Effects of Boron Stress on the Anatomical Structure of *Medicago sativa* L.

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Abstract

Effects of Boron (B) excess and deficiency on the anatomical structure of *Medicago sativa* L. have been investigated in hydroponic cultures (–B, Control, +B). According to the findings, it was shown that B deficiency (-B) mostly effected the root cortex parenchyma and then xylem and cambium tissues in the stem. In –B plants, the cambium increased in the stem thickness by dividing irregularly, however the differentiation to xylem decreased. Whereas the differentiation of cambium to xylem did not decrease after +B treatment (25 ppm B). There was a significant reduction in all tissues of plants grown in +B as compared to control, and a meaningful decrease occurred in the diameter of roots and stems. The cambium tissue in the stem was the one least affected by +B treatment. As the B content in the medium increased, the number of stomata and epidermis cells per surface area (mm^2) increased while the size of the cells decreased in the leaves. According to the observations and the data which is obtained by calculating the Degree of Xeromorphy, it is found that *M. sativa* L. developed xeromorphic structure after +B treatment.

Keywords: *Medicago sativa* L., Boron (B), stress, anatomy, xeromorphy degree.

Introduction

Medicago sativa L. is a very important economic crop in Turkey agriculture as a high quality animal feed, a deep rooting plant that enhances soil structure and a crop that can help prevent excess soil nitrate. Boron (B) is an essential micronutrient for higher plants, but the primary function of B still remains unknown. B deficiency (–B) and B excess (+B) are stress factors. B toxicity symptoms in the early vegetative phase (Çetin 1994) and also B deficiency symptoms in the late vegetative phase (Çetin 2004) were indicated in the previous investigations. The effect of excess B on the germination and development of *Medicago sativa* L. as well as the distribution of B in different organs of the plants was also established by Çetin (1994). Longbin et al. (2008) studied whether B in mature leaves in *Lupinus albus* can be retranslocated into the young reproductive organs, in response to short-term (3 d) interrupted B supply. According to their results, previously acquired B in the shoot was recirculated to the root via the phloem, transferred into the xylem in the root, and transported in the xylem to the shoot. In another study (Çetin et al. 2000), B was found playing an important role in the structure and germination of pollen grains. Likewise, the relationship among –B stress, peroxidase (POD) and polyamines (PAs) were presented in the B deficiency treatment in sunflowers (Palavan-Ünsal et al. 2000). In this study, according to the findings, the effects of negative and positive B, of –B and +B, on the root, stem and leaf tissues have shown differences.

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Material and Methods

Medicago sativa L. seeds were germinated and developed in a growth chamber (25°C±1 / 18°C±1 and 12 h light-dark photoperiod and 65 % relative humidity and 7000 lux). Hydroponic culture of Bucholz, which contains 5 ppm B, was used as control (C) treatment. –B plants, which took B element only from seeds were grown in –B solution. +B plants were grown in 25 ppm B nutrient solution. B was used as boric acid (H_3BO_3). In order to study the effects of –B and +B on the anatomy of *M. sativa* L., 75-day-old plants were used. While the anatomical structure of the root, stem and leaf was examined, care had been taken manually to secure sections from the same part of the plant. Root transverse sections were taken from the middle segment after the root length was measured, stem transverse sections, from the internodium lying between the 4th and 5th nodes, and leaf transverse and surface sections, from the middle part of the leaflets of the trifoliate leaves in the 5th nodes. Sections were put first into water, then passing through a gradually increasing glycerin series, they were coated with glycerin-gelatin dyed by safranin and were examined under the REICHERT light microscope. In the anatomical examination of *M. sativa*, microscopic measurements have been made on the transverse sections taken from the root, stem and leaf, and on the surface sections from the leaves. Their results were given by converting them into their real values. Measurements were taken on the radial direction of the transverse sections, and the photograph was taken with an OLYMPUS photomicroscope. After drawing the leaf surfaces taken from the 5th trifoliate of the 75-day-old plants, the leaf areas were measured by plan-meter and the mean values obtained.

Results

1. The Effect of B on the Root Anatomy of *M. sativa*

The most prominent change in the anatomical structure of plant growth in –B nutrient solution is a result of reduction in the rows of parenchyma cells of the cortex which is formed as part of the root diameter (Table 1). The root diameter of the plant has shown a significant decrease of 16.98 % in –B, and 24.81 % in +B as compared to the control. Although in the central cylinder there is not a significant change in –B, a decrease of 17.29 % has been determined in +B, as compared to the control (Fig. 1).

