Are more productive varieties of *Paspalum dilatatum* less tolerant to drought?

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Abstract

Paspalum dilatatum Poir., is a perennial C_4 grass widely distributed in the Argentinean Pampas. The response to water availability for materials developed with forage-production purposes is unknown. We hypothesized that genetic differences between commercial varieties are reflected in their regrowth capacity under water stress. The effect of five levels of constant water supply on three plant varieties (two derived from apomictic materials: 'Relincho' and 'Alonso' and one from sexually-derived material: 'Primo') were examined in the greenhouse. Leaf- and plant-response traits were followed during 38 d after a single defoliation event. Seven response variables were measured: three of them were morphogenetic (leaf elongation rate, leaf appearance rate and leaf elongation duration) and four were structural (number of live leaves, lamina length, tiller biomass and tiller production). The sexual material showed higher values for growth variables than the apomictic varieties (leaf elongation rate, leaf length and tiller biomass) across the environmental range. Apomictic varieties showed a proportionally similar drought response to the sexual material for the seven variables. No intra-specific trade-off (statistical interaction) was found between growth under high water availability conditions and drought tolerance.

Keywords: trade-off, phenotypic plasticity, *Paspalum dilatatum*, drought tolerance, productivity, plant breeding

Introduction

Growth and productivity in most grasslands of the world is limited by drought (Gastal and Durand, 2000).

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Water availability is highly heterogeneous in space and time, and this heterogeneity is related to the composition of plant communities (Mcnaughton, 1983; Medina and Motta, 1990). Plants respond to such variability through morphological and physiological changes. These changes represent a plastic, ontogenic response to the environment. As a result of biotic and abiotic stress, all plants, even tolerant genotypes, are affected in their performance. However, not all genotypes respond to extreme conditions in the same way (Bradshaw, 1965).

To assess grass production many authors define sward 'structural' variables. The main variables are lamina length, number of live leaves per tiller and tiller/tiller (number of tillers). In turn, these variables are determined by a number of leaf-level 'morphogenetic' growth variables, mainly leaf lifespan (LLS), leaf appearance rate (LAR) and leaf elongation rate (LER) (Figure 1; Chapman and Lemaire, 1993; Lemaire and Chapman, 1996; Hirata and Pakiding, 2002).

Morphogenetic and structural components of swards are not only associated with the genotype but also depend on growth conditions (Chapman and Lemaire, 1993). Namely, these traits are linked to developmental changes in response to environmental conditions. This is termed phenotypic plasticity (sensu Bradshaw, 1965). Plastic responses have been described in plants under drought. One of the traits most responsive to drought was found to be LER, and thus it represents a sensitive index of growth under drought (Hsiao, 1973; Onillon *et al.*, 1995). Grasses grown under drought conditions exhibit short stature, prostrate morphology, relatively steady tillering, and reduced tiller biomass (cf. Coughenour, 1985). The same general morphological changes are observed for palatable grasses in response to grazing (Milchunas *et al.*, 1988; Kotanen and Bergelson, 2000), not only as a plastic, but also as an evolutionary response (Quiroga *et al.*, 2009).

Stress not only reduces current leaf extension and growth, but can also impose a limitation on regrowth potential (Turner and Begg, 1978; Busso *et al.*, 1989). Recovery from defoliation depends not only on
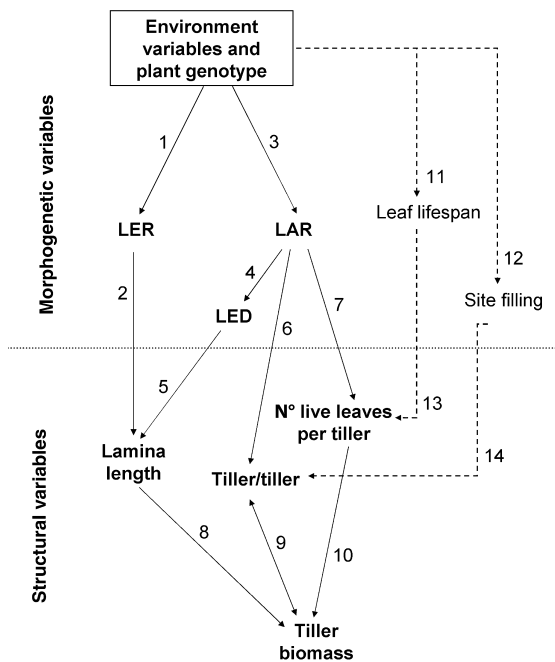
Figure 1 Relationship between morphogenetic and structural variables (modified from Chapman and Lemaire, 1993; and Hirata and Pakiding, 2002). Measured variables (arrows 1–10) are connected by *full lines*; other variables, which will be dealt with in the discussion (arrows 11–14), by *broken lines*.

