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Impact of water stress on the leaf tissues of two Moroccan varieties of durum wheat *(Triticum durum* Desf)

N. Assem*[1], L. El Hafid [2], F. Lamchouri [1]

[1] *Laboratory of Materials, Natural Substances, Environment and Modeling (LMSNEM)*
Polydisciplinary Faculty of Taza, Sidi Mohamed Ben Abdellah University of Fez, Morocco.
[2] *Laboratory of Water, Environment and Sustainable Development (LEEDD)*
Faculty of Sciences-University Mohamed First Oujda, Morocco

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- ✓ durum wheat;
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N Assem
najat.assem@usmba.ac.ma
(+212) 0630265652

Abstract

Cereals are the most important crops in Morocco. However, rapid population growth and climate warming are expected to advance the threat of drought, especially in areas with a semi-arid climate, as in the Mediterranean region. This work, we studied the impact of drought through irrigation stop on the anatomy and ultrastructure of leaves in two Moroccan varieties of durum wheat "Oum rabia" sensitive and "Marzak" resistant to drought. The results obtained showed that the thickness of the leaf and the anatomical features are influenced by the water stress. Indeed, the mesophyll thickness and the intercellular space decreases in the leaves of stressed plants compared to control plants. Cells chlorophyll parenchyma hydrated leaves are rounded while the cells of the stressed leaves are folded and deformed in both varieties. The study of ultrastructure of the variety "Oum rabia" under water stresss shows, in fact, a folded cell wall, a plasma membrane with invaginations, altered chloroplasts and disorganized thylakoid membranes. These changes are not visible in the drought resistant "Marzak" variety. This variety appears to have a very important membrane tolerance.

1. Introduction

Climate change has manifested itself in Morocco in the form of agricultural droughts from the beginning of the 80's. Drought may start at the beginning of the growing season from November to January where short periods of deficit water are frequent [1], followed by a second period of drought during the last phase of the season (March - April), and another longer from May to October [2].

Frequent droughts and temperature increase have caused severe damage to the agricultural sector, particularly for cereal production, which is dominant in Morocco.

The effect of drought at the ultrastructural level has been rarely studied [3, 4, 5].The first studies in drought conditions, in relation to alterations of the foliar ultrastructure, were carried out on the study of the enzymatic activity of lipases and phosphatases in the cotton plant, *Gossypium hirsutum L.*, drought-sensitive species and *Gossypium anomalum Waw and peyr.*, drought-resistant species [6 and 7].

The interest given to the effect of water stress is mainly on cytochemical studies. Consequently, it seemed interesting to undertake the evaluation of the impact of water stress on the anatomy and ultrastructure of the leaves of two Moroccan varieties of durum wheat, the sensitive variety "*Oum rabia*" and the resistant variety to the drought "*Marzak*".

2. Materials and methods

2.1 *Plant material and culture conditions*

The study was carried out on two Moroccan varieties of durum wheat "*Oum rabia*" sensitive and "*Marzak*" resistant to drought [8]. The selected seeds used were offered by the National Seed Marketing Corporation (SONACOS).

The Seeds are grown in greenhouse pots, in the laboratory of Cytology and Plant Morphogenesis of the University of Paris VI, Jussieu, France. Each pot filled with peat contains 15 plants and 4 pots are used in each experiment. The irrigation system used is spider (set at 1 minute of irrigation / day) with 15 hours of illumination, 9 hours of darkness and a temperature of 25 °C.

2.2 Structural and ultrastructural techniques

At the 3-leaf stage, treated plants are subjected to water stress by stop irrigation for 2 weeks and control plants are regularly watered. Thin sections from the top of the 3rd leaf were made for the
treated and control plants.

