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An anatomo-ecological experiment in *Austrostipa aristiglumis*, a lowland Stipoid species.

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Abstract

Leaf and sheath anatomy characteristic of an aquatic or amphibious plant is reported for *Austrostipa aristiglumis* (Gramineae). *Austrostipa* and the rest of the Stipoid grasses are normally considered to be classic xeromorphs. The degree of development of the hydromorphic characteristics appears to be related to the level of soil saturation. The ecological and phylogenetic significance of these observations is discussed.

Introduction

The Stipeae is a cosmopolitan grass tribe of c. 450 species, widely distributed and not clearly delimited (Barkworth & Everett 1987). The tribe has major centres of diversity in South and North America, Australia and Eurasia, with different genera dominating in each centre. The tribe is characterised by having florets with a single spikelet, the lemma usually being coriaceous or firmer, having comparatively large unicellular macrohairs, and a range of other epidermal features that help delimit the genera.

The species grow primarily in temperate or warm-temperate regions, and are dominant in many of the arid grasslands of southern Australia, South America, and Asia at varying elevations (0 –5000 m) and the ecological range includes arid, semiarid, semihumid and humid habitats (Barkworth & Torres 2001).

Within the tribe, there are recurring patterns of morphological and anatomical variation. Anatomy in the Stipeae has been the subject of numerous studies, with the majority on leaf anatomy being carried out for taxonomic purposes. Parodi (Parodi 1944; Parodi & Freier 1945) proposed that leaf anatomy was of value in determining generic limits in the tribe. Later studies in American species, have confirmed the importance of leaf (as well as lemma and palea) anatomical characters (Brown 1958; Matthei 1965; Reeder & Reeder 1968; Caro & Sánchez 1971,1973; Clifford & Watson

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1977; Thomasson 1978; Barkworth 1981; Scholz 1982; Watson & Dallwitz 1981, 1992; Arriaga 1982, 1983; Renvoize 1985; Roig 1964 [a & b], 1965; Torres 1993,1997 [a , b & c] ; Peñailillo 1996; Rojas 1997; Cialdella & Arriaga 1998; Arriaga & Barkworth 2000; Cialdella & Giussani 2002).

Taxa from south-west Asia were revised by Freitag (1985), and the Australasian stipoid taxa by Vickery et al. (1986), Jacobs et al. (1989) and Jacobs and Everett (1996).Vazquez (1992), Vazquez and Devesa (1996, 1997) and Vazquez and Barkworth (2004) treated the stipoids of Morocco and the Iberian peninsula, and Martinovsky (1976) those of central Europe. However, anatomical characters were not included in these studies. This study is part of a larger project being undertaken by the *International Stipeae Working Group*(1) to explore the diversity within the Stipeae with the ultimate goal of developing a strongly supported taxonomic revision of the tribe. The results could be used to predict how the distribution of stipoid species, and other grasses with similar characteristics, would be affected by changes in climate.

Stipoids are known to be species adapted to mesic and xeric environments. With the exception of *Trikeraia* (Clayton & Renvoize 1986), there is no mention of any relationship with water in the descriptions of the species habitat, nor any mention of hydromorphic characteristics, in the tribe.

In this contribution we describe the leaf and sheath structure of *Austrostipa aristiglumis* (F. Muell.) S.W.L. Jacobs & J. Everett, a species of semi-arid to sub-humid habitats that has hydromorphic characters that change with the level of soil saturation. While *Austrostipa aristiglumis* grows in a variety of habitats, it is most common on the intermittently-flooded, grey self-mulching clays or heavy alluvial soils of the westerly-flowing watercourses of the winter-dominant rainfall areas of the Murray-Darling Basin.

Methods

Six pots 190 mm internal diameter and 190 mm high were planted with diaspores of *Austrostipa aristiglumis* (RK24, voucher held at NSW) in November 2002, at Sydney, Australia as this is the time of year seedlings are found in the field. The pots were placed outside in five trays 6, 11, 44, 86, and 136 mm deep, and one pot (0 mm) retained as a control with no tray. The treatments were arranged linearly in random order from random number tables (0 mm position 4; 6 mm position 5; 11 mm position 2; 44 mm position 3; 86 mm position 6; 136 mm position 1). The pots were filled with a mixture of potting mix and clay. The trays were each maintained filled with water so that each pot had different levels of saturated soil. Each time the trays were filled the control was also watered and allowed to freely drain. The pots also received natural rainfall.

