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Effect of boron on the cell walls structure of sunflower

Wpływ boru na budowę błon komórkowych słonecznika

J. R. STARCK

Numerous investigators have indicated that the boron deficient plants reveal abnormalities in the tissue structure; the subject was reviewed by Shkolnik (1950), Gauch and Dugger (1954), Palser and McIlrath (1956), Stiles (1961), as well as by others. Lack of boron in nutrient medium results in a characteristic response of the young organs of plants. First symptoms are observed on root and stem apex. Boron deficiency cause the degeneration of the meristematic tissues and breakdown of the walls of parenchyma cells. Skok (1957) considers boron as functional in the phase of cellular maturation rather than the cell division. Reed (1947), has earlier shown that in boron deficient plants the subapical cells were first affected while the cells of the primary meristem were normal and become necrotic only after those below had failed. These observations would indicate that boron is required for cell elongation rather than for cell division.

A number of investigators have suggested that boron plays a role in the formation of cell wall constituents (Marsh and Shive (1941), Winfield (1945), Spurr (1957), Odhnoff (1957), Whittington (1958). Torssell (1956) suggested that the complexes between boric acid and carbohydrates may control the deposition of oriented cellulose micelles and accompanying stiffening of the cell wall.

The present investigation was undertaken to determine whether there are differences in the submicroscopic structure of cell wall of normal and boron deficient sunflower plants.

MATERIAL AND METHODS

Sunflowers *(Helianthus annuus* L. var. Mammoth Russian) were grown in plastic pots, each containing 5 kg. of quartz sand. Seeds were sown directly in moist sand in a greenhouse at Madison, Wisconsin, U. S. A. on June 1, 1960. At the beginning of the experiment one half liter of modified Hoagland's nutrient solution (Johnson at al. 1957) without boron was added to each pot. Two treatments were

instituted, namely: without boron and plus boron in amount 0.5 mg B/kg of sand. During the growing period the plants received an additional one liter of nutrient solution minus boron. Reagent grade chemicals and demineralised water were used.

Boron deficiency symptoms had become evident in the -B plants by about 18 days; these individuals were considerably stunted and the tips exibited chlorosis. Three days later stems were cut approximately 2 mm belowe the tips. The tips were then infiltrated with 6 per cent sucrose and fixed in a acetate-veronal buffered OsO_4 of pH 7.4. The fixed tissues were dehydrated in the graded ethanols, embeded in n-butyl methacrylate and stained 10 minutes with lead hydroxide (W a t s o n 1958). Thin sections were examined under Simens Elminoscop II electron microscope.

RESULTS

In the boron deficient tissue of stem tip outer layer of the cell wall appear denser than the adjacent inner layer. The cross section of cell wall does not reveal microfibrilar structure (Fig. 1) or reveal only scarce mesh of microfibrils (Fig. 2).

These microfibrils which would seem to be cellulose are interwoven throughout the wall and are not arranged in the layers. They seem to be transversaly oriented to the cell axis. Aggregates of high density are clearly seen in the vacuoles (Fig. 1, 2).

At normal level of boron (0.5 ppm) numerous layers of longitudinaly oriented microfibrils could be seen, especially on the outer surface of the cell wall (Fig. 3, 4). Closer examination of these walls reveals a difference in orientation of microfibrils between inner and outer surface. On the inner surface they are rather transversaly oriented and towards the outside they show greater tendency to longitudinal orientation. Staining density is highest in the region of middle lamella. In the cell walls of normal plants (Fig. 3, 4) contrast in the walls is much more pronounced than in the cell walls of boron deficient plants (Fig. 1, 2).

EXPLANATION OF PLATES

Electronmicrographs show transverse section through cell walls in the shoot apex of sunflower.