Fixation is carried out with a mixture of 1.5% paraformaldehyde and 2% glutaraldehyde in 0.1M sodium cacodylate buffer for 4 h; Postfixation is carried out in a mixture of 1% osmium tetroxyde in 0.1M sodium cacodylate buffer for 4 h. The samples are deshydrated by passing in successive baths of 30 minutes each of ethanol of increasing degree, 25°, 50°, 70°, 95°, followed by three baths of 30 minutes, in absolute ethanol [9]. The inclusion is made in an Epoxy resin [10].

2.2.1 *Photonic microscopy study*

Semi-fine sections (1 μm) were made using microtome and after staining with basic fuchsin and toluidine blue, the sections were observed under a photonic microscopy.

2.2.2 *Transmission electron microscopy study*

The ultrafine cuts are made with the ultra microtome and are collected on copper grids and then stained with uranyl acetate and lead citrate.

The observations are made using the Hitachi H300 transmission electron microscopy in Toulouse, France at Paul Sabatier University in the Center for Electron Microscopy Applied to Biology.

3. Results and Discussion

3.1 *Effect of water stress on leaf anatomy of the two Moroccan varieties durum wheat "Oum rabia" and "Marzak" (figure 1)*

The semi fine section show leaves that are characterized by bilateral symmetry,the existence of two epidermises: an upper or ventral epidermis and an inferior or dorsal epidermis) and the mesophyll (figure 1),

This leaf parenchyma cells dorso-ventral (figure 1) show a single chlorophyll parenchyma lacuna and homogeneous (mesophyll) characteristic of the leaves of plant species monocotyledonous.

The parenchymal cells are generally isodiametric or elongated, more or less rounded in the corners and leaving between them empty spaces called meats. The thickness of the cells of the epidermis is very variable, the outer wall being the thickest (figure 1). This has been observed by Jarrige and al [11]. The existence of conductive tissues (xylem and phloem) is also noted.

Water stress induced considerable changes in the anatomical characteristics of the leaves, in particular a considerable decrease in the thickness of almost all the histological components of the mesophyll for the two treated durum wheat varieties (figure 1, P2 and P4). The intercellular space of the stressed leaves (figure 1, P2 and P4) is lower than that of the irrigated leaves (figure 1, P1 and P3).

The reduction of the thickness of the mesophyll is due to the decrease of the intercellular space and the volume of the cells constituting this tissue. This modification affects the shape of the cell wall of the hydrous leaves of the variety "*Oum rabia*" which becomes folded and deformed compared to the hydrated leaves of the resistant variety "*Marzak*" (figure 1, P2).

These results are consistent with those obtained by studying the effect of water stress on leaf anatomy of two avocado cultivars [4]. These authors showed a mesophilic thickness and a smaller intercellular space of the stressed plant leaves compared to the control plants.

The cells of the hydrated leaves were characterized by rounding in the chlorophyll parenchyma cells, whereas the cells of the stressed leaves are folded and deformed [4].

Reducing the size of mesophyll cells under water stress is a mechanism of adaptation to drought [12]. Indeed, the arrangement of the cells of the chlorophyll parenchyma can be the result of the reduction of the diffusion of the conductance of the stressed leaves.

According to Sarda and al. [13], the plant adopts two types of strategies, depending on the extent and duration of the water deficit. The first response is manifested by the closing the stomata before the leaf water state of the leaf is altered. If the deficit is increased a second response, involving the synthesis of abscisic acid (ABA) which acts as a mediator in responses to water stress, mainly in stomatal movements [14]. This phytohormone synthesized at the roots is transported by the vascular system and conveyed by the sap to the leaves where it causes a decrease in transpiration by the closure of the stomata [15]. Indeed, water stress induces hormonal changes [16], alterations in the metabolism of carbohydrates [17], reduces the activity of certain enzymes [18] and weakens photosynthesis [19]. Thus, the reduction in mesophyll conductance due to the salinity of leaves of Citrus sp [20], Vitis vinifera [21], and Olea europea [19] contribute to the inhibition of photosynthesis.