One year later, after flowering, all the surviving plants from different treatments and the control (0mm, 11 mm, 86 mm) were preserved in 70% ethyl alcohol in November 2003, and herbarium voucher specimens prepared from the 11 mm and 86 mm treatments). This date was selected for harvesting because the plants had flowered and good vouchers could be collected.

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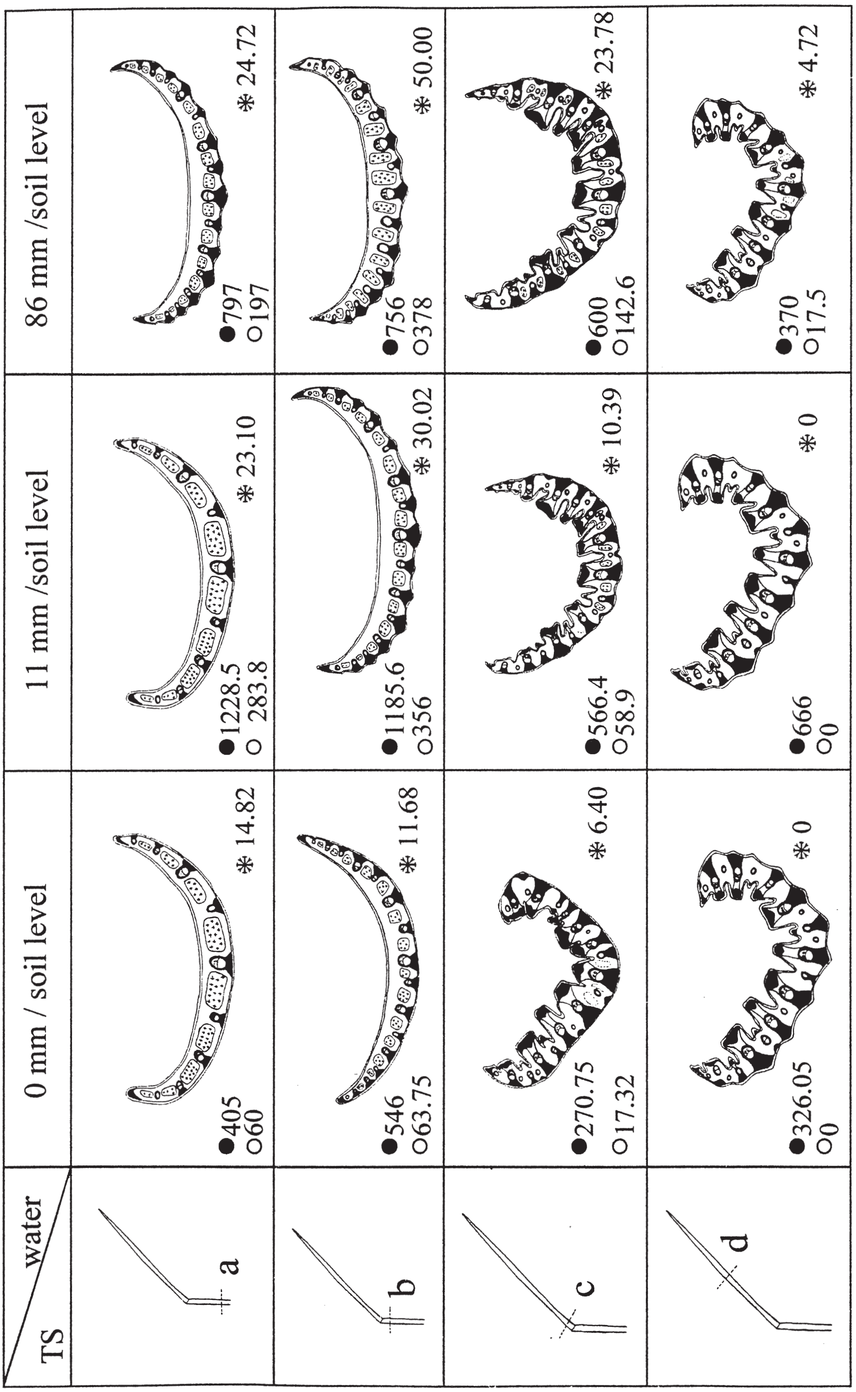