Fig. 1—2. Cell walls of boron deficient plants 29000 ×

Fig. 3—4. Cell walls of plants supplied with 0.5 ppm boron. 29000 ×

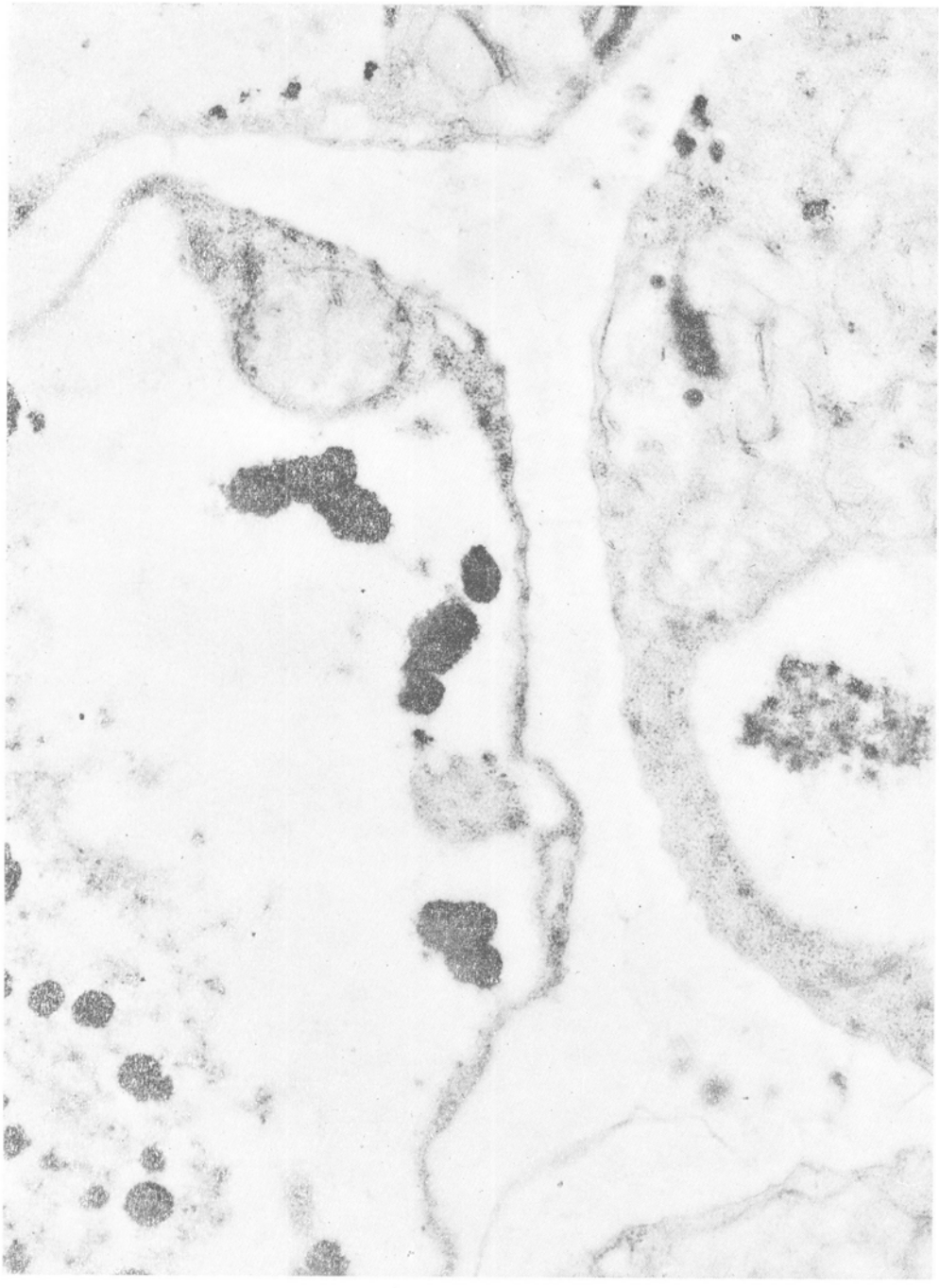

Fig. 1

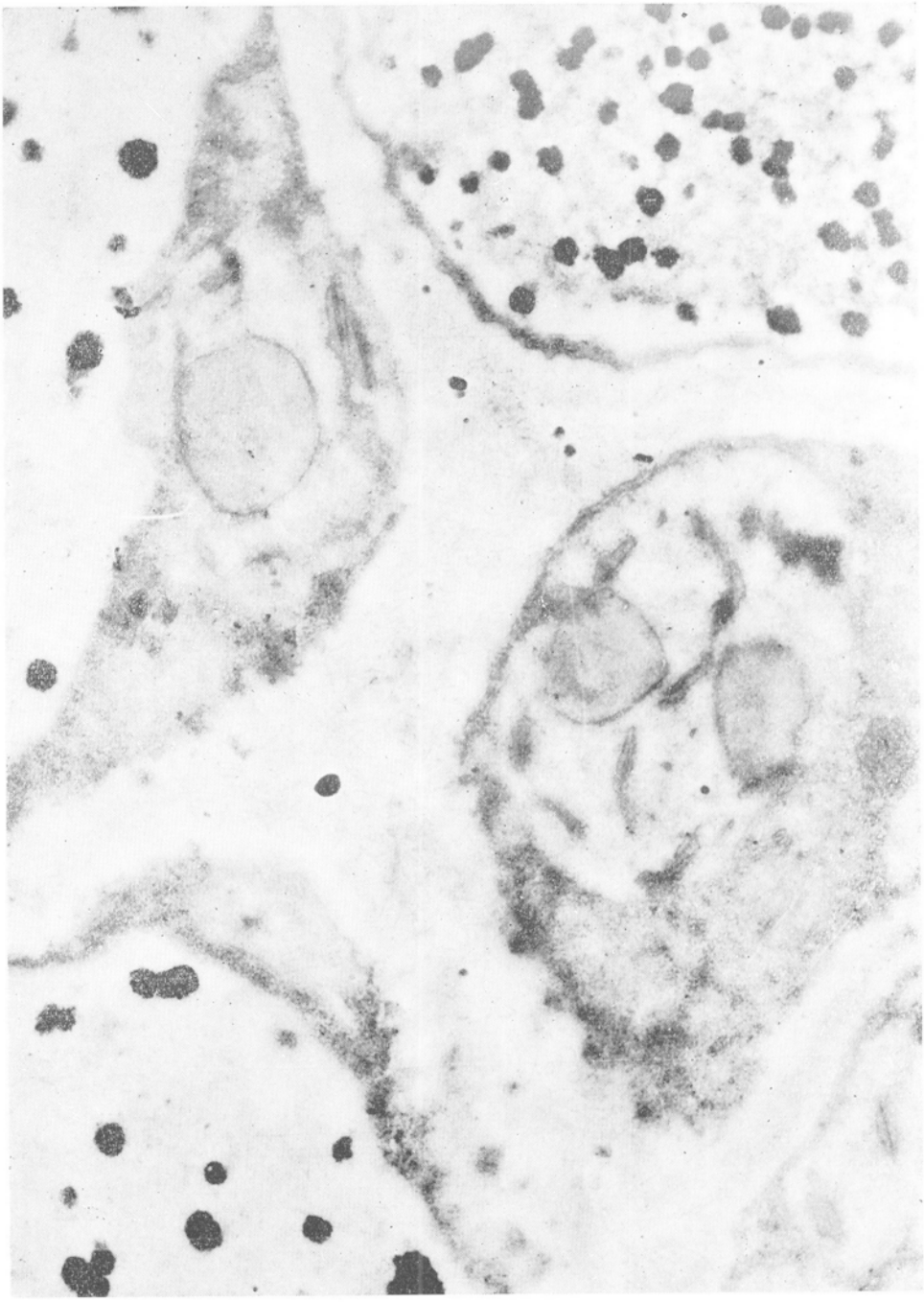

Fig. 2

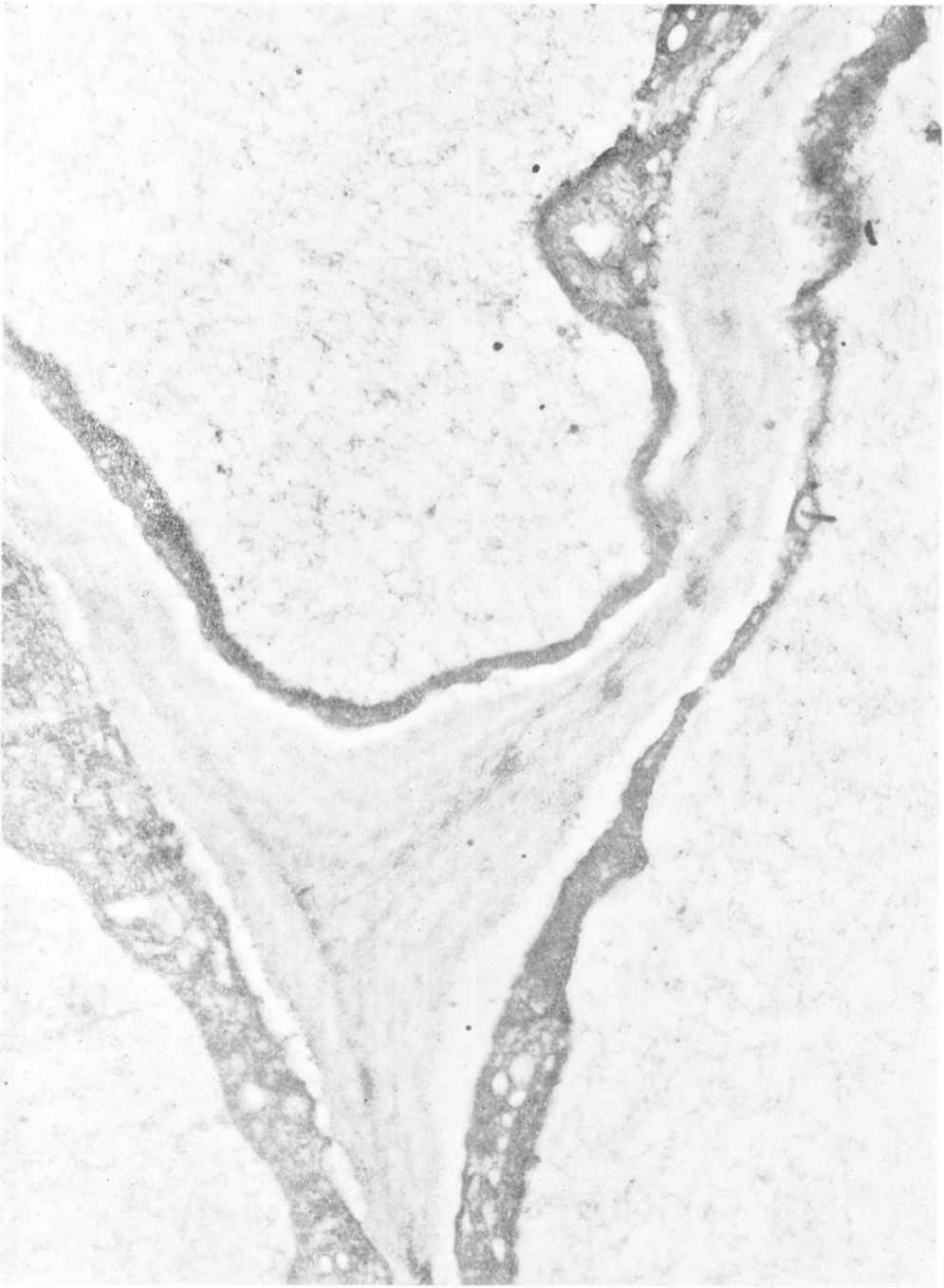

Fig. 3

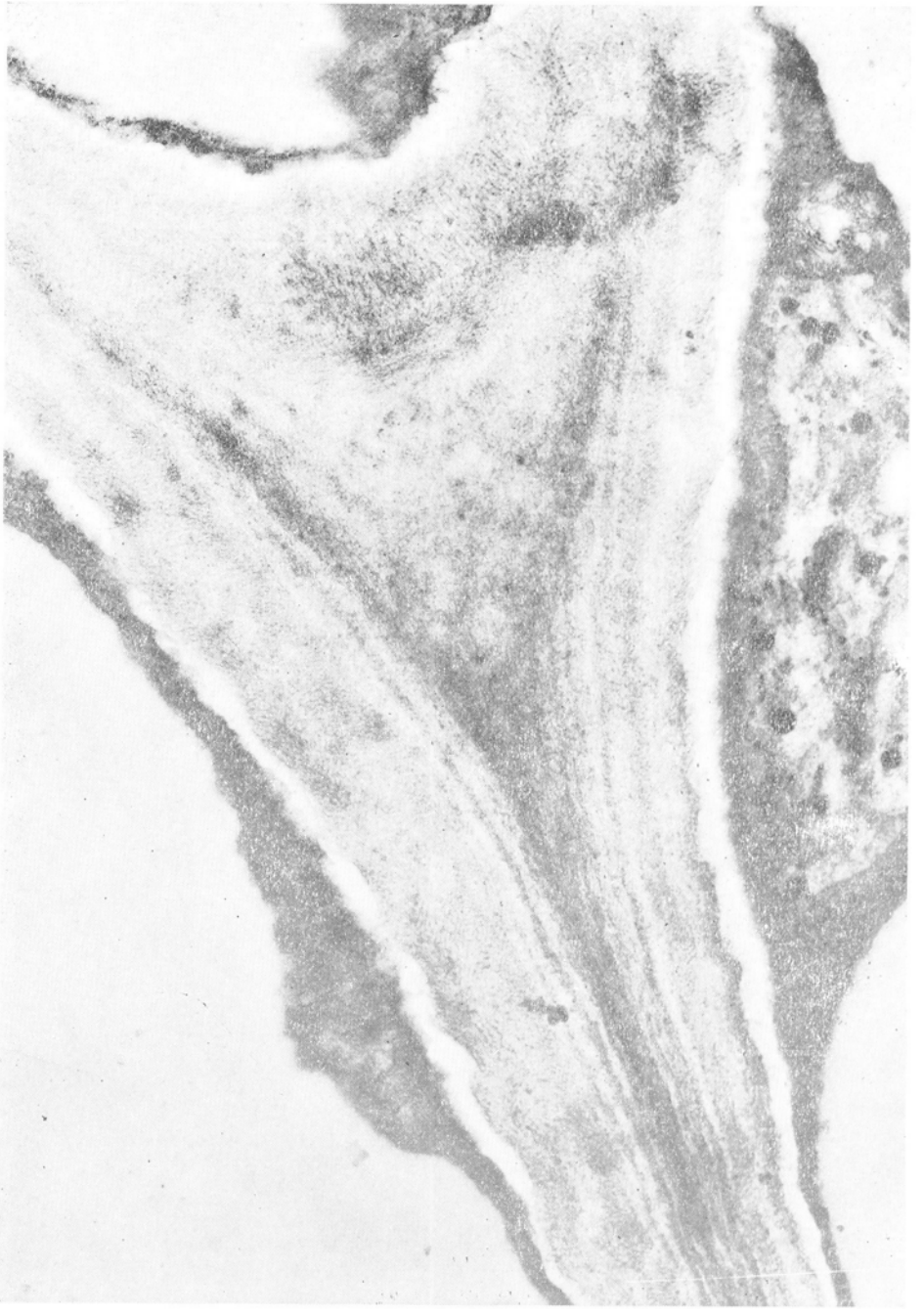

Fig. 4

DISCUSSION

One of the most characteristic symptoms of boron deficiency is brittlenes of young leaves and stems. It is presumedly associated with changes in cell wall composition or structure. Odhnoff (1957), assumed that boric acid primary influences the stretching