WATER STRESS-INDUCED CHANGES IN MORPHOLOGY AND ANATOMY OF FLAG LEAF OF SPRING WHEAT

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ABSTRACT

Flag leaves of wheat (drought hardened and non-hardened) were examined by light microscopy to determine whether the differences in leaf anatomy could be related to the known differences in dehydration tolerance. Plants exposed to water stress during tissue differentiation of flag leaves resulted in an irreversible reduction of leaf area and thickness, increased frequencies of stomata and higher number of bulliform cells with simultaneous decrease in number of intermediate veins and an increase in the share of the cell walls in total cell volume. The smaller leaf thickness was due to a diminished number of mesophyll layers and a decreased size of mesophyll cells. Such altered leaf anatomy indicated development of leaf xerophily. It was found that the irreversible changes in anatomy of wheat flag leaves play a decisive role in acquiring drought tolerance during wheat acclimation to drought.

KEY WORDS: Triticum aestivum, leaf anatomy, drought hardening

INTRODUCTION

The anatomical and morphological alterations associated with plant growth under water deficiency are well documented in the early literature reviewed by Maximov (1929), Levitt (1956) and Stocker (1960). In general, the increasing water deficit results in a decrease in the size of the epidermal, stomatal and mesophyll cells, an increased number of stomata, and palisade parenchyma layers and increased thickness of the outer epidermal cell walls. Further investigations were involved in quantitative description of plant development and dependence on environmental factors, including water stress (Nobel 1980, Hsiao and Bradford 1983, Lawlor and Leach 1985). However, we are only at the beginning of our understanding not only of the effects of drought but even of the regulation of leaf growth in general. Of particular interest in environmental influences on leaf anatomy and the accompanying consequences for photosynthesis is the flag leaf, a major contributor of photosynthesize to the developing grains in wheat ear (Patrick and Wardlaw 1984).

Grasses like wheat have unifacial leaves. Although leaves are amphistomatous, stomata are more dense on adaxial leaf surfaces. In the upper epidermis there are bulliform or motor cells (cellulae bullatae) lying midway between the veins (Esau 1965, Jordan 1983). The loss of water from the bulliform cells cause leaf rolling and adaxial surface becomes enclosed. Thus, the abaxial surface with lower stomatal density is the only surface that remains exposed to the atmosphere and the effective transpiration may be halved (O’Toole and Cruz 1980, Jordan 1983). Wheat has a uniform mesophyll arrangement that is not differentiated into the distinct palisade and spongy layers, although some cells beneath each epidermis appear to be elongated like palisade tissue (Esau 1965, Nobel and Walker 1985). The vascular system of the wheat leaf consists of three types of longitudinal bundles i.e. the midrib and six large laterals (three on either side of the midrib), small laterals and intermediates of various sizes (Patrick 1972). The intermediate veins are the most important distributors of water to mesophyll cells (Altus and Canny 1985) and they appear to function as loading, collecting and distributing reservoirs of assimilates (Altus and Canny 1982).

Our earlier studies have shown that water stress applied to spring wheat during flag leaf differentiation led to considerable increase in dehydration tolerance of that leaf (Zagdańska and Pacanowska 1979). Such ability of the flag leaf to hardening (acclimation) seems to be responsible for the continued dry matter production by the leaf under conditions of subsequent drought. Sustained growth under water stress may be facilitated by osmotic adjustment, increased cell wall elasticity and increased fraction of apoplastic water in the cell (Radin 1983). The only evidence supporting this suggestion is the correlation found between the amount of bound water and the cell wall volume (Boyter 1967). Moreover, Cutler and Rains (1977,1978) reported on the decreased cell size and thickening of the cell walls in the leaves of cotton pretreated with drought. The aim of this study was to check whether acclimation gained during water stress pretreatment complies with the modification of morpho-anatomical properties of wheat flag leaves.

MATERIAL AND METHODS

Spring wheat (Triticum aestivum L. var. lutescens cv. Kolibri) plants were cultivated in pots under greenhouse conditions. Control non-stressed plants were grown at high...
moisture level (60% of soil capillary capacity) throughout the whole experimental period. Other plants were drought-pretreated (drought-hardened) at the shooting phase (VNI stage of organogenesis according to Kuperman 1973) i.e. during flag leaf differentiation. All details of the experiment were described in the previous paper (Zagdańska and Pacanowska 1979). All measurements and observations were made on at least three fully matured, non-senescence flag leaves detached from control and drought-hardened plants.

Leaf area was measured by a planimeter (Automatic Are- ameter Hayashi Denkoh Co.).

Stomatal density was determined on abaxial and adaxial leaf sides by making replicas of stomata on silicone rubber. For each replica, the total number of cells and the number of stomata in a given area were recorded.

