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Influence of foliar fertilizing on stomata parameters in maize leaf (*Zea mays* L.)

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Abstract

In this research, the effects of foliar fertilizer Megegreen on stomata parameters of maize leaf (*ZP 677*) were studied. The experiment was performed on the experimental fields of the Institute of Agriculture, in Skopje, R. of Macedonia, during the 2008 and 2009. The foliar fertilizer was applied four times during the growing period in different concentrations of 0.3, 0.6 and 0.9% solution. Stomata density and size were measured on the adaxial and abaxial leaf surface from randomly selected plants from each replication in stage of silking. Stomata counts were made on the impressions from microscopic fields using the colodium method. Analyses of variance indicated that the application of foliar fertilizer has significant influence on stomata features on corn leaves. Results from research, show higher stomata density on adaxial (176,19-182,32 stomata/mm^2) and abaxial surface (289,12-293,12 stomata/mm^2) at variants 3 and 4. Variant 3 has the highest stomata length on adaxial surface (59,75 μm), without significant difference and the highest average length on the abaxial surface (63,00 μm), which is significantly different from the control variant. With the highest average width on adaxial leaf surface was variant 4 with 11,56 μm and on the abaxial surface was variant 2, with 13,49 μm. A positive significant correlation was observed between stomata number on the adaxial and abaxial surface of leaf (R^2= 0,856**).

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Introduction

Environmental factors cause diverse responses in plants, which can lead to the emergence of many morphological and anatomical changes. Previous studies shown that nitrogen, phosphorus and potassium, absorbs through the leaf, and can be transferred in different directions, so their activity is closer to the activity which they show when they absorb through the root (Sarić, 1971). Leaf structure, primarily the density and morphology of stomata and cuticle thickness of leaf is very important for ions absorbing through the leaf.

Stomata are small pores on the surfaces of leaves and generally comprised of two guard cells. Stomata were observed in the seeds, primary root, leaf and other parts of plants (Paiva *et al.,* 2006). They are the major gates for gas exchange of leaves (Brent and Ram, 2000). Stomata control the exchange of gasses between the interior of the leaf and the atmosphere (Hetherington and Woodward, 2003) and plays an important role in process of transpiration, (Sarić, 1991). Stomata also play an important role in plant innate immunity (Melotto *et al.* 2006).

Different cultivars of crop plants may have different gas exchange capabilities because they have various numbers of stomata per unit of leaf area and various stomata sizes (Farquhar *et al.* 2002). Stomata number is result of environmental conditions, like increasing concentration of CO_2, increasing temperature, drought and changes of the precipitation distribution (Zhenzhu and Guangsheng, 2008). The stomata number per unite area is a different not only between plant species but also within species, depending of the size, topography and age of the leaf. Lecoeur *et al.* (1995) showed that stomata density and stomata index may be influenced by cell growth, leaf development, age and position. Generally stomata are smaller when their number is higher and inversely.

High intensity transpiration besides the small number of stomata can be explained by the influence of some biogenic elements, especially nitrogen and potassium, which can influence capacity of stomata opening. At reduced availability with potassium or nitrogen, and other biogenic elements, cells very slowly react to changing environmental factors, resulting in increased intensity of transpiration (Levitt, 1968). Deficit of essential biogenic elements (N, P, S and Mg) has a huge influence on stomata formation and their morphological characteristics in maize leaf. As a result of the deficit of these elements from the nutrient solution leafy area was reduced (Kastori and Petrović, 1972a).

Plant stomata, the vital gate between plant and atmosphere may play a central role in plant/vegetation responses to environmental conditions, which have been and are being investigated from molecular and whole plant perspectives, as well as at ecosystem and global levels (Nilson and Assmann, 2007).

Water separation from the plant depends on the plant species, genotype and plant mineral nutrition regime. This is very important for the practice, because the selection of the genotype and the use of fertilizers can affect water consumption. In recent years, attention was directed to research on distribution and function of stomata. As an objective of this study was to analyse the influence of foliar fertilizing on distribution and morphology of stomata in maize leaf.

Materials and methods

Plant material

As a plant material, maize hybrid *ZP 677* was used. The hybrid belongs to the group of late hybrids, *FAO 600*, with potential yield above 15t/ha with high quality (http://mrizp.rs/zp-677).