phase. Thompson and Batjer (1950) showed that pollen grains of different fruit trees attained a higher per cent germination and a much greater pollen tube length if boron was included in germination medium than if it was omitted. It would indicated that boron is needed for elongating cells. Primary cell walls of young cells are usually high in non-cellulosic material mainly the pectic material and hemicelluloses Frey-Wyssling (1957). Busse (1959) suggested that the rate of elongation of cell wall depends on a balance between plastic substances and material capable of stiffening walls, which both are added during depossition. The stiffening materials would presumedly be a component of matrix since structural studies indicate that microfibrils, although reasonable rigid themselves, are able to slip past each other in the matrix of growing walls Setterfield and Bayley (1961). Probine and Preston (1962) came to conclusion that longitudinal creep must involve not only movement of microfibrils but also distortion of the molecular chain mesh of the incrusting substances and presume that the latter may be of great importance. They present experimental evidence that the rate of creep of longitudinal wall strips is greater if the strips were previously soaked in KCl than in $CaCl_2$ solution. However, there were notable differences between individual cells. Probine and Preston admit that the magnitude of the effect of divalent cations depends on the degree of estrification of the carboxyl groups. Also Bonner (1961) presented evidence that added calcium is a strong inhibitor of cell expansion. It is in accordance with Bennet—Clark (1956) hypothesis that calcium may form ionic binding between pectic carboxyl groups and reduce wall plasticity.