Table 1. The effect of B on the root anatomy of *M. sativa*.

Boron Concentration (ppm)	Root Diameter (μ)	Central Cylinder Diameter (μ)	Cortex Thickness (μ)	Epidermis Thickness (μ)	Endodermis Thickness (μ)	Trachea Diameter (μ)	Trachea Wall Thickness (μ)	Cortex Parenchyma Cell Row	Sclerenchyma Cell Row
-B (0)	286 ±2.7	121±0.8	81.9±2.6	4.8±0.3	11.9±0.7	19.0±2.6	3.0±0.3	3.7±0.1	12.0±1.0
C (5)	344 ±3.0	120±1.6	114.5±2.1	11.0±0.3	13.1±0.5	15.7±1.3	1.8±0.9	4.3±0.1	13.2±1.6
+B (25)	259 ±4.0	99±0.8	79.2±1.5	4.4±0.3	13.9±0.6	13.2±2.1	0.9±0.1	2.6±0.2	6.0±0.1

There has been a pronounced reduction of 28.47 % in the cortex thickness in –B, and a reduction of 30.82 % in +B. Although the diameters of parenchyma cells of the cortex are wider in –B, there has been a decrease in the cortex cell row. The rows of parenchyma cells of the cortex have shown a reduction of 13.95 % in –B, and 39.53 % in +B. The shape of the cortex cells which are observed as having smooth walls in +B, have been deformed in –B, and, moreover, some of them are disrupted. The reason for the root diameter being bigger in C suggests that the number of the row of the parenchyma cells of the cortex is multiple.

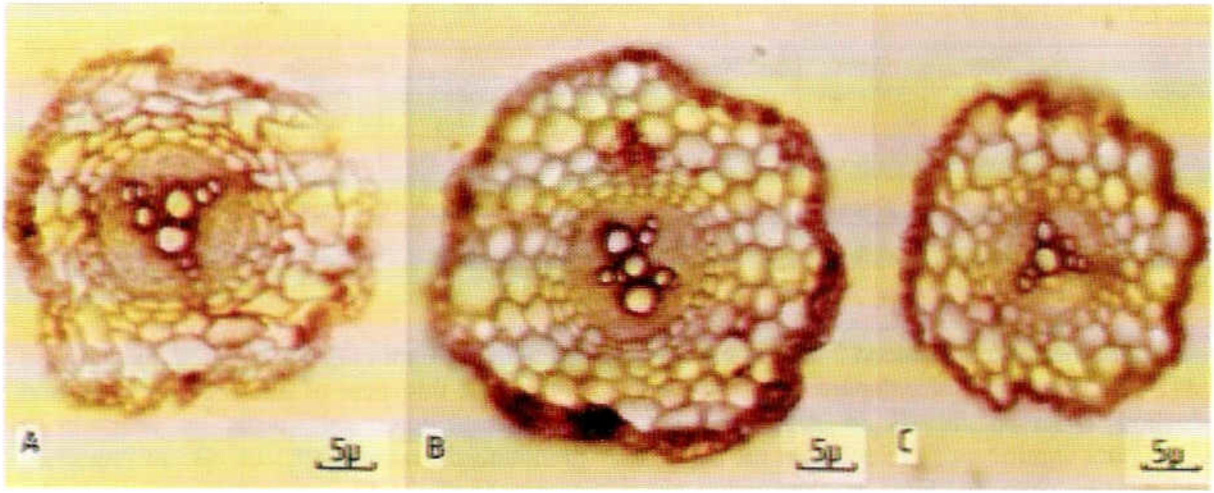

Figure 1. The effect of B stress on the root of *M. sativa*. A) –B; B) Control; C) +B.

A reduction has occurred in the cortex thickness in +B, as a result of the decrease in the cortex cell row. Cortex cells which seem regular in C and +B, have taken an irregular appearance in –B. Epidermis thickness has been reduced 56.60 % in either –B or +B as compared to C. Moreover, occasional bursts and irregularities are observed in the epidermis cells in –B. There has been no significant change in endodermis thickness. There has been no significant difference, in –B, between the findings and control, concerning trachea diameters, trachea wall thickness, and the number of sclerenchyma cells. The roots of plants grown in the solution containing +B, have been affected in their root development anatomically in a considerably negative manner.