Foliar treatments

Fertilizer Megagreen which was applied, is ecological foliar fertilizer made of calcite, micronized by a new tribomechanical technology. Main components of fertilizer are: $CaCO_3$: 82.3 %; SiO_2 5.56 %; MgO 3.02 %; CaO 41.7%; Fe 8783 mg/kg; Mn 156 mg/kg; Se 0.24 mg/kg.

Experimental variants of foliar treatments:
Variant 1. Control (without fertilizing);
Variant 2. Megagreen - 0.3% solution;
Variant 3. Megagreen - 0.6% solution;
Variant 4. Megagreen - 0.9% solution.

Foliar applications were made by back sprayer, with 4.8l solution for each variant. The foliar fertilizer was applied four times during the growing period, starting from the stage of 7-8 leaf (V7), in a intervals of 10-15 days.

Stomata analyses

Stomata density and morphology were measured on the adaxial and abaxial surface from randomly selected plants from each replication in silking stage. Leave material was collected from leaves under the cob. Stomata counts were made on the impressions from microscopic fields of for adaxial/abaxial surfaces using the colodium method (Sarić *et al.*, 1986). Stomata length (SL) and stomata width (SW) with guard cells (SWGC) were measured in micrometers on both surfaces from the impressions on 400 x magnification. Number of stomata (N) was calculated in one mm^2 per unite area of leaf with the Bϋrker net, and stomata area in one square millimeter of leaf was calculated as a product of SLxSWxN.

Statistical analyses

Analysis of variance was performed using SPSS 14.0 software (SPSS Inc., 2005), and correlation was calculated at 0.05 level.

Results and discussion

Analyses of variance indicated that the application of foliar fertilizer has significant influence on stomata features on the adaxial and abaxial surface on corn leaves (Table 1, 2 and 3).

Table 1. Stomata density (mm^2).

	No.stomata (adaxial)					No.stomata (abaxial)				
Variant	2008	CV	2009	CV	2008/09	2008	CV	2009	CV	2008/09
Control	158,67	12,19	183,68	9,01	171,17 c	245,28	11,92	335,04	4,31	290,16 ab
M 0.3%	152,39	11,18	171,52	8,89	161,95 d	256,80	10,58	295,84	4,78	276,32 c
M 0.6%	160,67	11,43	203,97	3,90	182,32 a	247,36	8,64	330,88	3,61	289,12 b
M 0.9%	166,62	14,50	185,76	6,45	176,19 b	246,56	11,29	339,68	3,77	293,12 a

*The means having common letter(s) are not significiently different at 0.05 level.

Table 2. Stomata length (μm).

	Stomata lenght (adaxial)					Stomata lenght (abaxial)				
Variant	2008	CV	2009	CV	2008/09	2008	CV	2009	CV	2008/09
Control	66,89	1.80	50,65	3.70	58,77 ***a***	57,83	3.69	56,37	2.23	57,10 ***c***
M 0.3%	65,51	5.70	44,76	1.36	55,14 ***b***	59,40	3.30	53,71	1.58	56,56 ***c***
M 0.6%	65,31	6.20	54,20	2.57	59,75 ***a***	60,28	0.94	65,71	4.26	63,00 ***a***
M 0.9%	65,67	7.66	45,59	3.14	55,63 ***b***	62,16	2.24	53,96	12.30	58,06 ***b***

*The means having common letter(s) are not significiently different at 0.05 level.

Stomata density on the adaxial and abaxial leaf surface

In our research with foliar fertilizing (Table 1), results show higher stomata density on adaxial and abaxial surface for variant 3 and variant 4. The variant 3 has the highest stomata density of adaxial surface, with 182,32 stomata/mm^2 statistically significantly at 0.05 level compared with control variant. Variant 4 has significantly higher average stomata density on abaxial surface compared with the control variant without statistical signification. Stomata density of variant 4 is 176,19 stomata/mm^2 on adaxial surface and 293,12/mm^2 on abaxial surface. Variation of stomata density is larger on both leaf surface in the first year of study compared with second year and range from 11,43 (variant 3) to 14,50 (variant 4) on the adaxial and 8,64 (variant 3) to 11,92 (control) on the abaxial surface of the leaf.

