LEAF GROWTH AND ANATOMY DURING WINTER DROUGHTING OF TETRACHNE DREGEI PLANTS

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ABSTRACT

The purpose of this work was to study the leaf growth and anatomy of *Tetrachne dregei* plants when they were subjected to a winter drought period. Plants were grown in semicontrolled conditions under two soil moisture regimes: irrigated and non-irrigated. Anatomical characterization was made using optical and scanning electron microscopy. No differences were found in stomatal index, stomatal length and epidermal cell size, between irrigated and non-irrigated plants. The adaxial epidermis of water stressed plants was more pubescent. Leaf abaxial epidermis of non-irrigated plants showed more intercostal plates of crystallized epicuticular wax than that of the irrigated ones. Leaf tissue distribution was not affected by the different moisture regimes. The effect of water deficit was neither detected on plant height, nor on leaf width and length. Except for the enhanced adaxial pubescence and abaxial wax crystal content, no other xeroplastic changes were noticed.

KEYWORDS

Tetrachne dregei, growth, anatomy, water relations, water stress

INTRODUCTION

Tetrachne dregei is a perennial grass growing in South Africa, and considered to be a good herbage contributor. Its leaves remain green throughout the winter period as well as maintaining a high protein level. These characteristics, together with good palatability, determine animal preference and uncontrolled use produced the diminution of the population in the original South African grasslands.

Although no information was available about *T. dregei* as a cultivated pasture, it was introduced into Argentina with the aim of improving the herbage quality in the temperate semiarid region, where winter and spring droughts are common. Several investigations were carried on climate analogies, germination, and sowing and harvesting methods (Milano and Rodriguez Sáenz, 1971; Veneciano *et al*., 1992 a and b).

Leaf anatomical characters, such as the size and the density of the stomata, are important in the regulation of the carbon dioxide metabolism and the water status of the plants. Leaf surface coverings, including epicuticular wax and hairs, can also affect plant water status by protecting it from radiation. Leaf movement may be a beneficial mechanism for reducing exposed leaf area under water shortage conditions or high irradiation, although it may reduce the rate of leaf photosynthesis. The size and density of the stomata vary among species and even between leaf blade surfaces (Miskin and Rasmusson, 1970).

The purpose of this study was to determine how the winter droughting affects foliar development and display, and the anatomical characteristics, including the epicuticular wax morphology of *T. dregei* plants.

MATERIAL AND METHODS

Plants of *T. dregei* were grown, from seed, in the experimental field at the Departamento de AgronomÌa, Universidad Nacional del Sur. Plots consisted of 1 m^3 containers filled with a loamy sand soil. Seeds were sown in spring, and thinned out to a final population of 30 plants per m^2. There were two treatments: the irrigated (I) or control plants were watered three times a week, and the non-irrigated (NI) or water stressed plants were deprived of water throughout the 60-day experimental period. Exclusion of rainfall was achieved with an automatic sliding roof. The experiment was a randomized block design with four replications.

Plant water status was defined by the relative water content (RWC) determined according to Turner (1981). Soil moisture was determined gravimetrically.

Total plant height as well as the length and the width of foliar blades were determined weekly.

At the end of the experimental period, 2 cm-long leaf samples cut from the proximal part of the distal third of the blade were fixed in formol-acetic acid-alcohol (FAA) for subsequent anatomical study. A stomatal survey was carried out, stomatal pore length was determined and the hairs on each epidermis were quantified after removing the other epidermis and most of the mesophyllic tissue. The Stomatal Index (SI) was calculated according to Salisbury (1927).

Samples, 5 mm-long, were dried at room temperature between slides for Scanning Electron Microscopy (SEM). After coating with a carbon/gold film, they were examined with a JEOL 35 SEM operated at 0.5 Kv.

Student Newman Keuls' test was used for comparison of treatment means.

RESULTS AND DISCUSSION

Non-irrigated *T. dregei* plants maintained their RWC at values similar to those of the irrigated ones (93-95 %) during the initial six weeks. Afterwards the RWC began to decline, reaching values of 79 % by the end of the experiment. By this time, the initial soil water content of 26 % had diminished by up to 5.98 % in the upper 20 cm of soil, 9.67 % between 20 and 40 cm deep and 11.37 % between 40 and 60 cm deep.

No significant differences ($p=0.05$) between I and NI plants were detected, neither in the final plant height (70 cm), nor in leaf blade width (4.5 mm) and length (45 cm). Water deficit produced the rolling of one of the leaf margins but no other exomorphic effect was noted.

When transverse sections of the blades were observed with the optical microscope, no differences were found in tissue distribution of I and NI plants. Under both treatments two types of veins were found; the larger ones, with highly developed metaxylem elements, ranged from 4 to 6 per blade and the smaller ones, alternating between the previously described, varied from 17 to 23 per blade. Chlorenchymatic cells radially surrounded the vascular bundles. Sclerenchymatic tissue formed strands to the abaxial epidermis in the smaller veins or to both epidermis in the larger ones.

The data for epidermal cell and stomatal number, SI and stomatal length are shown in Table I. No differences were observed between I and NI plants.

When macrohairs present in the adaxial epidermis were counted, a significantly (p=0.05) higher number were found in NI plants (112 per mm^2) than in the I plants (82 per mm^2). Another difference was that although most of the hairs of the NI plants measured the same as the average hair length found on the I ones (35 ± 10 µm), some particular ones reached up to 60 µm.

Observation of the leaf surface with SEM showed that the intercostal zones of NI plants were covered with crystalline wax deposits. Under higher magnification (4000 X), it became evident that the epicuticular wax of water stressed plants formed plate-like crystals, with irregular edges, mainly arranged perpendicularly to the leaf surface, while only amorphous wax covered the intercostal zones of the well-watered plants (Figure I).

The presented data suggest that the drought resistance of *T. degrei* is due mainly to pre-existent genetically fixed characteristics, as the short-term xeroplastic changes manifested during the experiment are reduced to a higher pubescence and a different form of the wax depositions. However, these rapid responses, detected after a winter drought period of nine weeks, may be important for the maintenance of the water status of the plants. The higher adaxial pubescence and the presence of plate-like wax crystals in the epidermal zone that corresponds to the bulliform cells may be related to the fact that, under water stress, practically no rolling or folding of the leaves was noted. The structural changes occurring in the protective tissues, epidermis and its appendage, are considered to be adaptive strategies of photosynthesizing organs in response to water stress (Shields, 1950; Pyykkˆ, 1966)

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Table I
Epidermal cell density (EC .mm-2), Stomatal density (S .mm -2), Stomatal Index (SI) and Stomatal length (SL, µm) in the adaxial and abaxial epidermis of Irrigated (I) and Non Irrigated (NI) Tetrachne dregei plants.

	$EC . mm^{-2}$		$S . mm^{-2}$		SI		SL, µm	
	I	NI	I	NI	I	NI	I	NI
Adaxial	694	671	54	54	7.22	7.45	30	30
Abaxial	800	826	56	57	6.54	6.46	30	30

No significant differences were found between the two treatments, for any of the epidermal characters presented in this table, at the 0.05 level, as determined by Student Newman Keulsítest.

Figure I
Epidermis of Tetrachne dregei. (A) and (B): Irrigated plant ; (A): adaxial epidermis, (B): wax photographed in the inter costal zone of A; (C) and (D): Nonirrigated plant; (C): adaxial epidermis, (D): wax crystals photographed in the intercostal zone of C