhizome of *P. wrightii*, and (D) leaf sheath of *P. modestum*. AS, air space; H, hypodermis.

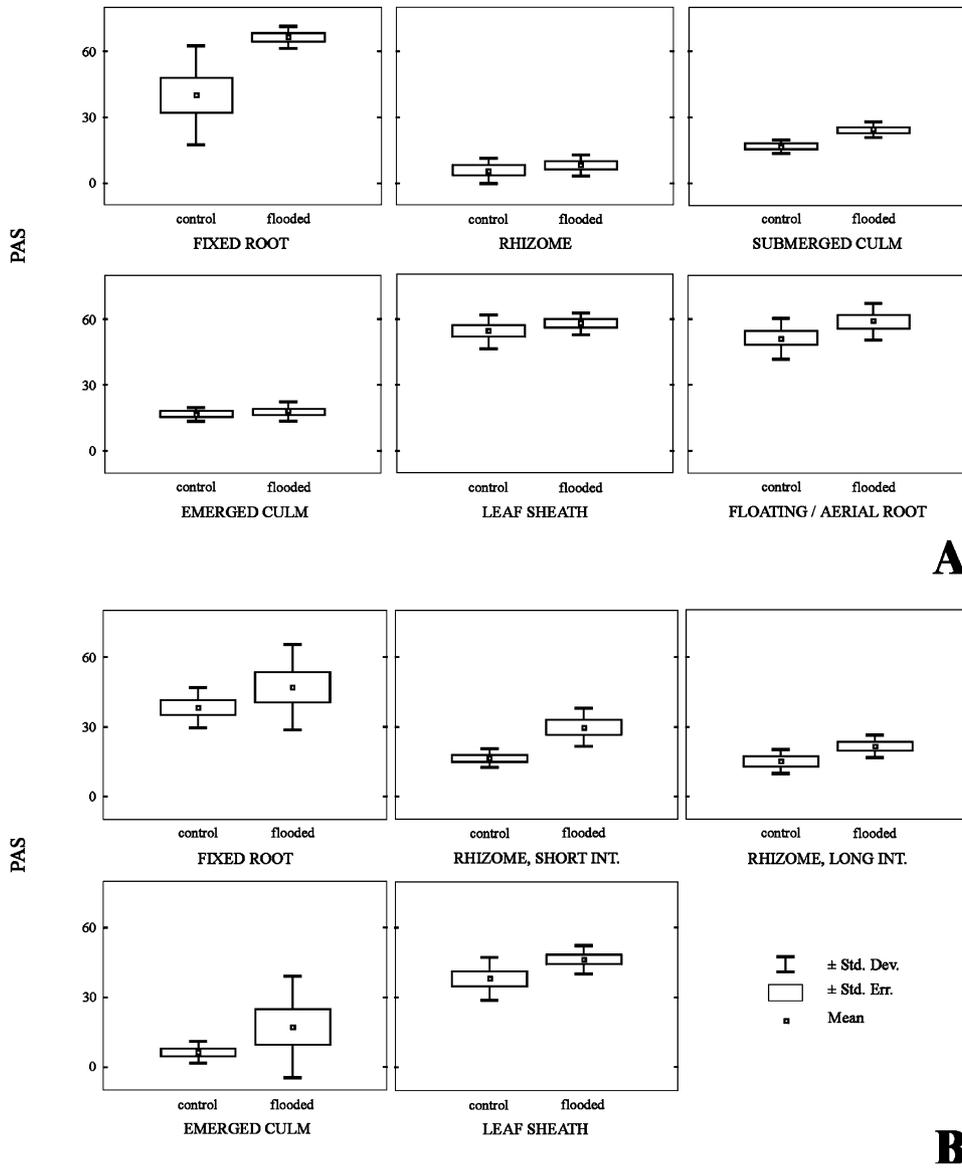
significant proportion of constitutive aerenchyma, is typical of plants able to survive and compete successfully in flooded habitats (Justin and Armstrong, 1987; Laan et al., 1989). The increase of aerenchyma formation in both species probably implies a relatively high efficiency in internal oxygen transport from aerial to submerged organs, in order to prevent hypoxia and facilitate gas interchange with the rhizosphere (Jackson and Drew, 1984; Armstrong et al., 1994; Jackson and Armstrong, 1999; Visser et al., 2003).

Although formation of aerenchyma is known to be controlled in many plants by ethylene activity (Jackson and Armstrong, 1999; Evans, 2003; and references therein), it remains unclear if it is also true in flooding-tolerant species (Smirnoff and Crawford, 1983; Jackson and Armstrong, 1999). In *P. modestum* and *P. wrightii*, the occurrence of large air spaces even under non-flooded conditions suggests that hypoxia

does not trigger the formation of aerenchyma. Formation of aerenchymatous tissues seems rather to be an outcome of endogenous morphogenetic processes, as suggested for rice (Jackson and Drew 1984), and hypoxia seems to function only as a regulator of their relative amount (Rubio et al., 1995).