Fig. 1. Transections of leaves and sheaths of *Austrostipa aristiglumis*. Schemes representing: **a**, TS at the middle of the sheath; **b**, TS of sheath below the ligule region; **c**, TS of leaf beneath the ligule region; **d**, TS at the middle of the leaf. Legend: ● Total leaf area expresed in µm^2; ○ Total cavities area expresed in µm^2; ❄ relationship of air cavities to total leaf area expressed in %.

Transverse sections were made from leaves and sheaths of preserved specimens. Sections, from the middle segment of lamina and sheath and from 5 mm over and under the ligule portion, of the second or third lamina of a sterile innovation, were obtained either freehand or by embedding the material in wax and sectioning with a rotary microtome. The sections were stained with Alcian Blue/Safranin (Cutler 1978) and observed and photographied using a Leica DMLB optical microscope with a JVC digital camera. Drawings of the cross-sections were prepared using a camera lucida. Measurements of total leaf area and total cavities area were made with the IM50 Leica' software, averaged from 10 measurements each.

Results

Leaf anatomy in transection

The transection (TS) outline is U-shaped (Fig. 1). Both semilaminae may be symmetric or asymmetric (differing in one rib). The upper surface has ribs and deep (>50% of leaf thickness) and narrow furrows. Ribs alternate between large and flat-topped, and smaller and round-topped; larger ribs are coincident with one 1st or 2nd order vascular bundle (VB); smaller ribs are coincident with 3rd order vascular bundles (Figs 1, 2). The lower surface varies from flat to slightly angular.

The midrib is scarcely distinguishable with only one 1st order VB located in the centre of the transection (Figs 1, 2). Neither colourless parenchyma nor transitional parenchyma are associated with the midrib. There are subepidermal strands of sclerenchyma under both epidermes in the midrib (Fig. 1). In specimens growing in more than 10 mm of water aerenchyma is associated with the midrib, situated in channels, laterally to the VB in TS made 5mm above the ligule (Fig. 2); aerenchyma is not present in TS made in the midrib at the middle level of the leaf. When present, aerenchyma (and the corresponding lacunae in TS which contains it) represents 20 –40% of the TS.

The semi-laminae have >6 VBs, alternating 1st, 2nd and 3rd order; 1st and 2nd order are situated in the centre of the TS or towards the lower surface; all 3rd order VBs are towards the lower surface (Figs 1, 2). No colourless parenchyma is present in the semilaminae. Sclerenchyma is present under both epidermes, fragmented, in girders; sub-epidermal transitional parenchyma is also present adaxially. Sclerenchyma and transitional parenchyma produced over all types of VBs, heavily over 1st and 2nd order (Figs 1, 2). Aerenchyma in the basal portions of the semi-laminae is present in leaves of specimens growing with >10 mm of water in the tray, channels between VBs are present in 60% of the semi-laminae.The channels with aerenchyma occupy 20 –40% of the depth of the TS. The 1st order VBs in the semilaminae are elliptic to ovate, with a strand of phloem adjacent to the xylem. First order VBs have an outer vascular bundle sheath, and are abaxially and adaxially interrupted by transitional parenchyma or sclerenchyma girders; and have an inner sheath complete around the VBs (Fig. 2). Walls of cells of the inner sheath in contact with phloem are more thickened than the other sheath cell walls, the thickening in "U" or "O" form. Second order vascular bundles are elliptic, with the outer sheath complete around the VBs or abaxially interrupted only by sclerenchyma; the inner sheath is complete. Third order VBs are circular, with complete outer and inner sheaths. No extensions of the bundle sheath were seen.

Leaf margins are acute in TS, with sclerenchyma or transitional parenchyma in the margins (Figs 1, 2).

