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BRIEF COMMUNICATION

Stomatal and chlorophyll fluorescence characteristics in European beech cultivars during leaf development

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Abstract

Changes in stomatal and chlorophyll fluorescence characteristics were analyzed in the course of leaf expansion in European beech (*Fagus sylvatica* L.) cultivars Aurea Pendula, Cristata, Rohanii, Rotundifolia and Viridivariegata. Stomatal length increased gradually from the second to the fifth phenological stage. Rotundifolia reached the highest mean stomatal length whereas Aurea Pendula and Cristata had the lowest values. Stomatal density for all cultivars decreased from the second to the fifth stage. Aurea Pendula reached the highest stomatal density in all phenological stages. The highest values of variable to maximum fluorescence ratio (F_v/F_m) were recorded in Rotundifolia, Rohanii, and the wild type, whereas Viridivariegata showed the lowest F_v/F_m. Similar trend was found in maximum to initial fluorescence ratio (F_m/F_0), but extremely low F_m/F_0 values were recorded in Viridivariegata in the last phenological stage. The highest potential electron capacity was found in Rohanii, Viridivariegata and the wild type and lowest in Cristata. This parameter increased in the course of early leaf development.

Additional key words: *Fagus sylvatica*, phenological stages, mutants.

European beech (*Fagus sylvatica* L.) is a drought-sensitive species that belongs to the major broad-leaved trees in Central and Western Europe. Beech also extends further southwards in the Mediterranean basin, where it is confined to mountainous regions where the rainfall is sufficient. Even in these sites, many beech stands can be considered as relicts, existing under conditions just barely within the limits of their ecological demands (García-Plazaola and Becerril 2000).

Global climate warming scenarios predict increasing temperature and prolonged dry summer periods for the next decades (Schär *et al*. 2004). Drought stress is one of the most important limiting factors for photosynthesis, as it induces stomata closing, and reduces gas diffusion between the mesophyll and the surrounding atmosphere. Stomatal density, apertures and their regulation are of key interest in the study of drought-adapatation in forest trees. Stomatal characteristics vary widely among beech genotypes and species (Fei *et al*. 1999, Denk 2003). It seems that stomatal characteristics and stomatal responses show evidence of adaptive acclimation and heritable variation. For example, stomatal size is smaller, stomatal density is higher and stomatal control more sensitive in xeric environments (Dunlap and Stettler 2001, Pearce *et al*. 2005). In addition, chlorophyll fluorescence parameters are frequently used to estimate resistance of the leaf photosynthetic apparatus to the extreme environmental conditions (Roháček 2002, Fracheboud and Leipner 2003). It is well documented that stomatal density, stomatal function as well as chlorophyll fluorescence parameters depend on the age of the leaf (Šesták and Šiffel 1997, Nesterenko *et al*. 2006).

The aim of this study was to evaluate the influence of genotype on stomatal characteristics and chlorophyll *a* fluorescence parameters in the course of leaf development of five selected European beech cultivars as well as to compare their sensitivity to the environmental conditions with the wild type trees.

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Abbreviations: F_m - maximal fluorescence; F_0 - initial fluorescence; F_v - variable fluorescence; PS 1 - photosystem 1; PS 2 - photosystem 2.
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The sampled trees of European beech grow at the Arboretum Borová hora of the Technical University, Zvolen, Slovakia. The site (latitude 48º36′N, longitude 19º09′E, altitude 306 - 338 m a.s.l.) is localized in a *Querceto-Fagetum* forest stand. The mean temperature in the growing season is 15.6 ºC and mean precipitation during the growing season is 399 mm. Five cultivars, representing mutants in leaf shape and colour and popular in horticultural planting, (Cristata, Rotundifolia, Aurea Pendula, Rohanii, Viridivariegata) together with the wild type were selected. Samplings and observations were made on leaves at a height of 1 m above the ground, at four different phenological stages of leaf development. Those stages are morphologically characterized as follows: 2nd stage - elongated bud, pigmented and unfolded at the apical end; 3rd stage - fully unfolded bud; 4th stage - growing young leaf; 5th stage - mature expanded leaf (Priwitzer and Minďáš 1998).