Stomatal index (SI) was calculated as follows:

\[ SI = \text{(no. of stomata/no of total cells)} \times 100 \]

For anatomical studies, samples were always taken from the midblade region. Hand-cut transverse sections were optically bleached, the content of the cells was dissolved with chloral hydrate, washed with 1% acetic acid and stained with alcan blue, SGS (Fluka), iodine green (Merck) and carmine alum (Merck). Observations and measurements were made with three leaves from which three cuttings were examined with an eyepiece micrometer under stereoscopic microscope MBS-301 and NU Zeiss.

**RESULTS**

Pretreatment of plants with water stress during the early stage of flag leaf development (i.e. during flag leaf differentiation) altered the final morphology and anatomy of hardened leaf. Hardening led to a marked reduction in leaf area and to an increase in stomatal density on adaxial and abaxial surfaces of the leaf (Tab. 1). At the same time, the ratio of adaxial to abaxial stomata frequency and stomatal index (stomata as a portion of a total of epidermal cells) remained unchanged (Tab. 1).

**TABLE 1.** Comparison of leaf area, stomatal frequency and stomatal index of flag leaves from control and drought-hardened wheat.

<table>
<thead>
<tr>
<th></th>
<th>non-hardened</th>
<th>hardened</th>
<th>LSD at 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf area (cm²)</td>
<td>21.2</td>
<td>13.6</td>
<td>0.9</td>
</tr>
<tr>
<td>Stomatal density (mm⁻²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(adaxial surface)</td>
<td>60.0</td>
<td>78.0</td>
<td>1.0</td>
</tr>
<tr>
<td>(abaxial surface)</td>
<td>41.0</td>
<td>50.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Stomatal index</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(adaxial surface)</td>
<td>15.0</td>
<td>16.0</td>
<td>1.0</td>
</tr>
<tr>
<td>(abaxial surface)</td>
<td>11.5</td>
<td>12.5</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Drought-pretreatment affected also the differentiation of the vascular system. In hardened leaf, the number of vascular bundles was reduced and the average distance between them tended to increase (Tab. 2). The number of intermediate veins, which are the last-formed and the smallest bundles arising in leaf primordium was reduced (from 42 to 33) in the hardened leaf. The reduction in the number (from five to two) of intermediate bundles is shown in Fig. 1. Other alterations in leaf anatomy induced by water stress are best seen in Figs 1-4 and Table 1 and 2. As has been shown, drought pretreat-ment affected not only the number of vascular bundles, but also their anatomy. In the case of midrib (Fig. 2) sclerenchyma fibres are more abundant on both sides of the midrib. The cells of the inner, thick-walled bundle sheath are larger in control leaf than in the hardened one. Hardening increased the number of bulliform cells from 4±1 for non-hardened control to 5±1 for hardened leaf (Fig. 1, 3-4). Bulliform cells were larger in the hardened leaf (Fig. 3).

Hardening treatment diminished mesophyll cells; they were smaller than in control leaves. The number of mesophyll layers was lowered from 4-5 in control leaf to 3 in hardened leaf. The hardened leaf thickness was reduced from 121 μm (control leaf) to 84 μm (hardened leaf) as measured in mid-way between each pair of adjacent bundles (Tab. 2). The walls of mesophyll cells in hardened leaf were thicker than in control leaf. Hardening led also to the formation of more thick-walled mechanical cells, mainly in the outermost edge of a leaf (Fig. 4) and directly above and below the veins (Fig. 2).

**TABLE 2.** Comparison of leaf thickness and number of veins if flag leaves from control and drought-hardened wheat.

<table>
<thead>
<tr>
<th></th>
<th>non-hardened</th>
<th>hardened</th>
<th>LSD at 0.05</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf thickness (μm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>at veins</td>
<td>143*</td>
<td>124*</td>
<td>5.0</td>
</tr>
<tr>
<td>inter veins</td>
<td>121**</td>
<td>84**</td>
<td>4.0</td>
</tr>
<tr>
<td>Intervenial distance (μm)</td>
<td>192***</td>
<td>211***</td>
<td>7.0</td>
</tr>
<tr>
<td>Vein type: number of veins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>midrib</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>large lateral (L₂, L₃, L₄)</td>
<td>6</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>small lateral (L₁, L₅)</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>intermediates</td>
<td>42</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>53</td>
<td>44</td>
<td></td>
</tr>
</tbody>
</table>

