

Acta Horticulturae et Regioteecturae 2  
Nitra, Slovaca Universitas Agriculturae Nitriae, 2015, pp. 45–48

## STOMATAL DENSITY IN MISCANTHUS LEAVES

Žaneta PAUKOVÁ,\* Zuzana JUREKOVÁ

Slovak University of Agriculture in Nitra, Slovak Republic

In this study, the number of stomata per unit area was determined in the perennial energy grass of two genotypes *Miscanthus × giganteus* (Greef et Deuter) and *M. sinensis* 'Tatai' on arable land in the South-western Slovakia. The dynamics of stomatal density per mm<sup>2</sup> was determined by non-destructive method in three randomly selected clumps. The number of stomata per mm<sup>2</sup> of area varied from 125 ±23 to 327 ±47, stomatal length ranged from 33.9 ±4.7 μm to 35.3 ±3.4 μm, and stomatal width ranged from 17.5 ±2.3 μm to 18.8 ±2.8 μm in the two genotypes. We confirmed ontogenetic heterogeneity of leaf area size and stomatal size parameters.

**Keywords:** energy plant, *Miscanthus*, stomata, stomatal density, cardinal points

The perennial grass *Miscanthus* with C<sub>4</sub> mechanism of photosynthesis is considered a suitable energy crop for cultivation in climatic conditions of Europe due to its strong growth, great productive potential and adaptability to environmental conditions.

The potential ability of the crop stand to form biomass depends largely on leaf area, anatomical, morphological and functional characteristics of leaves. Space that leaves occupy in the stand is determined by genotype and environmental conditions that allow bigger or smaller expansion of the leaf area (Volkenburgh, 1999). Stomatal density, as well as their size and the size of stomatal slit are considered to be relatively plastic features (Richardson et al., 2001) that are potentially adaptive to environmental changes. It was confirmed that stomatal density depends on the soil water content (Xu and Zhou, 2008), light (Thomas et al., 2003) and CO<sub>2</sub> concentration in air (Woodward, Kelly, 1995).

The aim of our study was to evaluate the density and localization of stomata in leaves of two genotypes of *Miscanthus sinensis* grown in field conditions on arable land. The stomata were studied on both sides of the leaf skin, depending on their ontogeny, position and size of the leaf area.

### Material and methods

The research was carried out at the field trial base belonging to the University Farm Holding of the Slovak University of Agriculture in Koliňany. The research plot is located 13 km from Nitra (48° 21' 20" N, 18° 12' 23" E). It belongs to the cadastral area of Koliňany. The code of the soil quality defined by BSEU is 0111002. The main soil type is gley fluvisol, in terms of grain structure it belongs to moderately heavy soils. In terms of exposure, the area is plain without an

expression of surface erosion (0° to 1°). Soils are deep (60 cm or more) and without skeleton.

The two genotypes used in the research were *Miscanthus × giganteus* and *Miscanthus sinensis* 'Tatai'. *M. × giganteus* Greef et Deu is a vital triploid hybrid (57 chromosomes) (Greef et Deuter, 1993). The planting material consisted of rhizomes provided by the company Hannes Stelzhammer, Austria. *M. sinensis* 'Tatai' is also a triploid hybrid (57 chromosomes). It was bred by crosspollination of *Miscanthus sinensis* genotypes. The planting material consisted of seedlings grown *in vitro* in the Power-H Kft, Hungary. Before planting, the seedlings were planted individually in rooting containers with a soil substrate (Jureková et al., 2012, 2013; Prčík a Kotrla, 2013).

The leaf area size A (mm<sup>2</sup>) was determined by the non-destructive method (Clifton-Brown, Lewandowski, 2000) directly in the field conditions. The dynamics of stomatal density per mm<sup>2</sup> in the leaves was determined by the non-destructive method in three selected clumps for each genotype in 2013. The analysis was performed on designated stem on juvenile, adult and senescent leaves located in the middle of a stand (approximately fifth leaf from the bottom), exposed to northern, southern, eastern and western direction by the micro-relief method (Pazourek, 1963). The samples were collected in the middle part of leaves outside the main vein on the adaxial (top) and abaxial (bottom) skin surface in May, June and July, 2013. The evaluation of the preparations was carried out by the optical microscope Axiostar plus, Carl Zeiss lens, CP-Achromat 40 × / 0.65, 10 × eyepiece PI / 18, Canon Utilities software Zoom Browser EX 4.6 and hardware Acer Travel Mate 4600, Canon Power Shot A 95. Totally, 144 microscopic view-fields were considered in the counting. Stomata length and width were measured in 88 randomly selected stomata. Statistical significance of the

