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INFLUENCE OF DIFFERENT SOIL MOISTURE ON ANATOMY OF MAIZE LEAVES AND ULTRASTRUCTURE OF CHLOROPLASTS

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Summary. The structure of the 7th, 8th and 12th leaf of maize plants (*Zea mays* L. cv. Knezha-611, 2L) grown under conditions of 80%, 60% and 40% of full moisture content has been studied. Data from anatomical analysis show that the gradual depletion of soil moisture does not provoke substantial histological changes. Analysis of the leaves' ultrastucture reveals that the water deficit (at 40% of soil moisture content) caused a typical destruction of thylakoids in the mesophyll chloroplasts. Chloroplasts in the bundle sheath show greater structural plasticity and stability. Functional differences between the studied tissues are probably the most important pre-requisite for the structurally different response of the plastid apparatus.

Key words: mesophyll, bundle sheath, chloroplast, water deficit, maize plants.

Introduction

Water deficit, as a typical natural factor of stress, causes substantial functional and structural changes in the photosynthetic apparatus. Most of the researches focused on functions (Goltsev et al., 1994; Yordanov et al., 1997; Yvanova et al., 1998). Functional deviations have their structural analogue in the plastids' apparatus. Structural studies have shown that the water deficit may provoke substantial modifications in the chloroplast's organization (Freeman and Dnisen, 1975; Maroti

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et al. 1984; Zuili-Fadil et al., 1990; Stoyanova and Yordanov, 1999). Stoyanova and et al. (1987, 1990) showed that the chlorocholine chloride and abscisic acid preserved in some extent the ultrastucture of chloroplasts of droughted sunflower plants. Most frequently subject of those studies are crops of economic importance to people. During the greater part of their period of vegetation, they are under condition of partial water deficit, which frequently extends beyond the limits of the optimal saturation and turns into a factor of stress. Maize, being an important crop, has also been subject to structural and functional studies, which have tried to determine the extent of the impact of water and temperature stress (Navari Yzzo et al., 1989; Ristic and Cass, 1991). Dekov et al. (2000) have studied maize plants in hydroponics and the effect of high-temperature stress and PEG-induced water stress, applied separately or in combination. The present study aims to determine the structural state of leaves as a photosynthetic organ of the maize plants grown as soil crops under conditions of varying moisture saturation of soil (80%, 60% and 40%). It also includes comparative anatomical and ultrastructural analysis of the chloroplasts in the photosynthesizing tissues, the mesophyll and the bundle sheath, of the 7th, 8th, and 12th leaves.

Materials and methods

Plants

Maize plants (*Zea mays* L., cv. Knezha 611 2L) were grown in pots, containing 6 kg air dry meadow-cinnamon soil, which is characterized by middle fertility in relation to nutrient content. It contains 2.5% organic mattwer and pH was 5.4. The plants were grown in green house, at 22–30 °C and natural photopriod. Two plants per pot were planted. When the 3rd leaf was fully developed the plants were divided into three groups in respect to soil water content, namely:

1. 80% of full soil moisture – i.e. weak soil waterlogging;
2. 60% of full soil moisture – normal water supply;
3. 40% of full soil moisture – water stress (soil drought).

In each group 20 plants were included. The necessary moisture was maintained by means of daily correction. After four weaks of drought, when plants were 38 days old the samples were taken simultaneously from all three groups for examination of leaf anatomy and chloroplast ultrastructure. After preliminary experiments, 7th, 8th and 12th leaves were taken for analysis.

Light microscopy

Segments from the middle part of the 7th, 8th and 12th leaves were fixed in 3% glutaraldehyde (pH 7.4) and used for light microscopy with an Amplival 4 microscope (Carl

Zeiss, Jena, Germany) studies. Cross sections were cut by hand. The thickness of the lamina between the bundles, the adaxial and abaxial epidermis, the mesophyll, and the bundles were measured. Ten morphometric measurements were repeated in triplicate.