Stomatal characteristics were measured on leaves of two representative trees for each cultivar and each phenological stage. Microscopic slides were prepared with abaxial leaf epidermis as described in Masarovičová (1991) and Tichá *et al*. (1999). Stomatal length observations were made on 60 squares, under a 1000× magnification, with a microscope *Olympus BH2*. Stomatal density was counted at a 400× magnification on 60 squares. Chlorophyll *a* fluorescence parameters were measured on both adaxial and abaxial sides of ten leaves per each tree. A portable fluorometer *Plant Efficiency Analyser* (*PEA*, *Hansatech*, Kings Lynn, UK) was used to determine the parameters of rapid kinetics of chlorophyll *a* fluorescence. The procedure of measurements is described in detail in Bolhàr-Nordenkampf *et al*. (1989). Before the measurement, the leaves were kept for 30 min under leaf clamps for a dark adaptation. Measurements were performed at irradiance of 2100 μmol m^{-2} s^{-1} at 1-s intervals. Three fluorescence parameters F_v/F_m, F_m/F_0, and "area" were determined. Duncan's multiple range test was performed to analyze statistically the measured data.

The highest mean stomatal length was observed in Rotundifolia (19.60 μm) which has the smallest leaves from all cultivars. Aurea Pendula and Cristata had the lowest mean stomatal length. With regard to the phenological stages, the wild type and Rotundifolia showed the highest values of stomatal length in stage 2, and Aurea Pendula had the lowest values. A similar trend was also observed in the third stage. In stage 4, Viridivariegata had the highest mean stomatal length whereas the lowest values were found again in Aurea Pendula. The fitfth phenological stage showed a trend different from the previous ones. Rotundifolia and Rohanii had the highest length whereas the lowest values were measured in Viridivariegata and the wild type (Table 1). Masarovičová *et al*. (1996) reported mean stomatal length for shade leaves of wild type European beech trees ranging from 25.7 μm to 28.2 μm. The results from our experiments show lower values in the wild type (23.84 μm for stage 5). This difference may be attributed to the influence of altitude as well as different provenance. The stomatal length gradually increases with the elevation along the altitudinal gradient (Fei *et al*. 1999). This fact explains why the lengths recorded in the wild type in our experiments performed at 306 - 338 m a.s.l., reached lower values than those presented by Masarovičová *et al*. (1996) that were observed in trees growing at 470 - 490 m a.s.l. Our data are in accordance with the data reported by Denk (2003) for *Fagus sylvatica* with mean stomatal length of 23.50 μm. In general, stomatal length increased gradually from the second to the fifth phenological stage.

With regard to mean stomatal density, Aurea Pendula reached the highest values in all phenological stages (Table 1). In other cultivars, density values significantly decreased in the order: Rohanii, Viridivariegata, Rotundifolia, wild type, and finally Cristata with the lowest value. For the second phenological stage, the highest stomatal densities were measured in Aurea Pendula and Rohanii and lowest in Cristata. In stage 3, stomatal density decreased in following order: Aurea Pendula, Rohanii, Viridivariegata, wild type,

Table 1. Stomatal length and stomatal density measured in four different phenological stages. Data represents mean values ± SE, $n = 60$. Means followed by the same letters in a column are not significantly different at $P = 0.05$ (Duncan's multiple range test).