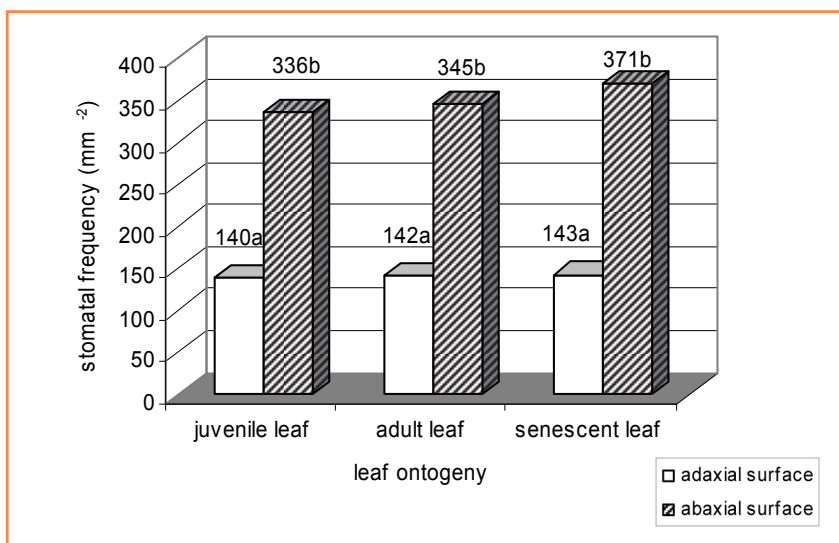
Contact address: Ing. Žaneta Pauková, PhD., Slovak University of Agriculture in Nitra, Faculty of European Studies and Regional Development, Department of Ecology, Mariánska 10, 949 76 Nitra, Slovak Republic  
e-mail: [zaneta.paukova@uniag.sk](mailto:zaneta.paukova@uniag.sk)

differences was evaluated by the LSD-test in the Statgraphic Plus software.

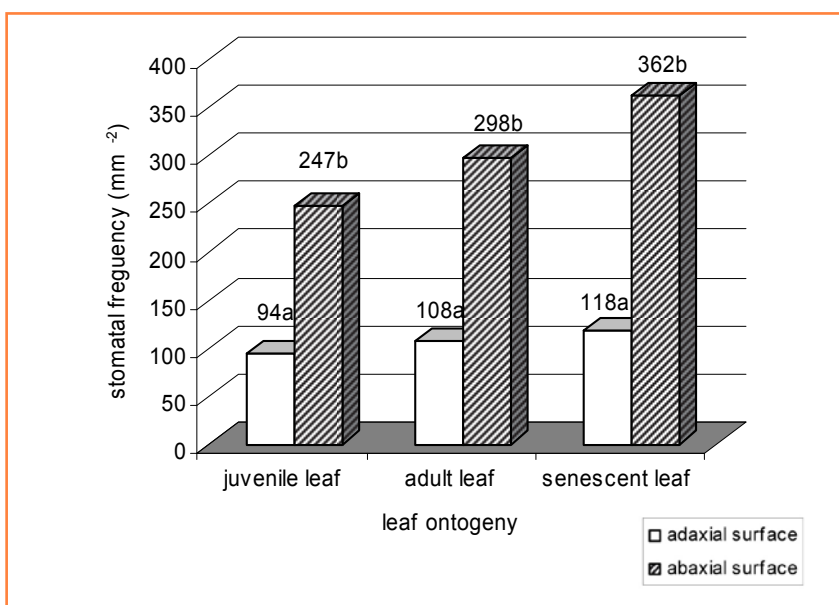
### Results and discussion

In stands, plant leaves adapt their growth to environmental conditions and thus affect the functional properties and characteristics of the whole plant. The leaves grow during their individual ontogeny, maturity and age according to the position on stems. Basal (oldest) leaves wither already during July. The leaves of the studied *Miscanthus* genotypes grew differently (Table 1) and differed also in the growth of the leaf area according to the cardinal directions. Young leaves of *M. × giganteus* created in juvenile growth stage larger area in the northern orientation, the leaves of *M. sinensis* 'Tatai' created larger area in the eastern orientation. The leaves oriented to the north did not grow in the maturity stage, unlike the leaves with the eastern orientation. The leaf area size of senescent leaves of the both genotypes decreased regardless of the position in relation to the cardinal directions. The leaves gradually dried up and withered.