the inherent capacity of the plant and the type of defoliation, but also on the biotic and abiotic environment (Maschinski and Whitman, 1989). After a defoliation event, the plant enters in a transient phase of rapidly changing carbon and nutrient allocation patterns (Richards, 1993). Defoliation and drought reduce a plants' carbon input, but carbon allocation priority turns out to be conflicting. Drought induces an increase of carbohydrates allocated towards roots, whereas with defoliation it is towards shoots (Gastal and Durand, 2000). Mueller and Richards (1986) and Busso *et al.* (1989) suggested that bud activation after defoliation would be comparatively more important for tiller production than bud number or viability. Drought can also reduce tiller production in defoliated and undefoliated plants (Busso *et al.*, 1989; Bullock *et al.*, 1994; Briske and Hendrickson, 1998).

Paspalum dilatatum Poir. is a palatable C_4 warm-season grass native to South America that grows in humid and temperate grasslands. In Argentina, it is distributed across a wide drought-flood gradient (Burkart *et al.*, 1990), and several studies have evaluated its responses to water supply (Loreti and Oesterheld, 1996; Colabelli *et al.*, 2004; Mollard *et al.*, 2008), and also estimated improvement due to its high forage value (Schrauf *et al.*, 2003). However, this has been difficult

because of the apomictic reproduction characteristics of this species, until crossing of *P. dilatatum* sexual ecotypes was made with *Paspalum urvillei* Steud., another species from Argentina which grows under different water regimes. The varieties thus obtained and used in this study were: Relincho and Alonso (apomictic) and Primo (sexual). Relincho was selected for its superior establishment and forage quality (germination speed, initial growth and low stem/leaf ratio, high density of small tillers, and high capacity for regrowth), at least without water restrictions. Alonso comes from a drier and warmer area compared with Relincho, and was selected for high forage production. Primo was selected for resistance to *Claviceps paspali* and for high forage production potential. It is a variety with a *Paspalum urvillei* introgression (grass from wetter and warmer area and lower forage quality, with a higher stem-to-leaf ratio than *Paspalum dilatatum*). The water stress response of all these materials is unknown (Schrauf *et al.*, 2003).

When genotypes with higher yield are grown close to optimal conditions, they allocate a large proportion of resources to biomass production. In turn, genotypes with an evolutionary history in limited-resource environments will have a greater proportion of resources allocated to tolerate these limitations (e.g. more root than shoot growth), and it is expected that these costs would result in a lower production even under conditions close to optimal. That is to say, there could be a trade-off between the ability to tolerate resource scarcity and to grow when resources are abundant (Chapin, 1980; Huston, 1994; Bazzaz, 1996). According to this trade-off model, genotypes which have higher growth under optimal conditions will have lower growth under resource scarcity. Alternatively, the null hypothesis (no trade-off) predicts that all genotypes will show a proportional decrease in their growth when under stress conditions (cf. Fernandez and Reynolds, 2000).

In this paper, the results of a greenhouse experiment are reported. It was designed to study *Paspalum dilatatum* varieties' regrowth after defoliation, measuring structural and morphogenetic variables. As stated above, the response of these three varieties to water stress is unknown but, in addition, no quantitative information is available either on their response under non-limiting resources.

This work has two objectives. The first was to quantify in a common environment the varieties' traits under both water restriction and unrestricted conditions. The second was to evaluate the regrowth of this palatable grass after defoliation under drought conditions to find out whether the genetic differences between selected varieties are associated with their drought tolerance. According to the trade-off hypothesis, it was expected that genotypes from regions with

water deficit or with structural characteristics associated with drought tolerance such as Alonso would be less affected by water stress than Relincho. Besides, Primo from more humid region and with more mesophytic characteristics was expected to present greater sensitivity to drought than both apomictic materials.