The epidermal cells in TS are smaller and thicker-walled over the VBs. Bulliform cells occur between all VBs, at the same level or slightly above the level of the other epidermal cells (Fig. 2). Epidermal hairs are absent.

Sheath anatomy in transection

The outline of the transection is permanently folded. The upper surface is flat; the lower surface is flat or ribs and furrows are slightly developed. There are more than 6 VBs in TS, alternating 1st, 2nd and 3rd order, all located at the same level, in the centre of the TS or towards the lower surface (Figs 1, 2). No colourless parenchyma is present in the TS. Abaxially there is fragmented sclerenchyma below the epidermis, exceptionally also adaxially. Sclerenchyma or transitional parenchyma is associated with all types of VBs. Aerenchyma is present between all VBs, in lacunae, situated in the centre of TS, or closer to the abaxial surface occupying 40 –70% of the depth of TS, sometimes >70%.

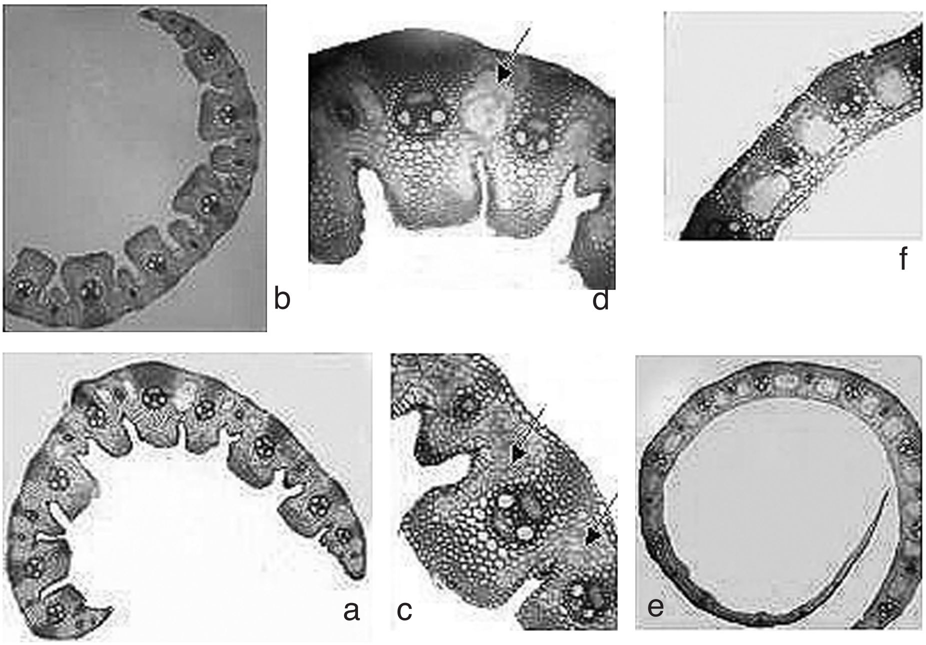

Fig. 2. Photomicrographs of *Austrostipa aristiglumis*. **a,** TS below the ligule region, showing air cavities developed on a leaf under 11 mm saturated soil treatment (35×); **b**, half of a TS below the ligule region, with no air cavities developed on a leaf under 0 mm saturated soil treatment (35×); **c**, TS of leaf showing bigger inter-cellular air spaces (arrows) in the first stage development of an air space cavity (70×); **d**, TS of leaf showing remains of cell walls from cells that have disintegrated to form an air cavity (100×); **e**, TS of sheath beneath the ligule region of a leaf under 86 mm saturated soil treatment (35×); **f**, detail of previous transection (70×).

First order VBs are ovate, with a strand of phloem adjacent to the xylem; the outer vascular bundle sheath abaxially is interrupted by sclerenchyma girders; inner vascular bundle sheaths are complete around the bundle. Second order VBs are elliptical, with an outer VB sheath abaxially interrupted by sclerenchyma girders, the inner sheath complete around the VB. Third order VBs are circular, with an outer sheath abaxially interrupted by sclerenchyma girders, and the inner sheath complete around the VBs.