Parameter	Cultivar	Stage 2	Stage 3	Stage 4	Stage 5
Stomatal length [μm]	Cristata	14.04 ± 0.21 cd	14.20 ± 0.31 cd	19.45 ± 0.26 bc	24.40 ± 0.23 bc
	Rotundifolia	15.69 ± 0.32 a	17.01 ± 0.30 b	20.37 ± 0.23 b	25.32 ± 0.24 a
	Aurea Pendula	13.55 ± 0.26 d	13.85 ± 0.19 d	16.00 ± 0.27 d	24.99 ± 0.22 ab
	Rohanii	14.84 ± 0.26 b	17.01 ± 0.34 b	18.63 ± 0.26 c	25.24 ± 0.23 a
	Viridivariegata	14.75 ± 0.25 bc	15.01 ± 0.20 c	21.61 ± 0.37 a	24.21 ± 0.23 c
	wild type	15.85 ± 2.27 a	18.20 ± 0.40 a	18.32 ± 0.23 c	23.84 ± 0.18 c
Stomatal density [mm^{-2}]	Cristata	390.42 ± 9.58 c	358.33 ± 6.71 f	182.08 ± 5.08 e	160.83 ± 4.18 d
	Rotundifolia	522.50 ± 16.72 b	350.42 ± 8.94 e	220.00 ± 6.92 d	208.33 ± 5.81 b
	Aurea Pendula	699.58 ± 12.19 a	578.75 ± 18.19 a	415.00 ± 10.16 a	242.08 ± 4.77 a
	Rohanii	685.83 ± 13.32 a	532.08 ± 12.68 b	375.00 ± 7.08 b	209.17 ± 4.07 b
	Viridivariegata	549.17 ± 9.98 b	437.92 ± 8.90 c	292.08 ± 6.84 c	193.33 ± 4.93 c
	wild type	527.50 ± 7.07 b	386.25 ± 14.5 d	158.75 ± 3.98 f	165.83 ± 3.36 d

Table 2. Parameters of chlorophyll *a* fluorescence (F_v/F_m, F_m/F_0, "area") measured in four different phenological stages. Means ± SE, $n = 20$. Means followed by the same letters in a row are not significantly different at $P = 0.05$ (Duncan's multiple range test).