The observation of the ultrafine cuts by the transmission electron microscopy (figure 2) made it possible to better visualize the effect of the dryness on the cellular organelles, in particular the chloroplasts.

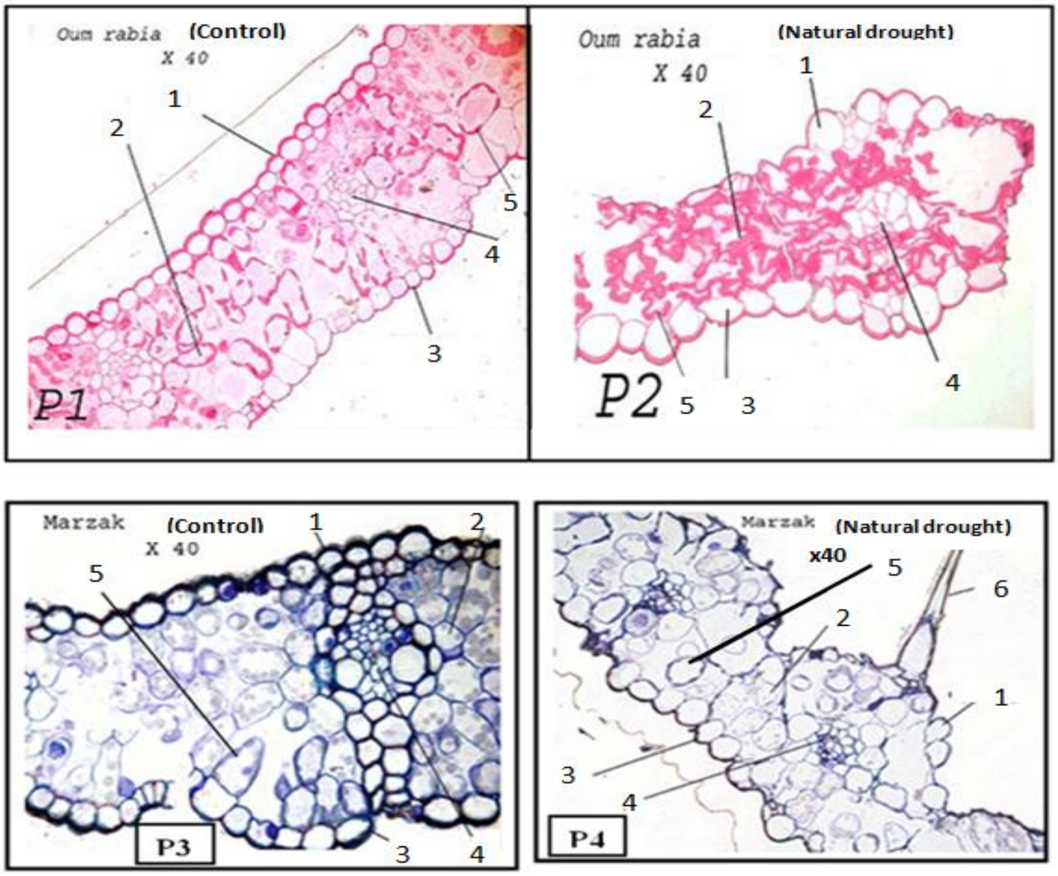

Figure 1. Cross-sections for the two Moroccan varieties of durum wheat "*Oum rabia"* **(P1, P2** and "*Marzak*" **(P3, P4) observed** under the photonic microscopy (GX40)
1: Upper epidermis or ventral; 2: mesophyll; 3: lower epidermis or dorsal; 4: vascular bundles; 5: chloroplast; 6: trichome

3.2 *Effect of water stress on the ultrastructure of the leaves of two Moroccan varieties durum wheat (figure 2).*

Ultrastructural changes accompanying hydration and dehydration were studied at the 3rd leaf level in the two varieties of durum wheat (*Marzak* and *Oum rabia*).