Similarity in aerenchyma placement and distribution among flooded and control plants seems to indicate that the pattern of cell death which leads to lysigenous spacing is associated to cell position rather than to cell ontogeny (Jackson and Armstrong, 1999; Drew et al., 2000; Evans, 2003).

As stated above, aerenchyma-forming cells in the root cortex of *P. modestum* and *P. wrightii* are arranged in a radiate pattern, what is a common feature among grasses (Justin and Armstrong, 1987). As usual among monocots inhabiting flooded areas, roots of *P. modestum* and *P. wrightii* bear one or two hypoder-



**Fig. 3.** Variation of percentage aerenchymatous space (PAS, i.e. the ratio between the area occupied by aerenchyma and the total cross-sectional area) in different organs of plants of: (A) *P. modestum* and (B) *P. wrightii* cultivated under two different hydric conditions.

mal layers composed of cells with thick, lignified cell walls (Smirnoff and Crawford, 1983). Nevertheless, occurrence of Casparian bands in such cell layers has not been demonstrated, so the exodermal nature of them (Peterson, 1988; Peterson and Perumalla, 1990) remains uncertain. The loss of cortical tissue in aerenchymatous roots make them vulnerable to changes in hydrostatic pressure (Jackson and Armstrong, 1999). Lignification and suberization of hypodermal cell walls contribute to avoid root collapsing, as well as function as a barrier preventing oxygen loss and excluding soil toxins which can be frequent in flooded soils (Končalová, 1990; Armstrong et al., 1991; Peterson, 1992).

In addition to the relatively high proportion of constitutive aerenchyma in roots and leaf sheaths, a plastic response of PAS to soil water conditions occurred. Nevertheless, such plasticity was partitioned differently among organs in both species. In fact, whereas in *P. modestum* substratum-fixed roots were largely responsible for the increase in aerenchymatous spacing, in *P. wrightii* the increase of aerenchyma production was homogeneous among different organs.

The contrasting patterns of aerenchyma formation in *P. modestum* and *P. wrightii* in response to flooding are expectable when considering the different hydric conditions of the habitats in which each of these species grows. In fact, areas inhabited by *P. modestum* are

nearly permanently flooded (Carnevali, 1994), so the abundant constitutive aerenchyma can be regarded in this species as an adaptive hygromorphic trait. Except in substratum-fixed roots, the PAS varies only slightly in response to fluctuating hydric conditions, within the limits imposed for our experimental design. It remains unknown to what extent the PAS in culms and leaf sheaths varies in response to deeper flooding.

On the other hand, *P. wrightii* inhabits areas in which flooding is annually followed by a striking dry period (Allem and Valls, 1987; Schessl, 1997; Pozzobon and Valls, 2003), during which the profuse rhizome system of this species remains alive in the dry soil. When the flooding season returns, the basal portions of the plants become submerged. The coordinated plastic response of PAS in all submerged organs allows the plants of *P. wrightii* to suit to the annual drought-flooding cycle.

Some graminoids growing in shallow water show root dimorphism, probably indicating a division of work between root types. In fact, the thick and poorly branched soil roots behave as anchor organs, whilst the profuse and finely branched aquatic roots function mainly in nutrient uptake (Končalová, 1990). In *P. modestum*, the limited increase of aerenchyma formation in the relatively slender superficial roots when compared with those fixed in the substrate are probably related to a higher ability to take in the oxygen dissolved in superficial water, and to advantageously exploit such resource because of a high surface/volume ratio (Armstrong et al., 1991). In contrast, the erect culms of *P. wrightii* bear no floating roots, but the development of profuse slender roots into submerged containers suggests that this species is able to renew its aerenchymatous tissues by annually replacing its root system at the begin of the flooding season (Jackson and Drew, 1984).

Finally, we are aware that, although the individual plants of *P. modestum* and *P. wrightii* used in our study displayed responses to flooding as described above, further assays including at least several genotypes of each species are still necessary to assess the hereditary nature of such phenotypic plasticity (Thompson, 1991; Via et al., 1995).

## Acknowledgements

We are indebted to P. Insausti for critical review, and to J.J. Valla and the staff of the “Lucien Hauman” Botanical Garden for keeping the material under cultivation. Collection of *P. wrightii* in the Brazilian Pantanal was facilitated thanks to the collaboration of Rosângela M.T. Magalhães-Schessl, Miramy Macedo, and the authorities of the IBAMA at Cuiabá, MT (Brazil). This investigation was supported by the grants AG-007 and JG-21 of the Buenos Aires University, Argentina.

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