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# THE EFFECT OF WATER POTENTIAL ON ACCUMULATION OF SOME ESSENTIAL ELEMENTS IN SUGARBEET LEAVES (*Beta vulgaris* ssp. *vulgaris*)

ABSTRACT: An investigation has been conducted on the effect of reduced water potential in nutrient solution on the accumulation of some essential macro- and micronutrients in the aboveground parts of young sugarbeet plants. Plants of 8 different sugarbeet genotypes were exposed for 21 days to a nutrient solution whose water potential of 0.1 MPa was regulated by PEG. Contents of N, P, K, Ca, Mg, Fe, Mn, Cu and Zn declined in all genotypes under water deficiency, but the intensity of reduction varied among the genotypes. The results indicated that some harmful effects of water deficiency could be attributed to disturbances in plant mineral nutrition, especially the lack of N, P, and Mg, as well as to impaired ratios between the contents of particular elements, especially K/Ca.

KEY WORDS: sugarbeet, PEG, drought, N, P, K, Ca, Mg, Fe, Mn, Cu, Zn

#### INTRODUCTION

Content of mineral elements in plants (mineral composition of plants) depends on numerous internal and external factors which have to be taken into account while optimizing plant nutrition. One of the ecological factors that affect plant provision with essential elements is water deficit. Drought is the most limiting factor of yield worldwide, our country included, and it often reduces crop quality. The impact of drought is complex, as there is no process in plant metabolism that remains unaffected by water deficiency. In addition to various levels of water requirement, plant species and genotypes differ in their ability to adapt their metabolism to water deficiency. The problem in drought investigation is that there is no single indicator of drought tolerance that could serve as a reliable criterion for evaluation of genotypes. It is therefore necessary to monitor different indicators of plant water and osmotic status (e.g. fresh and dry matter, leaf area, characteristics of stomata), biochemical parameters (e.g. content of osmotically active substances, activity of particular enzymes) as well as eventual changes in the elemental composition of plants. Analysis of such data and their interactions may lead to a conclusion about the degree of tolerance/sensitivity to drought in a particular genotype. Differences in plant mineral composition are the result of I) genotypic differences in plant mineral composition, and 2) different levels of tolerance towards water deficit exhibited by different genotypes, as manifested through differences in the uptake and accumulation of particular mineral elements.

Sarić and Kovačević (1981) and Petrović and Kastori (1990) reported that genotypic specificity plays an important role in sugarbeet mineral nutrition. Petrović et al. (1991) reported that the mineral composition of sugarbeet plants changed under drought conditions. Balanced mineral nutrition of sugarbeet has also been mentioned as a factor affecting the contents of various elements and the ratios between essential elements in plant tissues: K/Ca, S/N, P/Zn, P/Ca, P/Fe, Fe/Mn, Mg/Mn, etc. (Kastori et al., 1996). It was shown that water deficiency causes disturbances in biochemical pathways such as the contents of free proline, DNA and RNA, activity of RUBISCO, and others (Krstić et al., 1997; Kevrešan et al., 1998).

The aim of this investigation was to monitor the influence of long-lasting mild water deficit on the accumulation of several essential mineral elements in leaves of eight sugarbeet genotypes. The genotypes, taken from the breeding program of the Institute of Field and Vegetable Crops, had shown variability in drought tolerance under field conditions. Experiments were conducted in semi-controlled conditions. Preliminary tests showed that the osmotic value of 0.1 MPa in the nutrient medium provokes symptoms of water deficiency in sugarbeet plants, but at the same time it does not completely inhibit plant growth. The water potential of 0.1 MPa was therefore chosen for testing the effect of water deficit over a period of time during which young sugarbeet plants could exhibit their capacity to adapt to this stress.

### MATERIALS AND METHODS

Of the eight sugarbeet genotypes included in the experiment, seven belonged to *Beta vulgaris* ssp. *vulgaris* (genotypes 1 to 8), and one to *Beta vulgaris* ssp. *maritima* (genotype 9): 2nmm "O"1102-3-7 (line 1234, p. 399) (1), 2nMM C-39 (line 3484, p.25) shows quantitative resistance to rhizomania (2), 2nmm "O"11547 originates from Ukraine (K-2, p. 574) (3), 2nMM C-78 (line 3486, p. 27) (4), 2nMM MTRB (line 3465, p. 6) shows quantitative resistance to rhizomania (6), 2nmm "O"21223 (3412, p. 553) shows resistance to Cercospora (7), 2nmm "O" GRRT (line 3416, p. 555) shows qualitative resistance to rhizomania (8), and 2nMM C-51 (line 3493, p. 34) shows resistance to rhizomania, 2nMm, *B. mar* (9). These genotypes had come from the breeding program of Sugarbeet Department of the Institute of Field and Vegetable Crops Novi Sad and they had shown variability in drought tolerance under field conditions. The experiment was done under semi-controlled conditions. Plants were grown for 30 days in the complete nutrient medium and additional 21 days under conditions of water deficiency. After that, the contents of some essential macro- and micronutrients were determined in sugarbeet leaves.