Methods

Plant material

The plant varieties studied (Relincho, Alonso and Primo) originated from two *P. dilatatum* subspecies with different degrees of ploidy and different phenotypic characteristics. Relincho and Alonso are *P. dilatatum* ssp *dilatatum*, apomictic and pentaployd. These materials come from flooding and no-flooding communities (Burkart *et al.*, 1990). Relincho is a cultivar selected for its high establishment ability and initial growth. Alonso is an experimental material selected for its high productive potential and summer growth. Primo, *P. dilatatum* spp *flavecens* is sexual and tetraploid; it comes from no flooding communities with warmer summers. Of the three varieties studied, Primo is the one with the highest potential production. It was obtained by a 'backcross method' (Harlan and Pope, 1922), aimed to incorporate *Claviceps paspali* resistance into *Paspalum dilatatum*. It is an introgression of *Paspalum dilatatum* ssp *flavescens* ecotype Virasoro with one accession of *Paspalum urvillei*. Both are native to warmer and more humid climates than *P. dilatatum* spp. *dilatatum*, while *P. urvillei* is resistant to *Claviceps paspali*.

Drought treatments

Five different levels of drought were imposed by a subirrigation method which permitted a constant and uniform water potential in pots. This technique was first proposed by Haan and Barfield (1971) and then developed by Snow and Tingey (1985) and used in several studies (see Fernandez and Reynolds, 2000 for references and a diagram). As with any well-controlled method, conditions differed from the more variable ones encountered in the field but, on the other hand, allowed a greater degree of certainty regarding plant physiological responses (vs. for example, diverse rooting patterns). The subirrigation device comprises a column of porous commercial Styrofoam (Smithers-Oasis) by which the water rises through capillary action to the base of each pot. Individual plants were grown in pots sitting on the Styrofoam columns, which in turn were placed inside large plastic containers. The pot bases were permeable to water flux, and thus the water rose from the water container to plant roots. The water depth determined the distance from the water surface to the base of the pots (a sort of 'water table depth'), and thus the level of water availability to plants.

Experimental design

This was a greenhouse, split-plot experiment. The main plots corresponded to water supply treatment (five levels). The subplots corresponded to *Paspalum dilatatum* commercial varieties (three levels: Relincho, Alonso and Primo).The main treatment was imposed on fifteen plastic containers (5 water levels × 3 blocks × replication). Twenty-seven pots were placed in each container on top of Styrofoam columns as explained above. The total number of pots in the experiment was 405 (27 × 5 drought levels × 3 blocks).

The five levels of water availability (water table depth) were: 5 cm (control), 10, 14 and 18 cm (intermediate drought), and 22 cm (extreme drought). Each of the five plastic containers from each block was randomly assigned at one level of water supply. A diluted standard nutrient solution was used to ensure that water was the only limiting factor.

Nine genotypes (each from the same mother plant) were obtained from each variety in order to better represent their variability. For each of the nine mother plants [genets (sensu Kays and Harper, 1974)], fifteen samples (ramets) were obtained. Each of these were transplanted into individual pots and assigned randomly to one of the fifteen plastic containers. Thus, nine subsamples were included for each variety, leading to a constant genetic variability within each water-supply treatment. The pots were filled with sand and fitted at their base with a permeable cloth.

Plants were subjected to 90 d of one of the five levels of water availability after 12 d of acclimation (high resource availability). After 52 d aboveground biomass was harvested to simulate grazing (with scissors at 2 cm above ground). Results shown correspond to 38 d of regrowth (before plants were pot-bound) and describe the varieties' response under different water availability. In the glasshouse, light availability was measured as an average PAR (photosynthetically active radiation) of 1100 μmol m^{-2} s^{-1}, and average maximum and minimum temperatures were 28 and 18°C respectively. Tillers and leaves which emerged from day 53 until final harvest (day 90) were identified and marked. New tillers and emerged leaves were marked twice a week and length of each marked leaf was measured from the moment of emergence until the end of elongation (when the ligule appears). Number of live leaves per tiller, date of appearance of new leaves on each tiller, and total number of tillers were also recorded twice a week. These data were measured for each pot and then seven variables were calculated. Three of them are 'morphogenetic': leaf appearance rate (LAR,

leaves/tiller/d), complete leaf elongation duration (LED, d), and leaf elongation rate (LER, cm/leaf/d); and four are 'structural' variables: final lamina length (cm), number of live leaves per tiller, tiller/tiller (increase in number of tillers from the initial rating) and tiller biomass (mg/tiller at final harvest) (see Figure 1).