There is considerable evidence of interrelationship between boron and calcium metabolism. Marsh and Shive (1941) have shown that the total calcium content of maize is independent of the amount of boron supplied but the soluble calcium content runs parallel with both the soluble and total boron content of the plants. Similar results obtained McIlrath at al. (1960) with Siberian millet.

All these observation give renewed interest to the claim of Winfield (1945) that boron, calcium and pectic metabolism in higher plants are related.

Boric acid easily forms the methyl ester. It may be suggested that boron affects the transfer of CH_3 groups and regulates the degree of

pectin estrification. Lack of boron would cause decrease of methylation of pectic substances and increase ionic binding between pectic carboxyl groups by divalent ions, particularly calcium. Increasing amount of calcium binding may reduce wall plasticity and thus inhibit growth as suggested by Bennet—Clark (1956). According to multinet growth theory (Mühlethaler 1961), microfibrils which are deposited on inner surface in the transverse direction, during elongation are gradualy pulled out into longitudinal orientation. Stiffening of pectic matrix cause by boron deficiency would probably strongly inhibit this process and raise difficulties in deposition of microfibrils. It may probably explain why in cell walls of boron deficient plants (Fig. 2) microfibrils are transversaly situated and in cell walls of normal plants longitudinaly oriented in outer layer (Fig. 4).

SUMMARY

To study the effect of boron on cell wall structure thin sections of sunflower stem tips were examined under the electron microscope. The cross sections of cell walls of boron deficient plants did not reavel the microfibrilar structure or reveal only disorganised, scarce mesh of fibrils (Fig. 2). Vacoular agregates of unknown substances were clearly seen.

In the cell walls of normal plants numerous layers, specially on the outer surface could be seen. Contrast in those walls is much higher than in boron deficient plants. Structure of the cell walls of normal plants seem to be more organised than boron deficient plants.

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Department of Soil Management and Fertilization
Central Colege of Agriculture
Warsaw, Rakowiecka 8

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STRESZCZENIE

Materiał roślinny (słonecznik) przygotowano w kulturach piaskowych. Część roślin otrzymała pełną pożywkę z borem w ilości 0,5 mg B/kg piasku, a część pożywkę pozbawioną boru.

Po wystąpieniu symptomów braku boru na roślinach w kombinacji bezborowej, ucięto z roślin obu grup wierzchołki łodygi długości 2 mm. Z wierzchołków

tych po odpowiednim spreparowaniu sporządzono cienkie skrawki, z których zrobiono zdjęcia pod mikroskopem elektronowym.

W przekroju poprzecznym błon komórkowych z roślin pozbawionych boru nie stwierdzono struktury mikrofibrylarnej (fot. 1) lub tylko rzadką siatkę mikrofibryli, przypuszczalnie celulozowych (fot. 2). W soku komórkowym stwierdzono obecność licznych, silnie zaczernionych agregatów niezidentyfikowanych substancji.

W błonach komórkowych roślin, które otrzymały bor, stwierdzono obecność licznych warstw, szczególnie na stronie zewnętrznej błony (fot. 3, 4). Bliższe zbadanie tych błon wykazało, że w warstwach zewnętrznych mikrofibryle ułożone są podłużnie do osi komórki (w przekroju poprzecznym widoczne w kształcie drobnych przecinków). W warstwach wewnętrznych błony mikrofibryle ułożone są poprzecznie do osi komórki (w przekroju poprzecznym widoczne jako drobne niteczki).

Stwierdzone różnice w budowie błon komórkowych roślin otrzymujących bor i roślin pozbawionych boru wywołane są przypuszczalnie wpływem boru na procesy wydłużania się błon komórkowych poprzez wpływ na metylację pektyn.

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