Kastori and Petrović (1972a), found that the total stomata number on adaxial surface (x10^3) after 15 days with the deficit of N, S, P, Mg, was: N-893; P - 1113; S -1255; Mg - 1241; and after 60 days , were : N-65; P - 80; S - 233 Mg - 126 stomata for the total area from the fourth leaf. On the abaxial surface on same impressions > N-2140; P - 2647; S - 3092 Mg - 3068 stomata, ie N-165; P - 221; S - 586 Mg - 236 stomata were obtained.

Table 3. Stomata width and stomata width with guard cell (μm).

Variant	Stomata width (adaxial)					Stomata width (abaxial)					Stomata width with guard cell (adaxial)					Stomata width with guard cell (abaxial)				
	2008	CV	2009	CV	2008/09	2008	CV	2009	CV	2008/09	2008	CV	2009	CV	2008/09	2008	CV	2009	CV	2008/09
Control	12,67	15.2	8,53	3.76	10,60 ***b***	12,87	7.67	13,58	2.00	13,23 ***a***	29,04	7.83	22,08	0.86	25,96 ***c***	29,52	5.10	31,29	2.78	30,41 ***a***
M 0.3%	11,84	6.02	10,47	12.10	11,16 ***a***	12,63	3.75	14,34	4.34	13,49 ***a***	28,02	2.41	21,08	15.00	24,55 ***d***	28,89	2.49	28,29	9.39	28,59 ***c***
M 0.6%	11,73	9.86	10,34	1.56	11,04 ***a***	12,41	1.52	13,16	6.13	12,79 ***b***	28,20	8.55	27,74	4.18	27,97 ***a***	29,28	2.94	29,72	2.96	29,50 ***b***
M 0.9%	13,24	4.94	9,87	2.96	11,56 ***a***	11,91	5.54	14,52	1.48	13,22 ***b***	28,67	3.02	24,95	0.57	26,81 ***b***	28,16	4.05	30,13	2.92	29,15 ***c***

*The means having common letter(s) are not significiently different at 0.05 level.

Stomata are more presented on the reverse side of the leaf (on the middle leaf at later ZP 677 53.93 stomatas per square mm, on the last leaf at the same hybrid 72.37 (Angelov *et al.* 1995). Our research results show higher stomata density on abaxaial surface correlated with results reported by Angelov *et al.* (1995).

According the Maherali *et al.* (2002), the influence of the stomata size, structure and distribution on leafy surface from abiotic factors may depend on the plant species varieties, plant cultivation, crop density and other environmental factors. Miranda *et al.* (1981) showed variability of stomata number on corn leaf (*Zea mays*) and according with them stomata density, except their length, which varies across all leaf surface.

Stomata dimensions on the adaxial and abaxial leaf surface

Foliar fertilizing has no effect on adaxial surface for stomata length in the first year of application. The control variant was with the highest length on the adaxial surface (66,89 μm), opposite results from abaxial surface (57,83 μm).

Table 4. Correlation matrix for stomatal characters of corn leaf.

Character	No. stomata (adaxial)	No. stomata (abaxial)	SL (adaxial)	SL (abaxial)	SW (adaxial)	SW (abaxial)	SWG (adaxial)	SWG (abaxial)	Stomata area (adaxial)	Stomata area (abaxial)
No.stomata (adaxial)	1	,856(**)	,342	,183	-,493(*)	,361	-,188	,334	,465(*)	,871(**)
No.stomata (abaxial)		1	,157	-,159	-,760(**)	,629(**)	-,503(*)	,434(*)	,125	,940(**)
SL (adaxial)			1	,530(**)	-,059	-,123	,090	-,210	,877(**)	,296
SL (abaxial)				1	,257	-,569(**)	,523(**)	-,014	,618(**)	,081
SW (adaxial)					1	-,510(*)	,658(**)	-,407(*)	,242	-,674(**)
SW (abaxial)						1	-,493(*)	,417(*)	-,206	,630(**)
SWG (adaxial)							1	,111	,325	-,357
SWG (abaxial)								1	-,208	,478(*)
Stomata area (adaxial)									1	,275
Stomata area (abaxial)										1

* SL - stomata length (μm)
* SW stomata width (μm)
* SWG stomata width with guard cell (μm)

** Correlation is significant at the 0.01 level (2-tailed)
* Correlation is significant at the 0.05 level (2-tailed).

Highest average length of 62,16 μm showed variant 4 on abaxial surface with the highest CV of 7,66. In the second year, at variant 3 the highest stomata length on the adaxial (54,20 μm) with CV 2,57 and on the abaxial surface (65,71 μm) with CV 4,26 were observed. Average for the both experimental years (Table 2) showed that with the highest length on adaxial surface, was variant 3 (59,75 μm), but without significant difference compared to the control variant. Variant 3 has highest average length on the abaxial surface, with 63,00 μm, which is significantly different from the control variant.