Transmission electron microscopy (TEM)

Samples for electron microscopy were taken from the middle part of lamina, fixed in 3% glutaraldehyde in phosphate buffer (pH 7.4) for 12 h at 4°C and post-fixed in 2% OsO_4 for 4 hours at room temperature. After dehydration, the material was embedded in Durcupan (Fluka, Swizerland) and cut with Tesla (Prague, Czech Republic) ultramicrotome. Ultrathin sections were stained with lead citrate. Observations were carried out with JEOL 1200 EX (Japan) electron microscope.

Results and discussions

Leaf structure.

Maize leaves (*Zea mays* L.) possess all typical properties distinguishing the representatives of *Poaceae*. They are of the panicoid type, amphistomatic to hypoamphistomtic. Adaxial epidermis is built of basic epidermal and bulliform cells. In the maize variety used in this study, bulliform cells are organized in a fan - shaped group (from 3 -5 cells), lying higher than the basic ones. The mesophyll is homogeneous, typically structured for wheat. Mesophyll cells are comparatively smaller and are laid almost densely. In the cereals, the lower the leaves are located, on the stem, the less specialized are the tissues. Leaves from 7th, 8th and 12th leaves in maize possess all histological characteristics of a leaf with the typical for this plant species leaf structure.

The 7th leaf of studied maize variety has anatomical structure typical for the C_4-type plants. Average thickness of the leaf lamina in plants grown at 80% of the soil moisture is 129,50±3,85 μm (Table 1). The epidermis consists 29% and the vascular bundle, about 37%, of the total thickness. (the ratio of thickness of lamina and that of tissues in %). The thickness of the adaxial epidermis varies considerably because of its heterogeneity (basic and bulliform cells). The thickness of tissues between vascular bundles (mesophyll) comprised 65% of of the lamina thickness. Growth of plants at 60% and at 40% moisture led to a certain modification in the tissues' ratio. At 60% of the soil moisture content, the average thickness was about 38% lesser in the 7th leaf. After comparison of morphometric data in Table 1, it becomes apparent that anatomical parameters diminished their mean values. At the same time, the percentage of epidermis and the vascular bundle increased to 35% and 40% respectively, while

Table 1. The thickness [µm] of leaf lamina and its tissues grown under different soil moisture (80%, 60%, 40%) in 7th, 8th and 12th leaves of maize plants.

	Lamina (between bundles)			Adaxial epidermis			Abaxial epidermis		
	7th	8th	12th	7th	8th	12th	7th	8th	12th
80%	129.50±3.85	133.58±1.62	153.42±2.70	21.14±0.74	25.06±0.78	23.75±0.75	16.25±0.73	18.00±0.51	16.33±0.60
60%	79.33±2.42	150.58±2.36	135.83±1.86	15.08±0.65	18.33±0.70	19.08±0.70	12.83±0.48	16.50±0.49	14.83±0.41
40%	120.58±2.72	125.25±1.66	136.58±1.87	21.17±0.85	19.58±0.64	17.92±0.62	16.83±0.74	15.58±0.33	14.25±0.38
	Mesophyll			Bundle					
	7th	8th	12th	7th	8th	12th			
80%	85.08±3.04	90.50±1.37	113.00±1.15	47.58±2.05	53.67±1.35	66.50±1.37			
60%	61.42±1.77	117.67±2.43	101.92±1.50	38.50±1.21	66.42±1.63	64.50±1.87			
40%	83.00±2.02	89.17±1.54	101.75±2.52	45.33±1.12	54.17±0.42	56.58±2.19			

the mesophyll has remained relatively stable (65%). At 40% of the soil moisture content, all parameters increased their mean values compared with plants grown at 60% of the soil moisture but were lower compared with 80% soil moisture The analysis of the share in the lamina thickness shows that the epidermis consists about 30% of the whole thickness.The vascular bundles are 40%, and the mesophyll – 69%. Data show that under conditions of water deficit the most mobile part of this leaf is the epidermis. It responses sharply, and at first sight, without any regularity regarding the change of water conditions.