2. The Effect of B on the Stem Anatomy of *M. sativa*

Stem development is hindered both in –B and +B, as compared to C (Table 2). According to the findings, stem diameter has shown a reduction of 4.34 % in –B, and 27.72 % in +B. That is, the stem diameter of plants grown in –B has shown more thickness than the ones grown in +B (Fig. 2). The cortex is developed best in control. An expressive reduction of 8.51 % in –B, and of 18.55 % in +B has been observed in the cortex thickness, as compared to C. Although there has been no significant difference in the pith diameter in –B, there has been a reduction of 29.08 % in +B. In –B, parenchyma cells of the pith are thinned and there are occasional disruptions. In other words, the fine structure of the cell walls is ordered, because B is the building block of the polyhydroxy-chain in the walls. It has been suggested that in the –B, the fine structure of the walls is disordered because of the –B quality of this substance.

Table 2. The effect of B on the stem anatomy of *M. sativa*.

Boron Concentration (ppm)	Stem Diameter (μ)	Cortex Thickness (μ)	Pith Diameter (μ)	Bundle Thickness (μ)	Tissues in the vascular bundle			
					Sclerenchyma (μ)	Phloem (μ)	Cambium (μ)	Xylem (μ)
-B (0)	1264±9.8	78.4±3.4	748.6±49.8	179.3±7.9	19.7±5.7	24.3±2.0	13.5±0.5	121.8±18.4
C (5)	1321±14.2	85.7±2.0	676.2±24.5	236.9±10.5	26.6±2.3	21.3±1.0	6.9±1.1	182.1±4.1
+B (25)	955±7.6	69.8±4.2	479.5±22.7	168.0±11.2	17.4±0.1	28.3±1.3	5.2±0.5	117.1±5.1

Bundle thickness is reduced 24.31 % in –B, and 29.08 % in +B as compared to C. The reduction observed in –B in sclerenchyma tissue is not significant (Table 2). On the other hand, as is seen in Fig. 3, the walls of sclerenchyma cells are thinned as a result of the reduction of lignin in –B. In Fig. 4, the increase both in the thickness of sclerenchyma isles and in sclerenchyma cell walls is seen in a pronounced manner in C. In +B, an important decrease of 34.58 % has occurred in the sclerenchyma tissue, as compared to C.

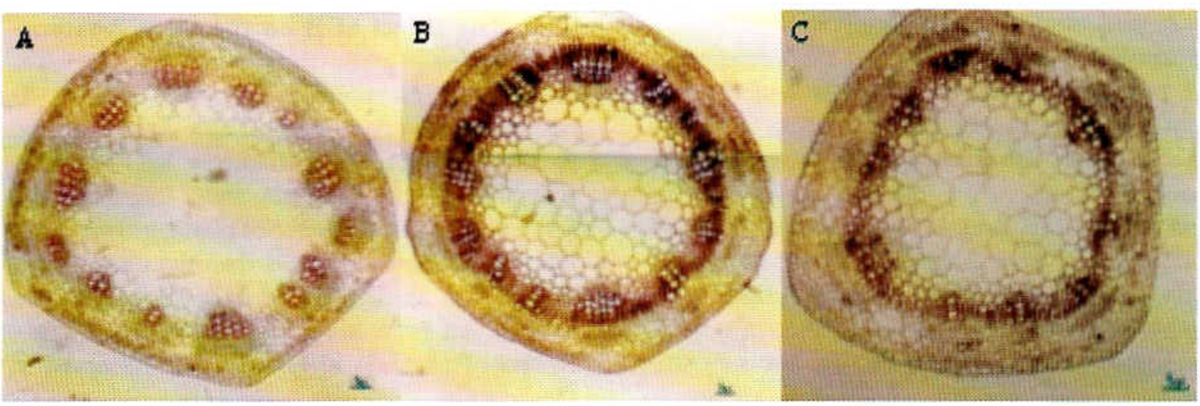

Figure 2. The effect of B stress on the stem of *M. sativa*. A) –B; B) Control; C) +B.

Phloem tissue has shown a pronounced increase of 24.73 % in +B, as compared to C. In –B, there has been an increase in the phloem wall thickness.