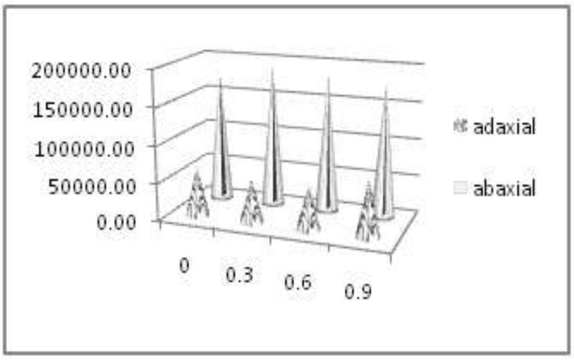

Fig. 1. Stomata area in 2008.

Only variant 4 in the first year, has highest stomata width (13,24 μm) compared with control variant on adaxial surface. In the second year, all variants has higher stomata width compared with control variant (8,53 μm) on adaxial surface. Comparing the results of Table 3, we can conclude that foliar application had a positive impact on the width on the adaxial surface because all variants have significantly higher width than the control variant. The highest average width on adaxial surface is determined in variant 4 (11,56 μm), and the lowest in the control variant (10,60 μm). In the first research year, the highest average width has control variant on abaxial surface. In the second year, in variants 2 and 4 was show higher stomata width from the control variant on abaxial surface. With the highest average (2008/2009) width on abaxial leaf surface is variant 2, with 13,49 μm. The other two variants (3 and 4) have a lower width compared to control variant.

Angelov *et al.* (1995) presented that stomata length of the adaxial surface of the middle leaf from the hybrid ZP 677 is 58.60 μm, and in the last leaf is 54.74 μm. On the abaxial surface stomata length on middle leaf of the same hybrid is to 53.82, and on the last leaf is 47.87 μm. Stomata width on the adaxial surface is 9.90 μm (on the middle leaf) and 11.74 μm (on the last leaf), while on the abaxial surface, width is 9.81 μm (on the middle leaf) and 9.33 μm(on the middle leaf). Stomata width, had minimal difference on abaxial and adaxial surface, compared with our results. Our results correlate with results of Angelov *et al.* (1995) showing that foliar fertilizer application positively influenced increase of stomata length on both sides of the leaf with a degree of significance. Pandey (2007) reported that the changes in the length and width of guard cell have no response to environmental conditions, which suggests influence of genotype. Francs *et al.* (2001) showed that guard cell length had no change when pore opens.

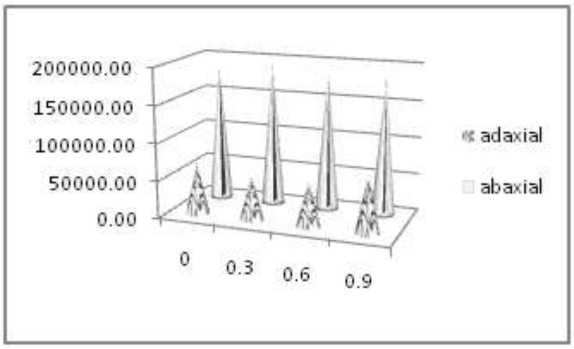

Fig. 2. Stomata area in 2009.

Stomata width with guard cells at our research was highest in control variant on adaxial and abaxila surface in first year and in the second year the highest stomata width with gard cells is observed in variant 3. On the adaxial leaf surface the largest average width of stoma with guard cells is determined in variant 3 (27,97 μm) and it was statistically significant. The foliar application had no effect on stomata width with gard cells on abaxial surface. Kastori and Petrović (1972a) in their study reported that stomata length ranged from 32-46 μm, and the width (with guard cells) ranged from 23 to 31 μm, comparable with our results. According the Holland *et al.* (2009) guard cell length generally increased with elevation. As can be seen from Figure 1 and 2, stomata area on adaxial leaf surface was highest at variant 4 (with concentration 0.9 %) and variant 2 (with concentration 0.3%) had the highest stomata area on abaxial leaf surface for both years. General conclusion is that the stomata dimensions on adaxial and abaxial surface show high variability in both years of our research.