Four hundred seeds of each genotype were soaked overnight in distilled water and subsequently sown in vermiculite. Trays with vermiculite and seeds were kept in a thermostat, in the dark, at the temperature of 24°C. After 8 to 10 days (depending on genotype) plantlets were replanted in plastic pots for water culture. The plants were grown in the complete nutrient medium after Hoagland  $\frac{1}{2}$  (H o a g l a n d and A r n o n, 1950). Eight plants of each genotype were planted in 12 replications (96 plants per genotype).

Polyethylene glycol (PEG) was used to induce water deficit. PEG is an inert, non-ionic polymer, which does not interfere with plant metabolism. PEG is often used in studies of plant water regime (Lawlor, 1970; Oertli 1985, 1986).

After 30 days of growth in the full nutrient medium, plants from 7 pots of each genotype were transferred to the full medium to which PEG 6000 was added in the concentration that provided the final osmotic value of the medium of 0.1 MPa (PEG). Plants of the remaining 5 pots of each genotype continued their growth in the full nutrient medium (control). The mass of PEG 6000 to be added to each pot in order to achieve the final osmotic value of 0.1 MPa was calculated by the regression equation  $y = 0.0028 x^2 + 0.0052 x$ , which was experimentally obtained for the concrete PEG, as described by M o n e y (1989), using automatic micro-osmometer Roebling, type 12/12 DR. Nutrient solutions were changed at 3-day intervals and aerated daily.

After 21-day treatment, plants were taken for analyses. Fresh matter was measured first, dry matter after drying the samples at 70°C to constant mass. Leaf area was measured by an automatic photoelectric meter LI-3000 (Automatic Area Meter LI-3000, LICOR, USA).

Total nitrogen was determined by the micro-Kjeldahl method, potassium content flamephotometrically, phosphorus content by the ammonium-vanadate-molybdate method. Contents of Ca, Mg, Fe, Zn, Mn and Cu were determined by standard atomic absorption spectrophotometry, using a Varian model SPECTR AA-10.

The obtained results were statistically processed by calculating means and the least significant differences (LSD) between the means, using the program MstatC (Michigan State University, USA).

## **RESULTS AND DISCUSSION**

Dry matter mass of the aboveground parts of plants grown in the conditions of water deficiency was 50% lower than that in the control (A r s e n i j e v i ć - M a k s i m o v i ć et al., 2002). Experimental results in this paper are expressed as content of mineral elements per plant. Nitrogen and P contents were significantly reduced in all genotypes exposed to water deficit. The reduction of N content was lowest in genotype 3 (13.8%), highest in genotype 7 (71.8%), with the average of about 50% (Figure 1). Generally, N content was



Fig. 1. Nitrogen content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

more variable in the control plants than in those exposed to drought. This can be explained by differences in growth rate during early stages of onthogenesis among the genotypes. Nitrogen content in leaves is considered as a better indicator of water deficit effects than N content in roots (F o s t e r et al., 1991). Reduction of N content under water deficit was reported for many plant species including maize, been, citruses, Festuca (B r u c e et al., 2002; F o s t e r et al., 1991; Z e k r i, 1995; H u a n g, 2001). Beside its effect on nitrogen content itself, water deficit affects N metabolism as well. This is evident through the inhibition of nitrate reductase activity and increase in nitrate and proline contents in young sugarbeet plants (P e t r o v i ć et al., 1987; P e t r o v i ć et al., 1991; A r s e n i j e v i ć - M a k s i m o v i ć et al., 2002). Genotypes tolerant to drought have higher N use efficiency than less tolerant genotypes (V a n d e n B o o g a a r d, 1995; F o s t e r et al., 1991).

Phosphorus content was least reduced in genotype 3 (18.4%), most reduced in genotype 8 (74.8%), while the average reduction was 50.5% (Figure 2). P uptake is sensitive to increased osmotic pressure in nutrient solution (K a s t o r i, 1976). It is well documented that P plays a role in drought tolerance in many plant species including sugarbeet (P e t r o v i ć, 1987), beans and sorghum (A1-K a r a k i, 1995) and wheat (G u t i e r r e z - B o e m and T h o mas, 1998). Under conditions of water deficit, disturbances in P provision as well as in P metabolism occur in sugarbeet, as indicated by a change in mineral and organic P ratio in favor of the former (P e t r o v i ć, 1987).



Fig. 2. Phosphorus content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.