A meaningful increase of 95.65 % has occurred in cambium in –B as compared to C. In +B, an increase of 24.63 % has not been considered significant. Both the rows of

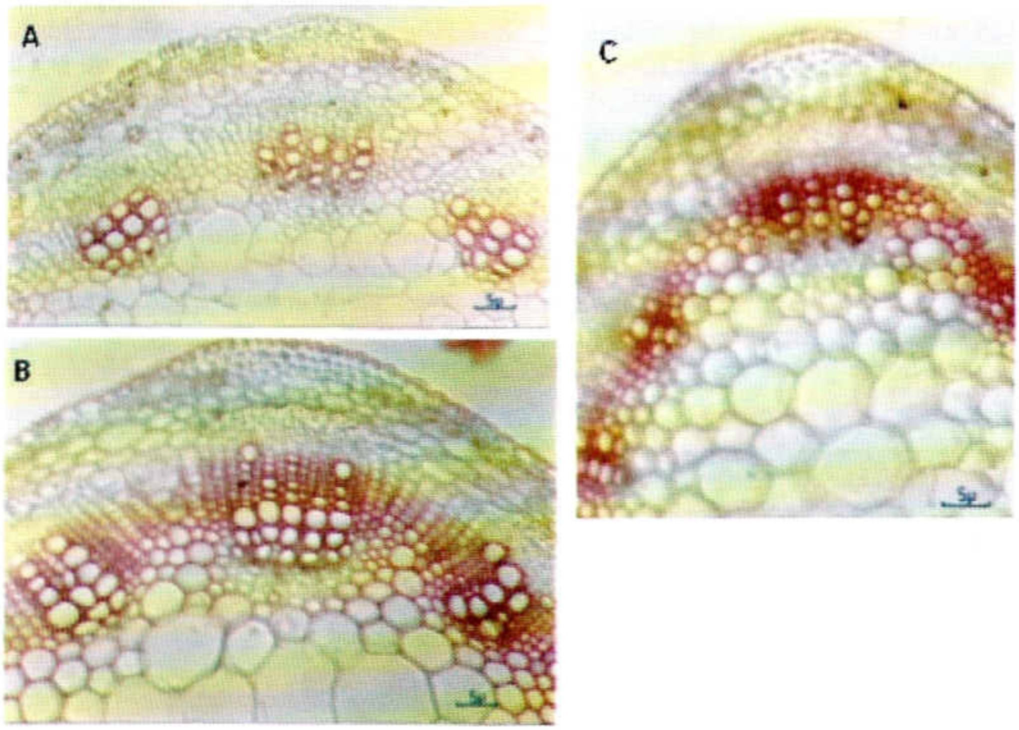

Figure 3. The formation of tissues in the transverse section of the stem of *M. sativa.* A) –B; B) Control; C) +B.

cambium cells and the cambium thickness which is in the radial direction are increased in –B. Although cambium has been thickened in –B, a reduction of 33.11 % has occurred in the xylem tissue of the vascular bundle as compared to C, since xylem differentiation is inhibited. +B has reduced the development of xylem tissue by 35.69 %, as compared to C.

3. The Effect of B on the Leaf Anatomy of *M. sativa*

The values about the leaf anatomy of *M. sativa* have been given in Table 3. The measured values of the transverse section (Fig. 4) and surfaces of the leaves belong to the trifoliate of 5^{th} node. Leaf thickness is decreased by 23.12 % in –B, and 46.45 % in +B, as compared to C. Leaf area has shown a significant reduction of 34.0 % in +B, as compared to C.

Table 3. The effect of B stress on the leaf anatomy of *M. sativa.* (XD) Xeromorphy Degree = stoma number / mm^2 x epidermis number/ mm^2 : 100.

Boron Concentration (ppm)	Leaf Thickness (μ)	Leaf Area (mm^2)	Epidermis Number (per mm^2)	Epidermis Size (μ^2)	Stoma Number (per mm^2)	Stoma Size Width (μ)	Stoma Size Length (μ)	Xeromorphy Degree (XD)
-B (0)	113.7±1.8	304.0±19.3	437.5±12.5	1644.4±98.4	132.8±4.9	15.7±0.6	23.0±0.1	576
C (5)	147.9±0.3	345.5±27.4	600.0±50.0	1333.6±54.8	147.5±5.0	14.3±0.1	21.7±0.1	882
+B (25)	79.2±1.3	228.0±13.5	825.0±52.0	972.0±30.4	212.5±2.6	12.8±0.2	19.9±0.3	1749

While in –B, the degree of xeromorphy (Önal 1978) which we have calculated according to the formula given in Table 3, is decreased by 34.69 %, as compared to C, it has increased by 98.29 % in +B. It has been found that the leaves of *M. sativa* are converted into a xeromorphic structure because of +B.