This study showed that control (irrigated) ultrafine cuts retain a quasi - normal structure for both durum varieties (*Marzak* and *Oum rabia*) (figures 2, a and d). The cell wall and the plasma membrane are intact. The chloroplasts have a normal shape and have more stromatic lamellae rich in thylakoides and scattered in orderly ways. A mass of lipid plastoglobules in the form of dense corpuscles with electrons is also observed (figure 2, a and d).

Under water stress by stop irrigation, the cell structure of durum leaves undergoes profound changes (figure 2, b, c and e). Chloroplasts are the organelles most affected by water stress. Plastidal stroma and grana disc stacks generally appear more dense to the electrons than those of the hydrated material and many plastoglobules can be observed. The plastid envelope and the thylakoids retain most often a normal structure. However, some plastids show dilatation of the granular and intergranial lamellae, which probably reflects the beginning of disorganization (figure 2; b, c and e).

The same transformations have been described in two *tea* cultivars, one sensitive and the other tolerant to drought. It has been shown that water stress has led to considerable structural alterations in mitochondria, chloroplast and vacuole [5]. These authors have shown that water stress has led to considerable changes in mitochondria, chloroplasts and vacuoles [5]. In *Maïze*, deep alterations of the plastidial structure were also observed during dehydration [3].

The ultrastructural transformations revealed during the dehydration of the two varieties of durum wheat are therefore very general. They are for the most part comparable to varying degrees, in the different cell types in the state of water deficiency of many plants. However, cytological changes are more pronounced in plants susceptible to desiccation than in the various tolerant plants studied (figure 2, a-e). In the susceptible variety "*Oum rabia*", the accentuated character of the plastid disorganization (figure 2, c) and the folding of the cell wall (figure 2, b) indicate poor preservation of cell integrity in anhydrobiosis. This degradation is also observed in various species during senescence [22] or in salinity conditions [23,24]. Water stress induces a disturbance of the lipid and protein composition in the cell membrane, thus affecting its stability [25].