No extensions of the bundle sheaths were seen (Fig. 2). Walls of the outer sheath cells are not thickened, walls of the inner sheath cells are "O" thickened, cells in contact with the phloem have thicker walls. Margins are acute in TS, with sclerenchyma or transitional parenchyma only in the margin.

Epidermal cells over the VBs are smaller and thicker in transverse section than the epidermal cells between. Epidermal hairs are absent.Tangential external walls of epidermal cells are thicker than internal walls. Stomata are in both epidermes or only in the adaxial epidermis.

The air canals have a lysigenous origin in both the leaf and the sheath. Close to the growing points, some cells separate from adjacents cells, developing larger spaces. Afterwards some cell walls begin to disintegrate and form cavities resulting in the building of big air channels along the sheath and up to the middle of the leaf. Remains of the cell walls can always be seen inside the air channels.

Discussion

Renvoize (1985) described the outstanding feature of the tribe Stipeae as the adaptation by many genera to dry habitats, reflected in the often xeromorphic nature of the leaf blade morphology and anatomy. Among the species listed at the end of his work is *Trikeraia hookeri* (Stapf) Bor but there is no mention of hydromorphic characters, despite *Trikeraia* being described as living by water (Clayton & Renvoize 1986), often on hummocks in bogs or swamps.

Austrostipa aristiglumis, on the other hand, is a species mostly growing on intermittently-flooded inland floodplains in semi-arid and sub-humid zones. These soils are dry most of the time.

Sculthorpe (1967) and Font-Quer (1970) described as amphibious those species that may live in dry soils flooded during a period of the year. One theory suggests that different groups or clades of angiosperms have retained different levels of vegetative plasticity that is manifiest in varying abilities to adapt to aquatic habitats (Arber1920; Sainty & Jacobs 1982; Jacobs & Wilson 1996). Amphibious species provide, in a structural and an ecological sense, a gradual transition between truly terrestrial and truly aquatic species (Sculthorpe, l.c.).

One characteristic of hydrophytous or amphibious species is the presence of air cavities that develop early in the different organs of the plant, while their number and size varies with the age and nature of the organ (Sculthorpe, l.c.).

The hydromorphic characters, stimulated by increasing soil saturation, presumably are an adaptation that allows *A. aristiglumis* to maximise its growth when water is available, even in "excess".

Aliscioni (2000) and Fabbri et al. (2006 in press) in their studies of the anatomy of the genus *Paspalum*, concluded that, under wet substrate conditions, species developed air cavities and aerenchyma and that this ability to develop air cavities represents a constant character. Aliscioni (2002) considered that this plasticity should be considered in systematic and phylogenetic analysis. We agree with that assessment, especially as *A. aristiglumis* represents the only known Stipoid species with hydromorphic plasticity, a characteristic that may have value in phylogenetic considerations.

Representatives of the *Stipeae* have been included in numerous molecular phylogenetic studies of grasses (Hsiao et al. 1999; Jacobs et al. 2000; Jacobs et al. 2006 in press); all show it to be a basal lineage within the *Pooideae*. Studies of the *Stipeae* molecular phylogeny (Jacobs et al. 2006 in press) support recognition of *Austrostipa* as a well-differentiated clade that includes *A. aristiglumis*. All the other species in that clade are adapted to live in xeric and semi-mesic habitats. Only *A. aristiglumis* has been observed to have the genetic adaptability and vegetative plasticity to capitalise on the intermittently-flooded habitat.

Conclusions

1) In *Austrostipa aristiglumis* the sheath structure in all cases (soil with normal water content or saturated soils) always has channels with aerenchyma; the channels increase in size with increasing saturation of the soil.

2) In *Austrostipa aristiglumis* the leaves show channels and aerenchyma in the lower part of the laminae, near the meristem and only in those samples living on the more saturated soils.

In any case, the channels and aerenchyma decreaeses and are completely lost towards the middle of the lamina.

3) In *Austrostipa aristiglumis* leaf and sheath air cavities have a lysigenous origin.

4) Most stipoid grasses have morphological and anatomical adaptations to mesic and xeric environments but *Austrostipa aristiglumis* is an unusual case of a stipoid grass adapted to intermittent flooding in inland south-eastern Australia, representing an amphibious plant.

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