During the research in 2013, we confirmed statistically highly significant dependence of the number of stomata on genotype in the skin of juvenile leaves (LSD<sub>0.05</sub> test ± 27.08) and in the skin of adult leaves (LSD<sub>0.05</sub> test ± 22.61). At the later stage, no differences were found. Similarly, Jureková et al. (2012) reported dependence of a number of stomata in the skin of juvenile leaves on genotypes of *M. sinensis* 'Tatai' from the same site in 2012. Fraser et al. (2009) found out that stomatal density of *Pseudoroegneria spicata* is not a phenotypically plastic plant trait but it is also genotypically differentiated.



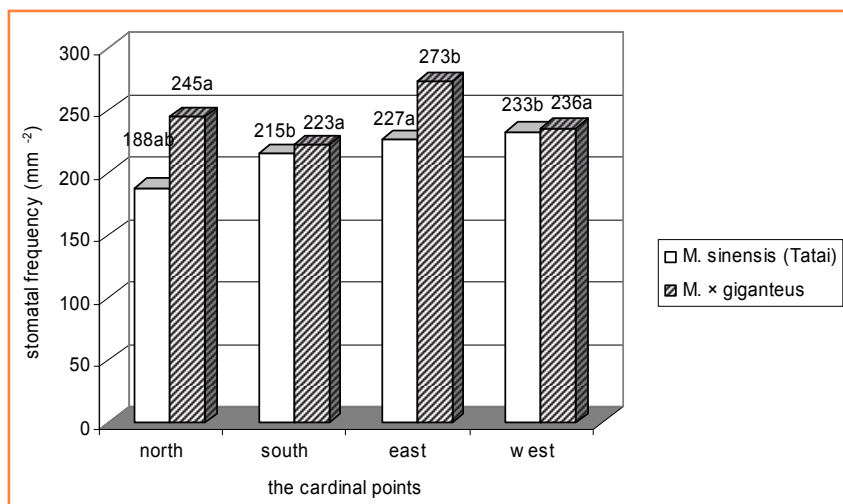
**Figure 1** Statistical evaluation of significant differences in the number of stomata per mm<sup>2</sup> of the adaxial and abaxial surface depending on the leaf ontogeny of *M. × giganteus*. Values with different letters (a, b) in columns indicate statistically significant difference according to the LSD test ( $P < 0.05$ )



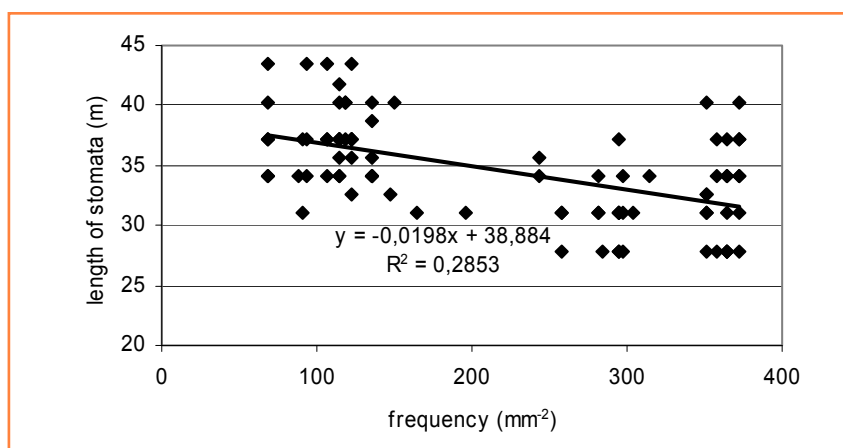
**Figure 2** Statistical evaluation of significant differences in the number of stomata per mm<sup>2</sup> of the adaxial and abaxial surface depending on the leaf ontogeny of *M. sinensis* 'Tatai'. Values with different letters (a, b) in columns indicate statistically significant difference according to the LSD test ( $P < 0.05$ )

**Table 1** Changes of the leaf area size in m<sup>2</sup> of the studied genotypes according to localization regarding to cardinal directions

Phase of leaf growth	Leaf area in m <sup>2</sup> – <i>Miscanthus × giganteus</i>		Leaf area in m <sup>2</sup> – <i>Miscanthus sinensis</i> 'Tatai'	
	north	east	north	east
Juvenile leaf	0.11375	0.09469	0.14534	0.13052
Adult leaf	0.12187	0.14632	0.13406	0.14872
Beginning of senescence	0.09828	0.14632	0.12268	0.12667
Senescence leaf	0.09201	0.10790	0.11154	0.12667



**Figure 3** Statistical evaluation of significant differences in the number of stomata per mm<sup>-2</sup> of the senescent leaves *Miscanthus* depending on the cardinal directions. Values with different letters (a, b) in columns indicate statistically significant difference according to LSD test ( $P < 0.05$ )