LER was measured as final elongated length divided by the time it takes to reach the final length (final lamina length/LED) (Bahmani *et al.*, 2000; Lemaire and Agnusdei, 2000; Bultynck *et al.*, 2004). LAR was estimated through the average time between the appearance of two successive leaves in a tiller. This estimation was used to estimate the number of leaves per day as 1/d between appearance of successive leaves.

Statistical analysis

Results were analysed using the 'InfoStat' statistical package (Universidad Nacional de Córdoba, Argentina). Analysis of variance (ANOVA) and Tukey mean comparison tests were performed with a significance level of 5%. ANOVA assumptions were checked, and lamina length was log-transformed to ensure normality. Interaction between block and treatment was always non-significant $(P > 0.3)$. Tiller production was analysed using ANCOVA using the initial number of tillers as covariate. Results were graphically presented as 'norms of reaction' (or reaction norms): the response variable based on an environmental gradient in this case levels of drought. Norms of reaction for a given genotype are determined by changes in the phenotype in response to environmental changes. If the norm of reaction of several genotypes are similar (similar slopes), this means that genotypes have similar plasticity and that there is no trade-off between performance and tolerance (Sambatti and Caylor, 2007). For clarity, results are shown as graphic 'reaction norms', but the corresponding statistical analyses are summarized in Table 1.

Results

Paspalum dilatatum varieties showed differences for all variables analysed. However, when studying the effect of drought, the results differed depending on the response variable (Table 1). Still, the interaction between drought and variety was not significant for any of the seven response variables.

The sexual variety behaved differently than the apomictic varieties for the three morphogenetic variables. Of these, LER was the only variable responsive to drought. LAR and LED turned out to be insensitive to our treatments (Figure 2).

Varieties exhibited different LERs with respect to drought level (Figure 2a). Primo exhibited 36% higher LER than apomictic varieties across all treatments. LER tended to converge between varieties as drought level rose. However, LER decreased when drought increased for all varieties. Apomictic varieties always exhibited higher LAR than the sexual variety (20%). LAR was not affected by water stress (Figure 2b). The sexual variety differed in LED from apomictic varieties. The former values were 25% higher than the apomictic values. LED exhibited no response to drought (Figure 2d). Sexual and apomictic varieties exhibited differences in two of the four structural variables (number of live leaves per tiller and lamina length). Tiller biomass was the only one of the seven measured variables for which all varieties differed (Primo>Alonso>Relincho). Notably, tiller biomass was the only variable unaffected by drought (Figure 3).

Relincho presented a higher number of leaves per tiller value than Primo in the entire gradient (23%).

Table I ANOVA results (*$P < 0.05$; **$P < 0.01$). Drought was applied to main plots, and varieties to subplots (see Methods). For definition of morphogenetic vs. structural variables see Figure 1.

|	Variety effect		Drought effect		Drought × Variety	
	F	*P*	*F*	*P*	*F*	*P*
Morphogenetic						
LER: Leaf elongation rate (cm/leaf/d)	6·94	<0·01**	4·79	0·029*	1·21	0·34
LAR: Leaf appearance rate (leaf/tiller/d)	4·83	0·019*	0·38	0·818	0·64	0·734
LED: Leaf elongation duration (d)	7·83	<0·01**	0·58	0·684	0·74	0·657
Structural						
No live leaves (No leaves/tiller)	12·19	<0·01**	4·55	0·033*	0·86	0·57
Lamina lenght (cm)	21·73	<0·01**	8·26	<0·01**	1·14	0·381
Tiller/tiller (No tillers)	3·36	0·056*	5·26	0·022*	0·27	0·967
Tiller biomass (mg/tiller)	25·42	<0·01**	1·1	0·421	0·96	0·493
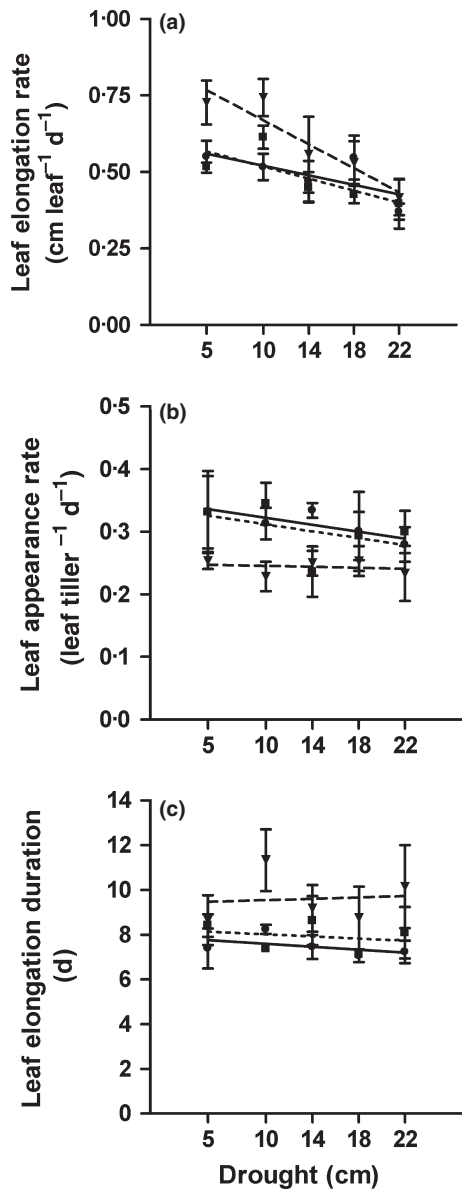
Figure 2 Morphogenetic variables under five levels of drought for three varieties of *Paspalum dilatatum*: Alonso (···■···), Relincho (–●–), Primo (–▼–) during 38 d after defoliation.