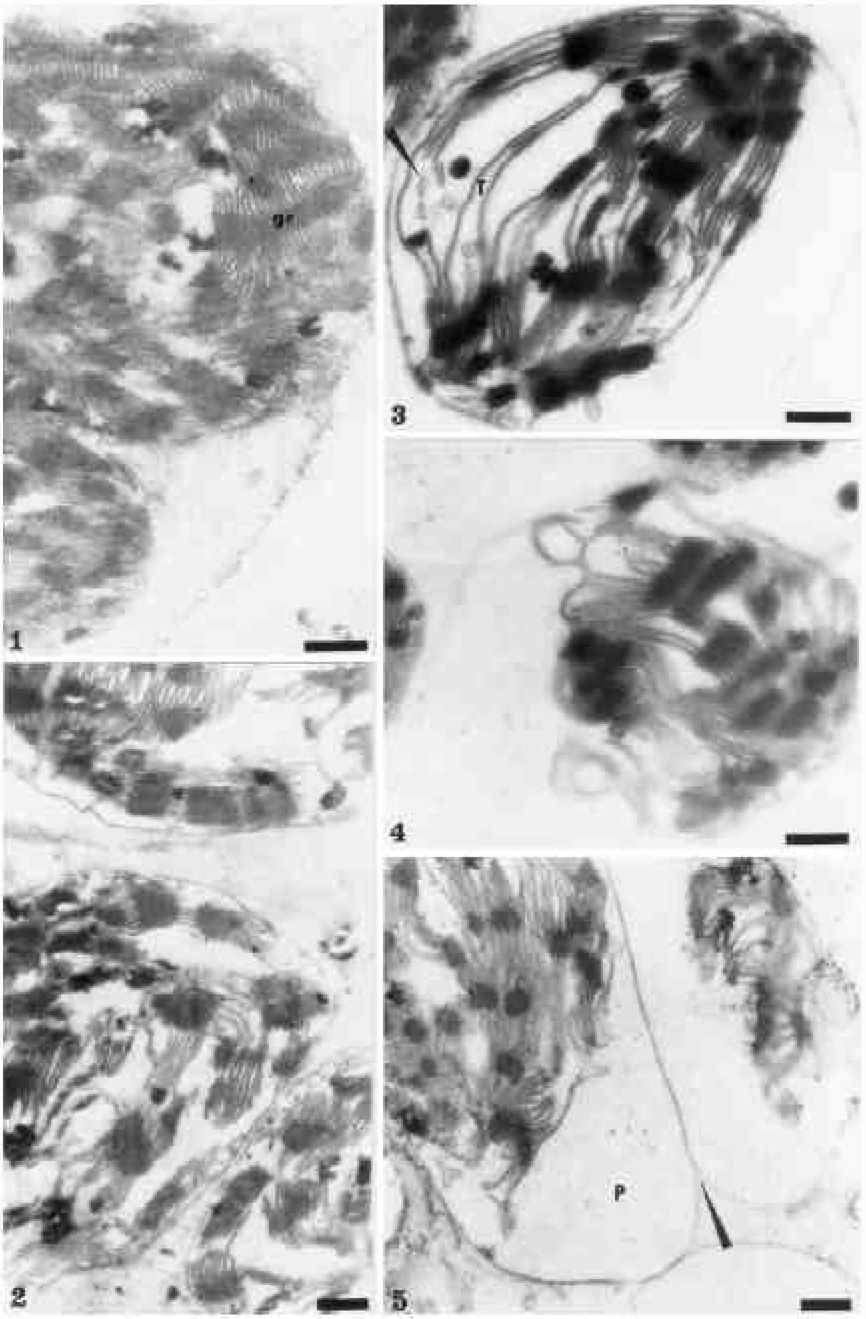
Along with the decrease of soil moisture to 60%, the thickness of the lamina of the 8th leaf increased by 12% as compared to the control plants and reached 150,58 ± 2,36 µm (Table 1). Enlarged thickness is due to the vigorously developed mesophyll and increased size of vascular bundles. The increase of mesophyll' relative share to 78% is not related to the increased number of the cell rows but to the larger volume of cells. At soil moisture of 40%, the total thickness of the eighth leaf decreased by 7% compared to the 80% and by 17% compared to the 60% soil moisture variant. This did not change substantially the ratio of its tissues.