Parameter	Cultivar	Leaf side	Stage 2	Stage 3	Stage 4	Stage 5
F_v/F_m	Cristata	upper	0.798 ± 0.003 a	0.804 ± 0.005 a	0.780 ± 0.016 a	0.780 ± 0.012 a
		lower	0.797 ± 0.004 a	0.794 ± 0.003 a	0.805 ± 0.003 a	0.777 ± 0.011 b
	Rotundifolia	upper	0.795 ± 0.003 b	0.797 ± 0.002 b	0.803 ± 0.002 a	0.804 ± 0.003 a
		lower	0.800 ± 0.003 b	0.803 ± 002 ab	0.809 ± 0.003 a	0.807 ± 0.004 ab
	Aurea Pendula	upper	0.814 ± 0.003 a	0.806 ± 0.002 a	0.786 ± 0.007 b	0.761 ± 0.011 c
		lower	0.811 ± 0.003 a	0.806 ± 0.004 a	0.777 ± 0.007 b	0.748 ± 0.015 c
	Rohanii	upper	0.800 ± 0.004 a	0.802 ± 0.003 a	0.779 ± 0.012 b	0.780 ± 0.006 b
		lower	0.797 ± 0.004 c	0.808 ± 0.003 b	0.802 ± 0.002 b	0.828 ± 0.002 a
	Viridivariegata	upper	0.744 ± 0.006 b	0.753 ± 0.005 ab	0.771 ± 0.005 a	0.689 ± 0.013 c
		lower	0.755 ± 0.007 b	0.766 ± 0.006 ab	0.782 ± 0.004 a	0.700 ± 0.014 c
	wild type	upper	0.780 ± 0.004 d	0.792 ± 0.004 c	0.801 ± 0.002 b	0.820 ± 0.001 a
		lower	0.790 ± 0.003 c	0.801 ± 0.002 b	0.803 ± 0.002 b	0.835 ± 0.002 a
F_m/F_0	Cristata	upper	4.988 ± 0.076 a	5.154 ± 0.105 a	4.797 ± 0.187 a	4.779 ± 0.217 a
		lower	4.961 ± 0.096 ab	4.886 ± 0.070 ab	5.150 ± 0.087 a	4.670 ± 0.201 b
	Rotundifolia	upper	4.899 ± 0.073 a	4.942 ± 0.062 a	5.095 ± 0.058 a	4.923 ± 0.094 a
		lower	5.012 ± 0.062 b	5.069 ± 0.045 ab	5.273 ± 0.076 a	5.021 ± 0.105 b
	Aurea Pendula	upper	5.417 ± 0.089 a	5.171 ± 0.067 a	4.763 ± 0.159 b	4.338 ± 0.182 c
		lower	5.313 ± 0.078 a	5.201 ± 0.104 a	4.557 ± 0.132 b	4.190 ± 0.200 b
	Rohanii	upper	5.044 ± 0.097 a	5.088 ± 0.085 a	4.665 ± 0.136 b	4.596 ± 0.110 b
		lower	4.968 ± 0.089 c	5.237 ± 0.079 b	5.061 ± 0.054 b	5.835 ± 0.054 a
	Viridivariegata	upper	3.954 ± 0.020 b	4.094 ± 0.099 b	4.408 ± 0.091 a	3.333 ± 0.139 c
		lower	4.159 ± 0.125 b	4.336 ± 0.112 b	4.625 ± 0.077 a	3.470 ± 0.156 c
	wild type	upper	4.579 ± 0.084 d	4.833 ± 0.079 c	5.045 ± 0.051 b	5.571 ± 0.039 a
		lower	4.791 ± 0.075 c	5.041 ± 0.052 b	5.095 ± 0.047 b	6.098 ± 0.082 a
"Area"	Cristata	upper	0.142 ± 0.011 b	0.120 ± 0.009 b	0.117 ± 0.015 b	0.245 ± 0.021 a
		lower	0.141 ± 0.011 b	0.102 ± 0.008 b	0.128 ± 0.010 b	0.215 ± 0.023 a
	Rotundifolia	upper	0.175 ± 0.011 b	0.193 ± 0.006 b	0.207 ± 0.010 b	0.412 ± 0.037 a
		lower	0.192 ± 0.012 b	0.181 ± 0.014 b	0.185 ± 0.008 b	0.284 ± 0.009 a
	Aurea Pendula	upper	0.219 ± 0.024 a	0.116 ± 0.007 b	0.141 ± 0.036 b	0.267 ± 0.024 a
		lower	0.228 ± 0.021 a	0.134 ± 0.012 b	0.097 ± 0.005 b	0.244 ± 0.020 a
	Rohanii	upper	0.302 ± 0.017 ab	0.339 ± 0.029 a	0.258 ± 0.014 b	0.355 ± 0.021 a
		lower	0.238 ± 0.021 b	0.188 ± 0.003 c	0.201 ± 0.007 c	0.309 ± 0.014 a
	Viridivariegata	upper	0.194 ± 0.011 c	0.241 ± 0.032 c	0.280 ± 0.022 b	0.327 ± 0.029 a
		lower	0.217 ± 0.010 b	0.210 ± 0.014 b	0.231 ± 0.010 b	0.355 ± 0.032 a
	wild type	upper	0.304 ± 0.009 a	0.245 ± 0.023 b	0.313 ± 0.016 a	0.272 ± 0.016 ab
		lower	0.283 ± 0.017 b	0.155 ± 0.009 d	0.343 ± 0.018 a	0.236 ± 0.016 c

Rotundifolia, and Cristata. In stage 4, cultivars showed a similar trend as in the previous stage with the significant differences among them. In the fifth stage, the highest value was found in Aurea Pendula followed by Rohanii and Rotundifolia, Viridivariegata, wild type and Cristata. Mean values of stomatal density reported for shade leaves of the wild type European beech trees range 103 - 135 stomata mm^{-2} (Masarovičová *et al*. 1996) or 144 - 151 stomata mm^{-2} (Lichtenthaler 1985). The numbers found in our experiments for stage 5 are not significantly different from the latter mentioned values. The differences in densities reported by Masarovičová *et al*. (1996) might be caused by the higher altitude of the experimental site. The significant influence of plant ecotype on stomatal densities is also well documented (Syros *et al*. 2006). Based on our results, gradually decreasing stomatal density during leaf development was observed.