The number of leaves was affected by water stress (Figure 3a). Both drought and variety effect were found when analysing lamina length. All the varieties presented the lowest lamina length (25%) in the dry end of the gradient. However, the sexual variety exhibited values 20% higher than apomictic varieties across the water treatment range (Figure 3b). Unlike the results observed with other variables, tiller production exhibited the higher values for apomictic varieties and lower

for Primo. Relincho exhibited 60% more tillers on average than the other varieties. Tiller production showed significant response to drought. For all materials, the tiller number was 52% higher in hydrated plants than in water-stressed plants (Figure 3c). Tiller biomass is the only response variable where significant differences were found between the three varieties (Tukey >0·05). The sexual variety exhibited the highest average biomass, followed by Alonso and Relincho presenting the smallest biomass/tiller. Tiller biomass provided no response to water stress (Figure 3d). Tiller production and leaf elongation rate were the variables most affected by drought stress (decrease of 52% and 36% respectively).

Discussion

Genetic differences did not affect traits associated with drought tolerance. Contrary to our expectations, the three *P. dilatatum* experimental varieties showed similar reaction norms (no interaction between drought × variety) for all response variables. In other words, no tradeoff was found between production of *Paspalum dilatatum* genotypes under unrestricted conditions and their production under drought.

Primo, the sexual variety, showed the largest tiller biomass. Resources were mainly used to produce longer leaves, but not in higher number. In contrast, Primo had the lowest number of live leaves per tiller (Figure 3). Therefore, it can be concluded that Primo had a higher carbon cost per lamina compared to the apomictic varieties. Primo showed the lowest tiller production of the three varieties. The high cost of maintaining leaves may have affected the amount of resources available to produce new leaves and tillers. Our results match previous work showing a tradeoff between leaf growth or lamina length and tiller production (Gautier *et al.*, 1999). LER, lamina length and LED were higher in the sexual variety than in the apomictic varieties. In accordance with Primo's longer laminae than Relincho and Alonso's, consecutive leaf appearance (LAR) was faster for the apomictic materials (Figure 2). As tillers originate from axillary buds, there is a necessary relationship between the number of leaves and potential number of tillers. Genotypes that exhibit high LAR often produce a high number of short leaves per tiller and a high tiller density. Conversely, genotypes that exhibit low LAR often produce few long leaves per tiller and a lower tiller density (Lemaire and Agnusdei, 2000). The apomictic variety Relincho (and to a lesser degree, Alonso) fit the first of these 'ideotype' models; whereas Primo, the sexual variety, fit the second model. Varieties employed in this research also fit a negative trend of tiller number vs. tiller biomass (cf. Sugiyama, 1995).
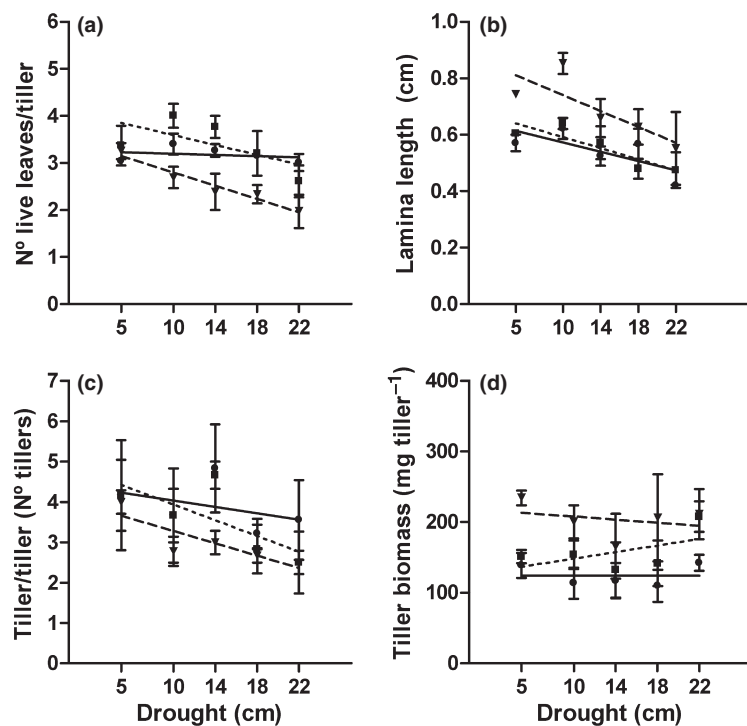
Figure 3 Structural variables under five levels of drought for three varieties of *Paspalum dilatatum*: Alonso (···■···), Relincho (—●—), Primo (—▼—) during 38 d after defoliation. Tiller biomass was measured in a final harvest.