Histological analysis of the 12th leaf showed that its lamina has the greatest thickness at 80% soil moisture content, equal to 153,42 ± 2,70 µm (Table 1). Growth of maize plants at 60% provoked reduction of its thickness by some 17%. In spite of the apparently lower values for each of the studied tissues, the mesophyll has preserved its share of 75%, while the bundle share has increased to 47%. Lowering the soil moisture to 40% the thickness of the leaf lamina and the tissue ratio has remained approximately unchanged: epidermis 28%, vascular bundles 45%, and mesophyll between bundles 70%.

Anatomical analysis of the 7th, 8th and 12th leaves showed that under conditions of gradually decreasing soil moisture no substantial histological changes occur that may change more or less profoundly the tissues ratio

Ultrastructure of chloroplasts

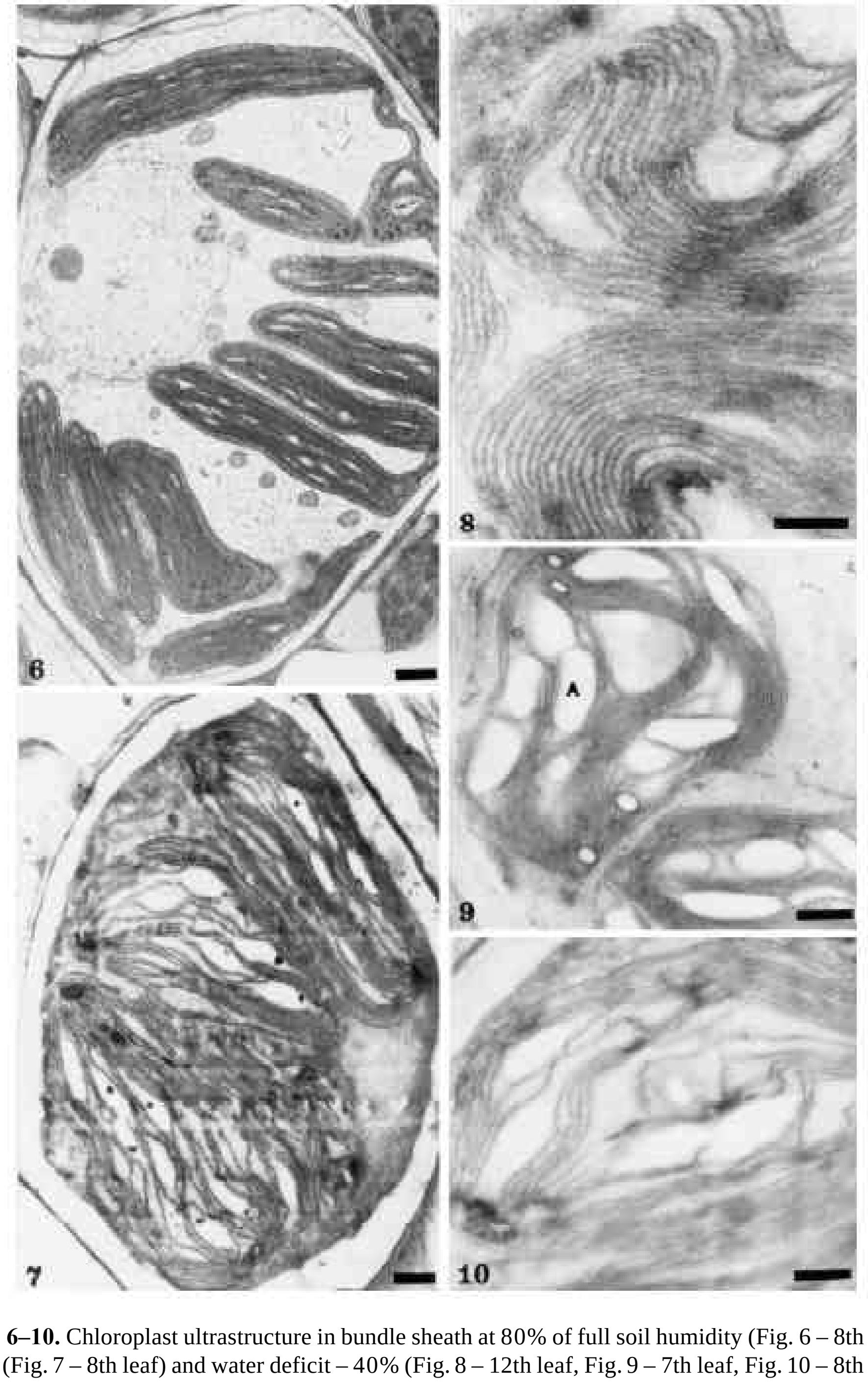
The electron microscope analysis showed that under conditions of water deficit, the most substantial changes take place in the mesophyll and the bundle sheath around the vascular bundles. The plastid apparatus determines the ultrastructural specifics of the cells from both tissues. The chloroplasts in the mesophyll cells to the 80% of soil moisture content have a well developed inner membrane system, occupying almost their entire volume. It is built up from extremely high grana (50 to 65 to 70 thylakoids), tied up to a very well developed stroma thylakoids. The chloroplasts do not contain starch. Plastid apparatus of the 8th and 12th leaves (Fig. 1) is structured in a similar way. In the chloroplasts' spatial orientation, they have formed extensive associative areas. Structural