**Figure 4** Linear dependence between the number of stomata per mm<sup>-2</sup> and the stomatal length in µm

The number of stomata in leaves was statistically highly significantly affected by adaxial and abaxial leaf surface of the skin in all three stages of ontogeny of *M. × giganteus* (LSD<sub>0.05</sub> test ± 54.54, ± 29.02, ± 43.79) (Figure 1) and *M. sinensis* 'Tatai' (LSD<sub>0.05</sub> test ± 38.89, ± 49.11, ± 32.90) (Figure 2). Ratio of stomata was 27:73 on adaxial and abaxial leaf surface, respectively.

The increase in the number of stomata in leaves during the ontogeny was also influenced by climatic factors in the studied year. The average precipitation during the observed months of May, June and July decreased significantly (78, 47 and 2 mm) and the average air temperature raised (15.6, 19.3 and 22.8 °C) (SHMÚ, 2013).

Fraser et al. (2009) found that reducing water supply increased the stomatal density of *Pseudoroegneria spicata*, but increasing water supply reduced the leaf area.

There were no statistically significant differences found in the number of stomata depending on the cardinal directions in juvenile leaves in both genotypes in 2013. Stomatal density of adult leaves of the genotype *M. × giganteus* oriented towards east was highly significantly greater compared with the other cardinal directions (LSD 0.05 test ± 29.62 north and east, LSD 0.05 test 17.77 ± south and east, LSD 0.05 test ± 23.70 east and west), whereas for the genotype *M. sinensis* 'Tatai' no significant differences were observed. The differences in stomatal density of senescent leaves

between south and east orientation and west and east orientation were found in *M. × giganteus* and were highly significant (LSD 0.05 test 26.82 ± south and east, LSD 0.05 test ± 35.72 east and west) (Figure 3). Between north, south and west each other there are no significant differences in the density of stomata. In the second observed genotype *M. sinensis* 'Tatai', the dependence of stomata from the cardinal directions was reflected only in senile leaves. The differences in stomatal density between south and east, south and west were highly significant (LSD 0.05 test ± 20.15 south and east, LSD 0.05 test ± 20.15 south and west) (Figure 3).

The number of stomata per mm<sup>-2</sup> of area varied from 125 ± 23 to 327 ± 47, stomatal length ranged from 33.9 ± 4.7 µm to 35.3 ± 3.4 µm, and stomatal width from 17.5 ± 2.3 µm to 18.8 ± 2.8 µm among the two genotypes. Correlations between the number of stomata per unit area and stomatal width ( $r = 0.49$ ), and between the stomatal length and width ( $r = 0.35$ ) were statistically significant. The values of the correlation coefficient between the number of stomata per unit area and the stomatal length ( $r = 0.54$ ) were evaluated as highly significant (Figure 4). Rayburn et al. (2009) compared the size of stomata of two *Miscanthus* genotypes in the USA and confirmed genotypic conditionality of the monitored indicator expressed by higher average length of stomata of *M. × giganteus* (24.4 µm) compared to *M. sinensis* 'Tatai' (21.8 µm). These data correspond to our observations where the average length of stomata of *M. × giganteus* was higher (35.3 µm) compared to *M. sinensis* 'Tatai' (33.9 µm). However, the width of stomata was higher in *M. sinensis* 'Tatai' (18.8 µm) than in *M. × giganteus* (17.5 µm). On the contrary (Moon et al., 2013) recorded in the soil and climatic conditions of Korea the average length of stomata of *M. × giganteus* shorter (21 µm) than that of *M. sinensis* 'Tatai' (22.5 µm).

## Conclusions

The results of the experiments with perennial rhizomatous energy grass *Miscanthus × giganteus* Greef et Deu and *M. sinensis* 'Tatai' grown in conditions of the South Western Slovakia provide following conclusions

about stomatal density. The number of stomata per mm<sup>2</sup> of area varied from 125 ±23 to 327 ±47, stomatal length ranged from 33.9 ±4.7 μm to 35.3 ±3.4 μm, and stomatal width from 17.5 ±2.3 μm to 18.8 ±2.8 μm in the two genotypes. Stomatal density of adult leaves facing to east was highly significantly greater compared to leaves facing the other cardinal directions. The differences in stomatal density of leaves facing to south and east, to south and west and to east and west in senescent leaves were highly significant. Correlations between the number of stomata per unit area and stomatal width ( $r = 0.49$ ) and between the stomatal length and with ( $r = 0.35$ ) were evaluated as significant. The value of the correlation coefficient between the number of stomata per unit area and the stomatal length ( $r = 0.54$ ) was evaluated as significant.