In all cases, LER decreased under drought conditions (arrow 1 in Figure 1). Drought also decreased leaf length; this effect was mediated by a reduction in LER and was unrelated to the duration of leaf elongation (arrows 2, 3 and 4 in Figure 1), since LED was insensitive to drought. This is what could have been expected for any developmental variable, as found, for instance, in LED for perennial ryegrass (Van Loo, 1992). In contrast with what was widely observed for changes in light quality, drought did not have an effect on LAR (arrow 3 in Figure 1). Earlier drought studies under field conditions allegedly showed a reduction in this variable (Norris, 1982). However, these results would be more attributable to high temperature than to water stress (Davies and Thomas, 1983; Van Loo, 1992). In our research, the lack of response of LAR to drought would explain the aforementioned lack of response to LED (arrow 4 in Figure 1).

Both tiller production and the number of live leaves were reduced by drought. However, such response cannot be attributed to LAR, the morphogenetic variable 'upstream' most directly associated with them (arrows 6 and 7 in Figure 1). One possible explanation for this apparent discrepancy would involve two other morphogenetic variables that also influence them, namely leaf lifespan (LLS) and site filling (arrows 11 and 12 in Figure 1) (Lemaire and Agnusdei, 2000). A faster senescence caused by drought would explain a reduction in the number of leaves per tiller, even for

the same LAR (arrow 13 Figure 1). Also, a reduction in site filling [relative increase of tiller number per leaf appearance interval (Neuteboom and Lantinga, 1989)], well documented for other cases (Barker *et al.*, 1985; Van Loo, 1992), would imply a lower number of tillers (arrow 14 in Figure 1).

All three study materials exhibited shorter, less abundant leaves under drought conditions. However, tiller biomass was similar in droughted vs. control plants (arrows 8 and 10 in Figure 1). Since tiller number was reduced, this would confirm the existence of a trade off between the production of new tillers and growth of existing ones (arrow 9 in Figure 1). The constancy in tiller biomass despite fewer and shorter leaves per tiller may be explained by changes in tiller allometry (shape). The most plausible changes are expected for specific leaf area (SLA) and its reciprocal leaf mass per unit area (LMA) (e.g. Van Loo, 92; Fernandez *et al.*, 2002; Poorter *et al.*, 2009). Production of thicker, denser tissues under drought, which besides conferring more physical resistance would reduce surface-to-volume ratio and thus contribute to water saving (Mcdonald *et al.*, 2003; Poorter *et al.*, 2009).

Conclusion

The three materials examined clearly differed in production potential, as evidenced from their differences in tiller biomass under control, unrestricted conditions.

However, at least under the imposed conditions, these differences did not translate to their responses to water stress. In other words, we did not find a trade-off between growth under conditions of high water availability and tolerance to drought. The tall material from humid environments (Primo) showed greater tiller biomass under all water-availability conditions. Varieties Alonso and Relincho, despite having traits that *a priori* seemed associated with drought tolerance (e.g. numerous, small tillers, short stature), did not show a superior performance under stress.

Acknowledgments

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