contact between organelles has happened without a change in their shape. Chloroplasts in the 7th leaf at 80% soil moisture content plants have grana of lesser height (35 to 40 thylakoids) and a lesser thylakoid density in their membrane system (Fig. 2). Approximately the same structural organization of chloroplasts in the studied leaves has been observed in plants grown at 60% soil moisture. Substantial structural changes in the plastid apparatus have been found in plants grown under conditions of 40% soil moisture. The mesophyll chloroplasts in 7th and 8th leaves in these plants have an inner membrane system built up of relatively small number not very tall grana (composed of 20 to 35 to 35 thylakoids) along with stroma thylakoids of different length (Fig. 3). Some of the peripherally located stroma thylakoids are very long and run across almost the entire length of the chloroplast. In them, one may observe partial fragmentation and vesiculation (Fig. 3, arrow). Disruption and swelling of thylakoids is the most universal structural response of the inner membrane system of cells under stress. It has been observed in wheat plants under water stress (Freeman and Duysen, 1975). It has also been found in bean plants under conditions of water deficit and thermal stress (Stoyanova and Yordanov, 1999), in barley plants under the impact of high concentrations of salicylic acid (Stoyanova and Uzunova, 2001), or under the impact of other stress factors (Yordanov et al., 1986; Pääkkönen et al., 1995). Our results are in agreement with those observed by Dekov et al. (2000) in water cultures under conditions of water stress with polyethilen gycole. Specific



