Leaf Anatomy and Ultrastructure of Poa Ligularis After Defoliation and Water Stress

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ABSTRACT

The objective of this study was to determine the effect of defoliation and water stress on leaf anatomy and the mesophyll cell ultrastructure of *Poa ligularis*. Anatomical differences were detected mainly at epidermal level. Under water stress, leaf roughness was enhanced since the epidermal-cell surface contours became conspicuous. Under defoliation the microscopic roughness decreased as a result of fewer epicuticular wax crystals being formed. Defoliation produced an enhancement of the interlamellar spaces in the irrigated plantís chloroplast. Under no defoliation conditions, chloroplast structure was not affected by water stress. When subjected to water stress, lightly defoliated plants presented chloroplasts with a bellowed outer membrane, irregular thylacoid distribution and the disorganization of peripheral grana. Severely defoliated plants showed disorganized internal chloroplast membranes and even the disappearance of the grana. No breakdown or disappearance of chloroplast external membranes was detected under the imposed growing conditions, suggesting that no irreversible changes were induced.

KEYWORDS

Poa ligularis, anatomy, ultrastructure, defoliation, water stress

INTRODUCTION

Water and grazing are the main factors regulating plant growth in the semi-arid region of Argentina known as El Caldenal, where the principal economic activity is animal production, mainly cattle breeding, with the natural vegetation as practically the only source of fodder. *Poa ligularis* is considered a good herbage contributor during the cool season, but has largely disappeared from grazed areas.

Studies on drought and grazing resistance show that the plant's ability to survive is associated with certain genetic structural characteristics (Ristic and Cass, 1992), but the short-term adaptative changes which they undergo are also important in evaluating the ability of the plants to grow under these stress conditions.

Foliar movements and the microroughness caused by leaf venation, stomatal depressions, epidermal-cell surface contours, wax crystals and trichomes, can affect leaf water status by protecting them from radiation or by decreasing transpiration (McWhorter, 1993; Premachandra *et al.*, 1991). Epidermal characteristics may be related to leaf palatability.

Changes in the water regime of plants play a very significant role in the form and function of cells. Water stress, as well as any other stress, causes structural disburbances of the cells and the degree of these changes may determine the resistance of the plant.

The aim of this study was to study the short-term changes on the leaf structure and ultrastructure of *P. ligularis*.

MATERIAL AND METHODS

Plants were collected from an ungrazed enclosure in the southern section of the temperate semi-arid region called El Caldenal, located in central Argentina (38° 45'S; 63° 45'W). Greenhouse experiements were conducted to evaluate the effect of three defoliation levels: UD: undefoliated plants; LD: lightly defoliated plants (cut down to 15 cm) and SD: severely defoliated plants (cut down to 7 cm)., on plants grown under two different soil moisture regimes: the irrigated (I) or control plants were watered three times a week and maintained near field capacity (26%), and the non-irrigated (NI) plants were submitted to three water stress cycles imposed by withholding water up to the observation of dawn wilting symptoms in the plants (22 days each). The experiment lasted 65 days after cutting the plants in August 1993 and 1994.

The plants' water status was defined by the relative water content (RWC) and soil moisture was determined gravimetrically.

Leaf display modifications were expressed as the ratio of projected width of folded leaves, to total projected width following unfolding (Carter and Smith, 1985). Foliar ridging was calculated on fresh hand-cut transverse sections, by measuring the angle formed by the sides of the adaxial epidermal ridges (Jones *et al.*, 1980).

At the close 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 the subsequent anatomical studies on hand-made sections, observed with optical microscope.

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

For ultrastructural studies, 2 mm^2 samples were fixed in glutaric aldehyde (2.5%) on cacodilate buffer (pH: 7.2) and post-fixed with OsO_4. Finally the material was dehydrated in a series of alcohol and acetone and embedded in resin. Sections were made on a LKB V 2088 ultratome, stained on grids with aqueous uranyl acetate and lead citrate and observed under a JEOL 100 CX Electron Microscope.

RESULTS AND DISCUSSION

The water content of the soil in irrigated pots was maintained between 23 and 26% throughout the experiment. The initial decline in the water content under water stress was rather higher in UD than in the defoliation treatments, reaching the final values of 2.3, 4.3 and 7.5% in UD, LD and SD treatments, respectively.

RWC of I plants was 86% under all defoliation treatments, and for NI reached 30% by the end of the experiment, with no significant difference between the defoliation treatments.

In well-watered plants folding of foliar blades presented no differences (p=0.05); the ratio of projected / total width being 0.90 on the first data and 0.80 for the remainder of the measurements. In water stressed plants, folding of foliar blades was smaller in defoliated than in UD plants throughout the stress period, the final projected / total width ratio being 0.68, 0.75 and 0.81 for UD, LD and SD plants respectively. The differences in these values were due mainly to folding by the midvein of the water stressed UD and LD foliar blades. *P. ligularis* presents bulliform cells along both sides of the main vein, and their loss of turgescence, when UD and LD plants are submitted to water deficit, could be detected by the diminution of the angle between the middle ridge and each of the two adjacent smaller ridges, from the initial values of 45-65° to nearly 0°.

Observations of transverse sections the leaf blades showed a highly developed chlorenchyma surrounding the vascular bundles, and abaxial or both abaxial and adaxial extensions of the sheaths reaching towards the epidermis.

When observed with SEM, the surface micromorphology of NI plants showed the consequences of the loss of turgescence, as evidenced by conspicuous epidermal-cell surfaces contours that enhanced roughness, specially in ND plants.

The presence of fewer epicuticular plate-like crystals of wax gave a smoother appearance to leaf surfaces of defoliated plants, when compared with the undefoliated ones (Figure 1).

In I plants defoliation treatments did not affect the general organization of the cells, but the chloroplasts presented enhanced interlamellar spaces.

Water stress produced the separation of the plasmalema from the mesophyllic cell walls and small vesicles appeared in the cytoplasm. In UD plants the chloroplast membranes remained organized. In LD plants, chloroplasts presented bellowed outer membranes, irregular thylacoid distribution and disorganized peripheral grana. SD plants showed disorganized internal chloroplast membranes and even disappearance of the grana.

No disturbance of nuclei or mitochondria was noted and no breakdown or disappearance of chloroplast external membranes was detected under the imposed growing conditions.

In view of the presented data it may be concluded that defoliation on its own does not affect the anatomy of the leaves of *P. ligularis*, nor the structure of the functional organelles.

When plants were submitted to water stress periods, the ND plants evidenced a higher loss of turgescence. Although final values of RWC were similar for all the defoliation treatments, during the experimental period, UD plants presented the lowest values of RWC. For this reason the highest folding and epidermal roughness was noted in NI-UD plants.

At an ultrastructural level, it could be seen in NI plants that the more defoliated the plants were, the more disorganized was the cell chloroplast. Nevertheless, no breakdown or disappearance of chloroplast external membranes was detected during the experimental periods, suggesting that no irreversible changes were induced.

P. ligularis seems to withstand grazing well, but care must be taken when soil water availability is deficient, as defoliation worsens the effect of water stress.

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Figure 1

Epidermis of irrigated plants of *Poa ligularis*. (A) and (B): Undefoliated plant; (A): adaxial epidermis, (B): hair photographed in the intercostal zone of A; (C) and (D): Light defoliated plant; (C): adaxial epidermis, (D): hair photographed in the intercostal zone of C.