#### Acknowledgment

This paper was supported by project "Build the research centre AgroBioTech" ITMS code 26220220180.

#### References

- CLIFTON-BROWN, J.C. – LEWANDOWSKI, I. 2000. Water use efficiency and biomass partitioning of three different *Miscanthus* genotypes with limited and unlimited water supply. In *Annals of Botany*, vol. 86, 2000, pp. 191–200.
- FRASER, L. H. – GREENALL, A. – CARLYLE, C. – TURKINGTON, R. – FRIEDMAN, C. R. 2009. Adaptive phenotypic plasticity of *Pseudoroegneria spicata*: response of stomatal density, leaf area and biomass to changes in water supply and increased temperature. In *Annals of Botany*, vol. 103, 2009, no. 5, pp. 769–775.
- GREEF, J. M. – DEUTER, M. 1993. Syntaxonomy of *Miscanthus × giganteus* GREEF et DEU. In *Angewandte Botanik*, vol. 67, 1993, pp. 87–90.
- JUREKOVÁ, Z. – KOTRLA, M. – PAUKOVÁ, Ž. 2013. Life cycle of *Miscanthus × giganteus* Greef et Deu grown in South-Western Slovakia conditions. In *Acta Regionalia et Environmentalica*, vol. 10, 2013, no. 2, pp. 40–43. DOI: 10.2478/aree-2013-0008.
- JUREKOVÁ, Z. – KOTRLA, M. – PAUKOVÁ, Ž. – PRČÍK, M. 2012. The growth and yield of different *Miscanthus* genotypes in the conditions of South-Western Slovakia. In *Acta Regionalia et Environmentalica*, vol. 2, 2012, pp. 29–34.
- SHMÚ. 2013. Slovak Hydro-meteorological Institute. Data from the meteorological station in the village Velké Janíkovce in 2013. In Department of biometeorology and hydrology, Nitra : Nitra, 2013.
- MOON, Y.H. – CHA, Y.L. – CHOI, Y.H. – YOON, Y.M. – KOO, B.CH. et al. 2013. Diversity in ploidy levels and nuclear DNA amounts in Korean *Miscanthus* species. In *Euphytica*, vol. 193, 2013, pp. 317–326.
- PAZOUREK, J. 1963. Study of leaf epidermis by microrelief method. In *Preslia*, vol. 35, 1963, pp. 210–216.
- PRČÍK, M. – KOTRLA, M. 2013. Evaluation of dry matter production of *Miscanthus sinensis* 'Tatai' in the condition of South-Western Slovakia. In *Acta Regionalia et Environmentalica*, vol. 10, 2013, no. 2, pp. 55–58. DOI: 10.2478/aree-2013-0012.
- RICHARDSON, A.D. – ASHTON, P.M.S. – BERLYN, G.P. – Mc GRODY, M.E. – CAMERON, I.R. 2001. Within-crown foliar plasticity of western hemlock, *Tsuga heterophylla* in relation to stand age. In *Annals of Botany*, vol. 88, 2001, pp. 1007–1015.
- RAYBURN, A.L. – Crawford, J. – RAYBURN, CH.M. – JUVIK, J.A. 2009. Genome size of three *Miscanthus* species. In *Plant Molecular Biology Reporter*, Springer Science, Business Media B.V., vol. 27, 2009, pp. 184–188.
- THOMAS, P.W. – WOODWARD, F.I. – QUICK, W.P. 2003. Systemic irradiance signaling in tobacco. In *New Phytologist*, vol. 161, 2003, pp. 193–198.
- VOLKENBURGH, E.V. 1999. Leaf expansion – an integrating plant behavior. In *Plant, Cell and Environment*, vol. 22, 1999, pp. 1463–1473. DOI: 10.1046/j.1365-3040.1999.00514.x
- WANG, Y. – CHEN, X. – XIANG, C. 2007. Stomatal density and bio-water saving. In *Journal of Integrative Plant Biology*, vol. 49, 2007, pp. 1435–1444.
- WOODWARD, F.I. – KELLY, C.K. 1995. The influence of CO<sub>2</sub> concentration on stomatal density. In *New Phytologist*, vol. 131, 1995, pp. 311–327.
- XU, Z. – ZHOU, G. 2008. Responses of leaf stomatal density to water status and its relationship with photosynthesis in a grass. In *Journal of Experimental Botany*, vol. 59, 2008, pp. 3317–3325.