LSD at 0.05: * = 5.0; ** = 4.0; *** = 7.0

**DISCUSSION**

The presented data show that water stress pretreatment of flag leaf tissues during the early stage of its development resulted in an irreversible reduction of the leaf area. The substantial reduction in final leaf size resulted likely from the water stress-induced inhibition of cell expansion and cell division. In grass leaves, cell divisions are limited entirely to the basal meristem so that lamina expansion in the nearby distal regions becomes entirely dependent upon cell enlargement (Kriedemann 1986). Since the rate of cell enlargement determines the frequency of cell division (Körner et al. 1989), water stress may also result in fewer divisions. Leaf area expansion is very sensitive to any water deficit in a tissue (Bradford and Hsia 1982) but it can recover after stress is relieved (Acevedo et al. 1971, Hsiao and Bradford 1983, Sakurai et al. 1986). However, the growth of flag leaf did not completely recover. The main reason for such irreversible effect of water deficit on the leaf area expansion may be due to the altered response of growth to cell turgor i.e. changes in the extensibility of the tissues and in the turgor necessary to initiate growth (Matthews et al. 1984). A decrease in cell size is a general characteristic of leaves subjected to water stress during development and is regarded as an adaptive response,
Fig. 1. Transverse sections (adaxial surface uppermost) of flag leaves of control (A) and drought hardened (B) wheat showing equivalent portions of the leaf blade (from the edge of the leaf to small lateral Ls vein), x125

Fig. 2. Transverse sections of flag leaves of control (A), and hardened (B) wheat showing the midrib, x287
Fig. 3. Transverse sections of flag leaves of control (A) and hardened (B) wheat between the midrib (M) and small lateral (L₁) vein, x108

Fig. 4. The edge of the blade of control flag leaf (A) and of the hardened one (B), x300
which may markedly reduce water loss and improve heat exchange and thus, increase plant drought tolerance.

The greater stomatal frequency in the hardened leaf is a common response of plants to water stress but its meaning to the increased drought resistance has not been fully elucidated. Studies carried out on isogenic lines of barley (Jones 1977, 1985) did not demonstrate a positive correlation between stomatal frequency and transpiration. Since leaf rolling contributes to reduced transpiration independently of stomatal closure, the higher number of bulliform cells in the leaf epidermis of hardened leaf may lead to a more efficient rolling and improved plant water balance (O'Toole et al. 1979, Begg 1980, O'Toole and Cruz 1980, Jordan 1983). Therefore, the observed reduction of cell and leaf sizes and the leaf rolling response seems to be more important for drought tolerance than the reduced stomatal frequency.

The adaptation of leaves to drought in our experiment was accompanied by a decrease in leaf thickness by 20-30% (at veins and intervines, respectively). This was caused not only by a smaller size of mesophyll cells but also by the reduced number of mesophyll layers. The thinner leaves and smaller cells occurred also in many other plants as a result of water stress (Nobel 1980). The size of mesophyll cells is strongly negatively correlated with photosynthetic rate in many plants (Jellings and Leech 1984) including wheat (Austin et al. 1982). We have found that this negative relationship is maintained in acclimated wheat leaves, since CO₂ uptake (expressed in mg CO₂ dm⁻²h⁻¹) was similar in both fully turgid, non-hardened and hardened leaves with smaller cell size (Zagdańska 1984).

The similar photosynthetic rate per unit area in the acclimated leaf in comparison to control may indicate that cessation of cell expansion could itself account for the accumulation of photosynthates. Photosynthates not used in growth of the acclimated leaf were probably responsible for the observed formation of thick-walled mechanical cells, thicker cell walls of epidermis, mesophyll and bundle sheaths. Thus, acclimation increased the share of cell walls in the cell volume. The same finding was made in developing cotton subjected to drought (Cutler and Rains 1977).

Acclimation reduced the number of intermediate veins and thus, increased slightly the distance between the veins. However, the reduced number of veins with the concomitant decrease in the number of mesophyll layers have no influence neither on water supply to mesophyll nor to the loading of assimilates.

The data presented above indicate that acclimation of wheat flag leaf to soil drought is associated with reduced growth and alterations in leaf anatomy, which may be regarded as adaptation favouring tolerance. These modifications indicate the development of leaf xerophily, which improves water conservation and overall drought tolerance of spring wheat.

ACKNOWLEDGEMENTS

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LITERATURE CITED


ZMIANY MORFOLOGICZNO-ANATOMICZNE LIŚCIA FLAGOWEGO PSZENICY JAREJ WYWOŁANE STRESSEM WODNYM

STRESZCZENIE

Stwierdzono, że susza glebowa występująca w czasie różnicowania się tkanek liścia flagowego prowadzi do nieodwracalnych zmian w jego morfologii i anatomii. Zmiany te wyrażały się zmniejszoną powierzchnią liścia, zwiększoną ilością aparatów szprzekowych na jednostce powierzchni i zwiększoną ilością komórek zawiasowych, a także redukcją ilości wątek przewodzących. Zaoberwano także zwiększony udział ścian komórkowych w całkowitej objętości komórki. Zmniejszona grubość liścia spowodowana była redukcją ilości warstw szprzeli i zmniejszeniem rozmiarów komórek mezofilu. Obserwowane zwiększenie ksaryzmu liścia flagowego wiązało się z podwyższoną zdolnością do tolerowania odwodnienia.

SŁOWA KLUCZOWE: Triticum aestivum, anatomia liścia, odporność na suszę.