A difference is observed between the formation and color of the old leaves taken from the 10^{th} node of *M. sativa* in the presence of –B and +B (Fig. 5). As is seen in the figure, the leaves which are grown in –B are smaller and are of dark green color, as compared to C. The occurrence of this dark color is related to the fact that, in –B, sugars are not transported to the younger leaves and remain in the old ones, and that starch accumulation has increased. In +B, manifestations like yellowing first and the later drying of the tips of the leaves, are the result of the toxic effect of the accumulated B because the transpiration has been more on the tips and edges of old leaves.

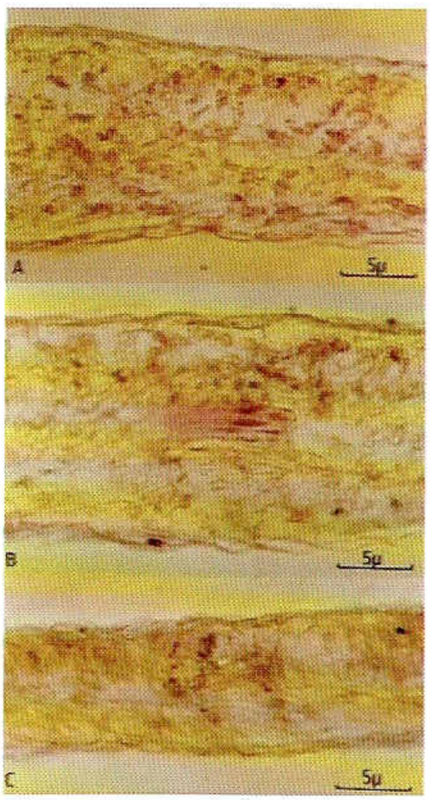

Figure 4. The effects of B stress on the leaf of the *M. sativa*. A) –B; B) Control; C) +B.

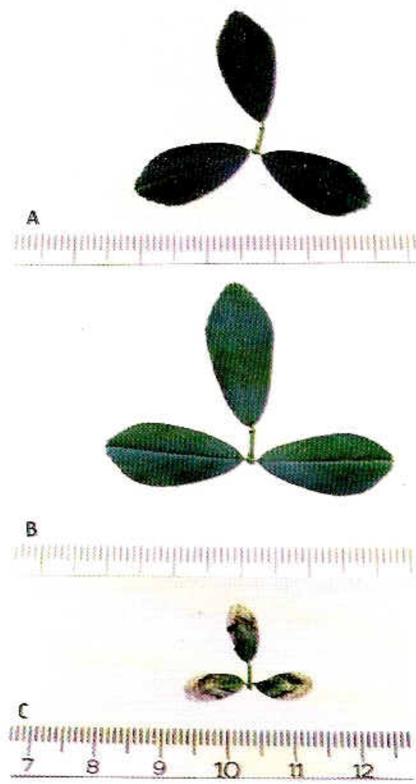

Figure 5. The effects of B stress on the leaf area and color in *M. sativa*. A) –B; B) Control; C) +B.

Drawings from the surface sections are seen in Fig. 6. While in –B the number of epidermis per square mm (mm^2) is decreased by 27.08 % as compared to C, it increases by 37.5 % in +B. While the size of the epidermis cell is increased 23.3 % in –B, as compared to C, it has decreased by 27.11 % in +B. B excess affects the epidermis and stoma cells in a pronounced manner. By the effect of B, the number of stomata per square mm. is increased, while stoma size, on the contrary, has shown a reduction by the action of +B.

According to the findings, the negative effects of –B and +B on the root, stem and leaf tissue show differences.