Figs. 1–5. Chloroplast ultrastructure in mesophyll at 80% of full soil humidity (Fig. 1 – 8th leaf, 2 – 7th leaf) and water deficit – 40% (Figs. 3 and 4 – 8th leaf). (gr – grana, t – stroma thylakoid, p – peristromium; bar = 500 nm)

formations in all the studied variants are the unilaterally set stroma zones free of any thylakoids. They are structured like peristromium and have been formed most likely in relation with the unilateral increase of the stroma volume without a change in the inner membrane system' spatial orientation. Miyane et al. (1987) have discussed in a similar way the stroma zones formed in spinach plants after having been affected by O_3 and SO_2. Formation of these stroma zones has also been related to the change in thylakoids' spatial orientation. As a structural response to a stress-inducing impact, this change has been relatively rarely recorded. Similar stromal zones were observed by Molas, (1997) in treated with Ni cabbage plants. This phenomenon is regarded as an adaptation to stress and is probably related to loss of water. The chloroplasts in the sub-epidermal mesophyll layer of the 8th leaf in plants also grown at 40% of soil moisture have different architecture of their inner membrane system (Fig. 4). It lacks the typical spatial orientation along the longitudinal axis. Part of the long peripheral stroma thylakoids lie in concentric circles. Probably the transformational changes were provoked by the water deficit, and are of adaptive nature with regard to the organelle's function. We found typical destructive changes in chloroplast's sub-epidermal mesophyll layer of the 12th leaf at 40% soil moisture. There, most of the stroma and grana thylakoids were destroyed (Fig. 5). Consequently, thylakoids in grana have merged, while in stroma thylakoids there was some peculiar 'dissolving of membranes'. This structure correlates well with the rate of photosynthesis suppression. We obtained similar results in bean plants exposed to combined stress: water deficit and high temperature (Stoyanova and Yordanov, 1999). In the studied variant with the maize plants (12th leaf, at 40% soil moisture content) the structural contact between the chloroplasts has been brought about through the formed stroma zones described above (Fig. 5 arrow). The extensive associative areas between stroma zones, without any thylakoids are indicative for functional deviations, among them disturbed plastids' transportation should be mentioned.

The plastid apparatus in the cells of the bundle sheath around the bundle in maize plants possesses all structural peculiarities of the Kranz (C_4) syndrome. The C_4 chloroplasts in the studied maize plants, grown at 80% soil moisture content are typically agranal, with well-developed thylakoids and comparatively big starch granules (Fig. 6). Under conditions of water deficit, the structural changes in the C_4 chloroplasts have been identified by a change in the thylakoids spatial orientation and by their swelling. These changes are most clearly expressed in agranal chloroplasts of the 8th leaf (at 60% soil moisture) and in the 12th leaf under conditions of water deficit (Fig. 7 and Fig. 8). In the 12th leaf, thylakoid membranes are not typical and strongly repand. In these chloroplasts, an increase in the volume of stroma has been observed, wich is localized around thylakoids. Such a change has caused an increase of the chloroplast' volume to their filling up the entire cell (Fig. 8). Starch in the C_4 chloroplasts was not observed only in this variant of the experimental plants. Studies of *Festuca vaginata* showed that chloroplasts in the bundle sheath during the drought and after recovery



Figs. 6–10. Chloroplast ultrastructure in bundle sheath at 80% of full soil humidity (Fig. 6 – 8th leaf), 60% (Fig. 7 – 8th leaf) and water deficit – 40% (Fig. 8 – 12th leaf, Fig. 9 – 7th leaf, Fig. 10 – 8th leaf). (a – starch grain; bar = 1µm – 6, 7 and 9; bar = 500 nm – 8 and 10).

from drought were also without any starch (Maroti et al., 1984). In the seventh and eighth leaves of plants grown at 40% soil moisture we found that in the stroma of the agranal chloroplasts, at the places where starch granules used to be, there were voids of the same shape (Fig. 9 and Fig. 10). We assumed that the water deficit provoked enhanced decomposition of starch, thus preserving the granule place without changing the chloroplast architecture. The same effect on the chloroplast structure of mesophyll was observed in been plants exposed to combined stress – high temperature plus water deficit (Stoyanova and Yordanov, 1999).

Structural organization of plastid apparatus in the mesophyll and in the bundle sheath under condition of water deficit depends directly on the functional characteristics of both tissues. The structurally- and functionally different specialization of the studied tissues is the most important factor affecting the chloroplast’s response to the impact of stress. Our results showed that water deficit induced the typical destruction of thylakoids in the mesophyll chloroplasts. Structural modifications in the bundle sheath chloroplasts can rather be regarded as an adaptive to the given environmental conditions. These chloroplasts show greater structural plasticity and stability. Studies carried out on C_4 chloroplasts in *Portulaca oleracea* after SO_2 impact have also shown that they are very stable structurally. The toxic effect was demonstrated only in the disturbance of the structural contact between the chloroplasts and mitochondria (Miyake et al. 1985). From this point of view, the plastid apparatus of the mesophyll compared to that of the bundle sheath is structurally more sensitive and unstable with respect to the water deficit under conditions of the experiment. Functional differences between the studied tissues are probably the most important pre-requisite for the structurally different response of the plastid apparatus.

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