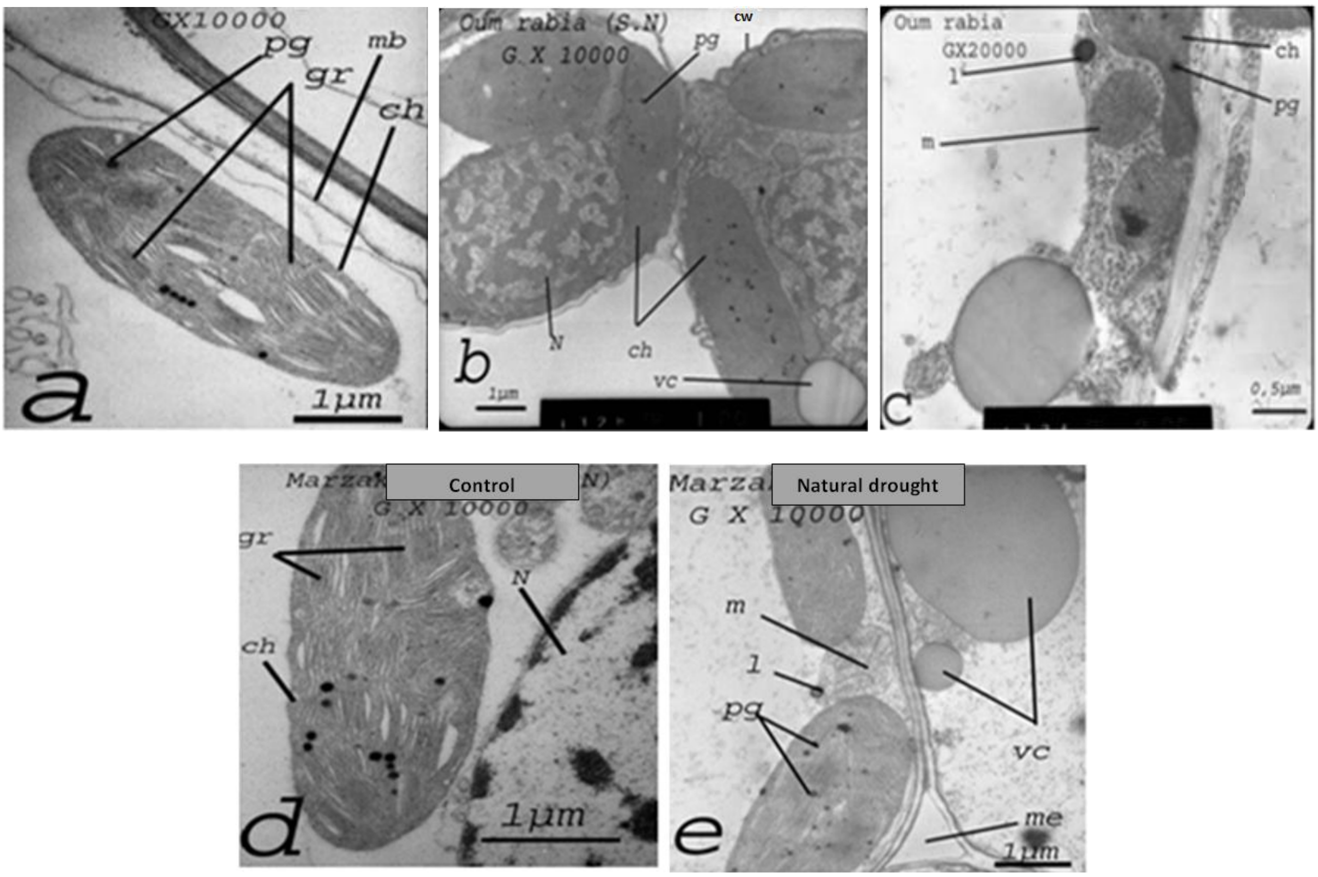

Figure 2: Infrastructural aspect of the leaves cells of the two Moroccan varieties of "*Oum rabia" durum wheat (a, b and c) and "Marzak" (d and e) subjected or not to water stress*

N: Nucleus, cw: cell wall, ch : chloroplast, gr :granum, mb : plasma membrane, me : meatus, pg: plastoglobule, vc :vacuole, l : lipid, m: mitochondria

a. control of the variety "*Oum rabia*" sensitive to drought,
b. treated of the variety "*Oum rabia",*
c. treated of the variety "*Oum rabia",*
d. control of the variety "*Marzak*" resistant to drought,
e. treated of the variety "*Marzak".*

In the tolerant variety "*Marzak*", on the other hand, the limited character of plastid disorganization, membrane and agronomic resistance [25] of the variety indicate a particularly good preservation of cell integrity in anhydrobiosis. Various systems for protecting membranes under stress conditions attempt to limit disturbances in the physico-chemical properties of membranes and to maintain an adequate level of stability [24]. This explains our results obtained in (figure 2, e) for the drought resistant *Marzak* variety. Indeed, the level of stability of cell membranes under stress conditions is an important factor in the determinism of their tolerance level [24].

Conclusion

The study of the anatomy and the foliar ultra structure of two Moroccan varieties of durum wheat "*Oum rabia*: sensitive and *Marzak*: resistant to drought" under conditions of water deficit, allowed to visualize the cell structures most affected by the drought and better understanding of wheat resistance mechanisms. These changes are more marked in the *Oum rabia*: sensitive variety compared to the *Marzak* resistant variety.

The ultrastructure of chloroplasts under the effect of drought induce disturbances in the photosynthetic function of the cells, especially in the "*Oum rabia*" variety sensitive to drought. The accentuated character of the plastid disorganization and the folding of the cell wall in the variety "*Oum rabia*" and the limited character of these two parameters in the resistant variety "*Marzak"*, indicate a particularly good preservation of the latter, the integrity of the cells in anhydrobiose.

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