The highest values of F_v/F_m were found in Rotundifolia, Rohanii and wild type (Table 2). In Rotundifolia and wild type, F_v/F_m values gradually increased on both adaxial and abaxial sides of leaves during the leaf ontogeny. In the adaxial leaf side of Rohanii, the values were maximum in phenological stages 2 and 3, but lower in stages 4 and 5. In Cristata, no significant differences among phenological stages were found, except the fifth stage and the abaxial leaf side. In Aurea Pendula, the decrease of F_v/F_m values was observed in the course of leaf expansion on both leaf sides. The lowest values of F_v/F_m were recorded in

Viridivariegata and especially in stage 5, they almost reached values indicating a water stress. The differences in F_m/F_0 values (Table 2) among genotypes were similar to those mentioned above for F_v/F_m ratio. The highest values were in Rohanii, Rotundifolia, and the wild type and the lowest in Viridivariegata.

The highest values of the "area" were recorded in Rohanii, Viridivariegata, and the wild type, but no significant differences were found among them (Table 2). This parameter gradually decreased in other three cultivars, Rotundifolia, Aurea Pendula, and Cristata. Viridivariegata responded specifically, it had the lowest F_v/F_m and highest "area" from all the cultivars. With regard to phenological stages, Cristata and Rotundifolia showed the same trend with the highest values in the last phenological stage. In Viridivariegata, this parameter increased with leaf expansion.

The ratio F_v/F_m is the most frequently used parameter in ecophysiological research, and it determines the maximum photochemical efficiency of PS 2. The ratio typically ranging between 0.75 - 0.85 is proportional to the effectiveness of light energy utilization under standard conditions of CO_2 fixation and to the quantum yield of photochemical processes (Demming and Björkman 1987). A decline in F_v/F_m is a sensitive and early indicator of change in photosynthesis and in the physiological status of the plant in general, resulting from various environmental stresses (Lichtenthaler *et al*. 1996, Stępień and Kłobus 2006). Cai *et al*. (2005) investigated photosynthetic properties in relation to leaf development in tropical trees. The increase of both chlorophyll content and maximum photosynthetic rate with leaf expansion indicated that total photochemical capacity increased as well. The proportion of absorbed photons that were not actually utilized in photochemistry decreased, resulting in a generally higher F_v/F_m rate in more fully expanded leaves. It is well established that ratio of activities of PS 1 and PS 2 declines during leaf ontogenesis (Šesták 1985). We found an increase in photochemical efficiency of PS 2 in the course of phenological stages in the cultivar Rotundifolia and the wild type. Their young leaves were light green and after full leaf expansion they turned green. The same trend was observed in Rohanii but only on the abaxial leaf side that changed the colour from red-purple to light green. On the adaxial side of leaves, the values of F_v/F_m ratio were lower in stages 4 and 5 in comparison with the first two stages. Red-purple colour on the upper leaf side is a reliable indicator of anthocyanin presence (Cai *et al*. 2005). The young leaves of Cristata, Aurea Pendula and Viridivariegata were light green and during leaf expansion they changed the colour from yellow to yellow-green. There is an indication that the cultivars Cristata, Aurea Pendula and Viridivariegata were more sensitive to environmental stresses which was reflected in lower values of chlorophyll fluorescence parameters that in other cultivars. Application of chlorophyll fluorescence parameters may be used for the selection of European beech cultivars with a commercial horticultural importance. However, it is expected that plants growing under long-term elevated CO_2 concentrations in the future atmosphere should increase the water use efficiency and probably survive under eventual higher drought stress (Pospíšilová and Čatský 1999). In case of European beech trees, a reduction in net photosynthesis or pigment content (mainly chlorophyll *a*) with simultaneous increase in antheraxanthin, zeaxanthin and tocopherol pools that play an important photoprotective role are well documented under drought-stress conditions (Tognetti *et al*. 1995, García-Plazaola and Becerril 2000).

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