Correlation analysis for stomata characters of adaxila and abaxial leaf surface

From the correlation studies (Table 4) among the stomatal parameters It was observed a positive significant correlation between stomata number on the adaxial and abaxial surface of leaf (R^2= 0,856**). This result implies that measuring the stomata density in one side of leaf is adequate for stomata density on another side of leaf. Also stomata number on abaxial surface had significant (P=5 and 1%) highly positive correlation with stomata area on the same surface of leaf (R^2= 0,940**). This correlation suggests that stomata area is strongly associated with the stomata density in one square millimeter of leaf.

Significantly positive correlation also was observed between stomata area and stomata length of adaxial leaf side (R^2= 0,877**). Negative weakly relationship was noted only between stomata number of abaxial side and stomata width on the adaxial surface (R^2=-0,760**). Gaskell and Pearce (1983) found that stomatal density was negatively correlated with grain yield and with stomatal size. Stomatal frequence in wheat was shown to be greater on the adaxial than on the abaxial surface.

References

Angelov I, Matvejeva J, Stankovic L, Kratovalieva S. 1995. Morphology and distribution of stomatas at some hybrids of maize (*Zea mays L.*). Macedonian Agricultural review **42(2),** 87-93.

Brent EE, Ram O. 2000. Analyses of assumptions and errors in the calculation of stomatal conductance from sap flux measurements. Tree Physiology **20,** 579589.

Farquhar GD, Buckley TN, Miller JM. 2002. Optimal stomatal control in relation to leaf area and nitrogen content. Silva Fennica **36(3),** 625-637.

Franks PJ, Buckley TN, Shope JC, Mott KA. 2001. Guard cell volume and pressure measured concurrently by confocal microscopy and the cell pressure probe. Plant Physiology **125,** 1577-1584.

Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. Nature **424,** 901-908.

Holland N, Andrew D, Richardson. 2009. Stomatal length correlates with elevation of growth in four temperate species. Journal of Sustainable Forestry **28,** 63-73.

Lecoeur J, Wery J, Turc O, Tardieu F. 1995. Expansion of pea leaves subjected to short water-deficit: cell number and cell-size are sensitive to stress at different periods of leaf development. Journal of Experimental Botany **46,** 1093–1101.

Levitt J. 1968. Stomatal opening: rolle of potassium uptake. Science **163,** 494.

Kastori R, Petrović M. 1972a. The effect of some biogenic elements on the stomatal apparatus in maize plants. Matica srpska Novi Sad **42,** 124-135.

Maherali H, Reid CD, Polley HW, Johnson HB, Jachson RB. 2002. Stomatal acclimation over a subambient to elevated CO_2 gradient in a C3/C4 grassland. Plant, Cell and Environment **25,** 557–566.

Melotto M, Underwood W, Koczan J, Nomura K, He SY. 2006. Plant stomata function in innate immunity against bacterial invasion. Cell **126,** 969-980.

Miranda V, Baker NR, Long SP. 1981. Anatomical variation along the length of the *Zea mays* leaf in relation to photosynthesis. New Phytologist **88,** 595-605.

Nilson SE, Assmann SM. 2007. The control of transpiration. Insights from *Arabidopsis*. Plant Physiology **143,** 19-27.

Paiva EA, Lemos-Filho JP, Oliveira DM. 2006. Imbition of Swietenia macrophylla (*Meliaceae*) seeds: The role of stomata. Annals of Botany **98,** 213-217.

Pandey R, Chacko PM, Choudhary ML, Prasad KV. 2007. Higher than optimum temperature under CO_2 enrichment influences stomata anatomical characters in rose (*Rosa hybrida*). Scientia Horticulturae **113,** 74-81.

Sarić RM. 1971. Plant Phisiology, Novi Sad.

Sarić M, Kastori R, Petrović M, Stanković Z, Krstić B, Petrović N. 1986. Practical physiology of plants, Scientific Book, Beograd.

Sarić M, Krstić B, Stanković ZJ. 1991. Plant Phisiology, Science, Beograd.

Zhenzhu X, Guangsheng Z. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. Journal of Experimental Botany **59(12),** 3317–3325.