Fig. 3. Potassium content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

Water deficiency provoked statistically significant reductions of K content in leaves in all genotypes except genotype 3 (Figure 3). The average reduction of K content was 41.8%, the highest reduction was registered in genotype 7 (59.27%). Potassium participates in osmoregulation and a change in its content is usually coupled with changes in the contents of sugar and amino acids (Jones et al., 1981; Ford and Wilson, 1981) which play a role in plant adaptation to the lack of water. Potassium is most probably important in keeping the balance with negatively charged amino acids (Jones et al., 1981). Calcium and magnesium contents declined significantly in all genotypes exposed to water stress, on average by 63% and 54%, respectively (Figures 4 and 5). The reduction of K and Mg contents was found in 7 different citrus genotypes suffering from PEG-induced water deficit (Zekri, 1995). The ratio between K and Ca contents in leaves of sugarbeet genotypes was disturbed under the experimental conditions. In the control plants, the average value of this ratio was 13.5 while in the plants exposed to PEG-induced water deficit it was 21.2, meaning that its average increase was 57.22%. Potassium and calcium are not equally mobile in plants: K is very mobile and easily reutilized, while Ca is poorly mobile and it is practically not reutilized at all. Since transpiration flow is the key factor in provisioning the aboveground plant parts with Ca, and since this flow is impaired under the conditions of water deficit due to reduction in water uptake and transpiration (K a s t o r i, 1968), a change occurs in the K/Ca ratio in the shoots of plants exposed to PEG. Changes of this ratio affect the functioning of stomata as well. Increase in Ca con-



Fig. 4. Calcium content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

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Fig. 5. Magnesium content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

tent in guard cells may be a sufficient signal for stomatal closure (L u a n, 1993), the process in which ABA (S c h a u f et al., 1987) and auxin (M a r t e n et al., 1991) are involved. Therefore, Ca deficiency could cause a disturbance in the stomatal control of transpiration.

The P/Ca ratio increased by 33.7% under the water deficit, suggesting that the Ca content in leaves declined to a higher extent than the P content, which again can be explained by poor mobility of Ca in plants.

Iron content declined significantly in all genotypes under the water stress (51.4  $\mu$ g on average) (Figure 6). The lowest relative decreases in iron content were found in genotypes 1, 3 and 8, the highest in genotypes 7 and 9. The P/Fe ratio changed only slightly (less than 10%) under the experimental conditions. This reduction in Fe content can therefore be assigned to the generally reduced uptake of iron from the nutrient solution.

The content of Zn was significantly reduced in all genotypes under the water deficiency conditions. On average, this reduction was 54.8% (Figure 7). The decrease in Zn content was relatively lowest in genotypes 1, 3 and 4 and highest in genotypes 7, 8 and 2. Although reduced under the water deficit, Cu content was not significantly changed in all genotypes (Figure 8). Cu content was not significantly reduced in genotypes 1, 3, 4 and 6, it was significantly reduced for " = 0.05 in genotype 9, while the reduction was significant for " = 0,01 in genotypes 2, 7 and 8. Similar to Fe and Zn, Mn content was also significantly reduced in all genotypes, with the average reduction of 42.86% (Figure 9). The lowest reduction was recorded again in genotype 3, the highest in



Fig. 6. Iron content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.



Fig. 7. Zinc content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.



Fig. 8. Copper content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.



Fig. 9. Manganese content in leaves of different sugar beet genotypes. Control, complete nutrient medium; PEG, nutrient medium with osmotic value reduced to 0.1 MPa by addition of PEG.

genotype 7. Manganese is involved in the process of photosynthesis and activation of several enzyme systems. It was shown that Mn deficiency inhibits cell elongation (M u k h o p a d h y a y and S h a r m a, 1991). Cu, Zn and Mn are components of superoxide dismutase (SOD), which exists in two forms: Cu/ZnSOD and Mn/SOD. It was found that the expression of genes coding for these enzymes can be induced by the lack of water and/or by rehydration of plants which had experienced drought. Also, an increase in corresponding mRNA content was recorded during the period of plant adaptation to changes in the amount of water available to plant (Perl-Treves and Galun, 1991; Wu et al., 1999).

### CONCLUSIONS

The contents of N, P, K, Ca, Mg, Fe, Zn, Mn and Cu in the leaves of young plants of all 8 sugarbeet genotypes declined under water deficiency conditions. However, the intensity of reduction differed among the genotypes. Therefore, it was not possible to establish a general pattern for the observed changes in the contents of the tested elements.

The results suggest that some detrimental effects of water deficiency were due to impaired mineral nutrition, especially the lack of N, P and Mg, and also due to disturbed ratios between the contents of certain elements, especially K/Ca.

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### УТИЦАЈ ВОДНОГ ПОТЕНЦИЈАЛА НА НАКУПЉАЊЕ НЕКИХ НЕОПХОДНИХ ЕЛЕМЕНАТА У ЛИСТОВИМА ШЕЋЕРНЕ РЕПЕ (Beta vulgaris, ssp. vulgaris)

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#### Резиме

Циљ рада је био да се испита утицај смањеног водног потенцијала хранљивог раствора на накупљање неких неопходних макро- и микроелемената у надземном делу младих биљака шећерне репе. Биљке 8 различитих генотипова шећерне репе су 21 дан гајене на хранљивом раствору чији водни потенцијал је подешен на 0,1 МРа помоћу полиетилен гликола (PEG). Садржај N, P, K, Ca, Mg, Fe, Mn, Cu и Zn је у свим генотиповима опао у условима недостатка воде, али интензитет овог смањења није био једнак код свих генотипова. Резултати указују да се неки од штетних утицаја водног дефицита могу приписати поремећајима у минералној исхрани, посебно недостатку N, P и Mg, као и измењеном односу између садржаја појединих елемената, посебно К/Са.