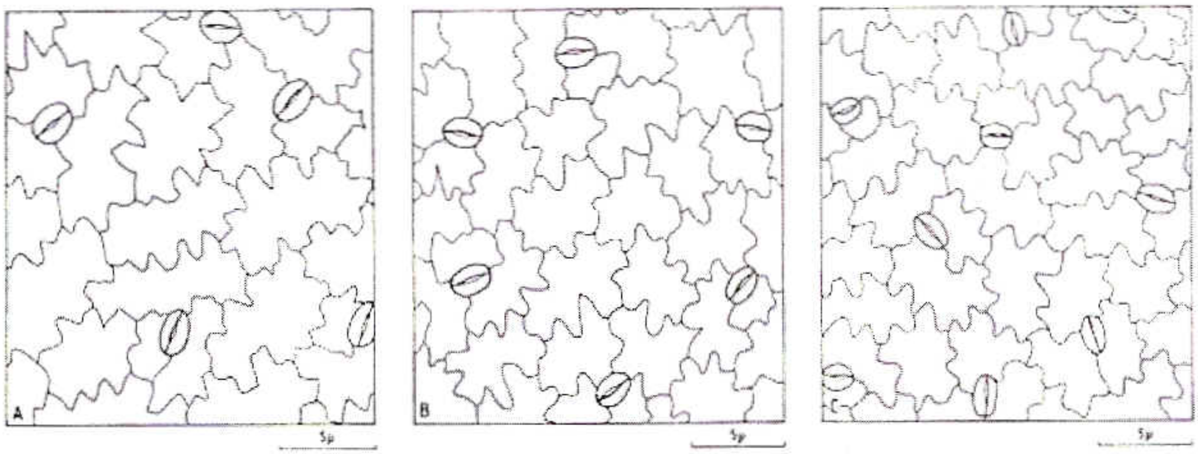

Figure 6. The effects of B stress on the number and size of epidermis and stomata in the undersurface of the leaf of *M. sativa*. A) –B; B) Control; C) +B.

Discussion

In this anatomical study, the effect of B on the root anatomy has been investigated and the optimal root growth is observed in the sections of plants grown in C. The most prominent difference in the anatomical structure of the plants grown in –B has been the cortex thickness which reduces root diameter significantly. In –B, although the cortex cell row has been reduced, the diameter of cortex parenchyma cells has increased, but their walls become thinner and some of them have been ruptured, destroying the cell shapes. Maevskaya et al. (1974) found similar results in sunflowers. Contrary to the findings of Bergmann (1983), in this root sections, an increase, though not significant, has been found in the wall thickness of trachea in –B as compared to C.

In this study of the stem anatomy, the significant reduction of the stem diameter in plants grown either in –B or in +B, as compared to C, has been related to considerable reduction of cortex thickness and the bundle thickness as that in the root. The shoot thickness suggested by Bussler (1964) and the leaves thickness suggested by Mengel (1972) in the symptoms of –B, have not reflected these findings. In this finding, the pith diameter in the stem has developed most in –B, but pith parenchyma cells are thinned and there are occasional ruptures in some places. That is, the structure of cell walls is deformed because B forms the building block of the polyhydroxy-chain in the walls. The fine structure of the walls is deformed in –B because of the deficiency of this substance (Spurr, 1957). The B content in the cell wall was affected by B concentration solution in the coffee leaf (Rosolem, 2007).

According to the finding, the difference in the bundle thickness of the stem has been caused by the failure of xylem tissue to develop well in –B, because the differentiation of cambium to xylem has been reduced. According to Bussler (1974), phloem does not develop well in –B. However, in this finding, there has been no meaningful difference in the development of phloem between –B and C. As to xylem tissue of the stem a significant reduction is measured both in –B and +B. Although there is an expressive increase in the cambium tissue of –B, xylem differentiation has been prevented (Krosing 1978). In cambium cells of –B, a significant increase in the radial direction as compared either to C or to +B has been found. We may relate this to the result of abnormal division in cambium cells, due to –B.

In these anatomical findings regarding the leaf, contrary to the findings of Bussler (1964), a significant reduction has been observed in the leaf thickness and leaf surface either in –B or +B, as compared to the control (Table 3). However, in –B, there has been a pronounced thickening as compared to the situation in +B (Mengel 1972). Furthermore, it has been observed that both spongy and palisade parenchyma cells are disorganized in +B (Sotiropoulos et al., 2002). According to these findings in –B, old leaves are smaller and they have a darker color. We may relate this dark color to the failure of sugars being transported to younger leaves, and to the increase of starch accumulation in –B. Bergmann (1983) related the dark color of the leaves in –B to similar results, and this supported our findings.

In this study, it has been established that, in +B, the number of stomata and epidermis cells per mm^2, in the leaves is increased, and that the cells of stomata and epidermis shrunk. As the amount of B in the plant increases, a xeromorphic structure has been encountered in the leaves. Since there is a reverse relation between the intensity of the xeromorphic structure and transpiration, we may accept this anatomical and morphological change as a physiological adaptation mechanism for the plants grown in +B.

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