

A preliminary study on physiological changes of Central European beech provenances in response to progressive drought stress

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Abstract

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Four-year seedlings of two European beech ecotypes (*Fagus sylvatica* L.) were grown under well-watered and drought conditions for 51 days. Two populations were from the same medium-wet climatic region, but they differed in altitude. The results showed that there were significant differences in responses to different watering regimes in both provenances tested. Drought reduced quantum yield of photosystem II (PS II), decreased electron transport rate (ETR) through photosystem II and photochemical quenching (qP). More dissipation of energy excess (qN) was found in PV1 under drought-stress conditions. Water-deficit was associated with increases in proline content. Moreover, drought-treated seedlings of both provenances had significantly decreased chlorophyll pigment contents (Chl *a*, Chl *b*, Chl *a* + *b*) and carotenoids (Car *x* + *c*). Drought also influenced the height and stem diameter of seedlings. However, the differences between the provenances were statistically significant in drought stressed plants only in case of proline content and fluorescence parameter qN.

Key words

beech provenances, chlorophyll fluorescence, drought stress, photosynthetic pigments, proline

Introduction

Growth and distribution of European beech (*Fagus sylvatica* L.) in Central Europe is considerably influenced by water availability. The current climate change is associated with more frequent drought periods (prolonged and repeated drought periods, primarily in spring and summer) – decreasing the tree tolerance against unfavourable environmental conditions. In relation to fact that European beech is rather sensitive to extreme drought, the identification of symptoms and effects of drought presence becomes extraordinary important in context of stability and further development of beech ecosystems. Eco-physiological approaches including tree growth assessments are necessary for determining the extent of drought-caused changes. As photosynthesis system plays a key role in plant life, evaluation of photochemical processes under progressive water stress

has a great importance. Chlorophyll *a* fluorescence is a measure of photosynthetic performance because it shows a strong relationship with the quantum yield of CO₂ assimilation (GENTY et al. 1989, OXBOROUGH and BAKER, 1997). Consequently, the processes of excessive energy dissipation, photo-inhibition avoidance and photo-oxidation under drought stress (BAQUEDANO and CASTILLO, 2006) can be monitored through chlorophyll fluorescence parameters; photochemical efficiency of photosystem II (PSII) – as good indicator of photoinhibition, and non-photochemical quenching (NPQ) – as good indicator of excessive energy dissipation.

Valuable information on the plant adaptation to drought can be gained by assessment of contents and ratios of photosynthetic pigments controlling the absorption and dissipation of energy in plants (BAQUEDANO and CASTILLO, 2006).

The aim of this study was evaluation of the (expected) differences in physiological parameters in response to drought stress in two populations of European beech. The objectives of this experiment were (i) to investigate progressive drought stress effects on growth, photochemical processes, and pigment and proline contents in the rather sensitive woody plant *Fagus sylvatica* at the seedling growth stage; and (ii) to evaluate the biochemical, ecophysiological and growth responses of two beech provenances from the Central-European region.

Materials and methods

Plant material and greenhouse experiment

Four-year-old beech seedlings, in plastic pots (7 dm³) containing substrate based on peat and composed bark, were grown under greenhouse conditions from July 2010 to August 2010 (51 days). The nutrient contents in the substrate were (DM in %): N 0.3–1.2; P ca. 0.1; K ca. 0.2; moisture content (max.) 65.0%, pH value (aqueous extract) 5.0–6.5. The characteristics of the studied seedlings descended from two different ecotypes of European beech (*Fagus sylvatica* L.) are shown in Table 1. By ten plants per each provenance were grown under fully irrigated conditions (variant *control* – *K*), and other ten were maintained under drought conditions (variant *drought* – *S*). The modes of humidity, air temperature, adequate air circulation and light conditions were controlled throughout the entire experiment.

The plant water potential (ψ) was recorded during the whole experiment – as an indicator water status in plant. The leaf water potential was measured by psychrometric method with a PSY-PRO (Wescor, USA) via psychrometric chambers C-52. The measurements of leaf water potential were performed once a week during the entire experiment.

Pigment analyses and proline concentration

Five leaf samples per a provenance and treatment were collected at the beginning and at the end of experiment. One sample was composed of ten leaf discs carved from

five leaves (two discs for a leaf). The leaf samples were wrapped in aluminium foil, quick frozen in liquid nitrogen and stored at –196 °C until the analysis in the laboratory. In the laboratory, the samples were extracted with a 80% aqueous solution of acetone, homogenised to suspensions and filtered. The contents of chlorophyll pigments (Chl *a*, Chl *b*, Chl *a* + *b*) and carotenoids (Car *x* + *c*) were determined with a spectrophotometer CINTRA 6.5, GBS, Australia. The methodology by LICHTENTHALER (1987) was used to calculate the concentrations of photosynthetic pigments. The pigment concentrations have been expressed in the unit weight of dry matter (mg g⁻¹).

The measurement of proline was carried out spectrophotometrically, according to BATES et al. (1973). This methodology works with the evaluation of proline color reaction with ninhydrin.

Chlorophyll fluorescence parameters

Chlorophyll fluorescence emission was measured with using three fully expanded leaves of ten plants per a provenance and a treatment, at the beginning and at the end of the experiment. The measuring device was a MINI-PAM (pulse amplitude modulated portable fluorometer by Heinz Walz, Germany). The maximum fluorescence yield (*F_m*) of dark adapted leaves (30 minutes) and the minimum fluorescence yield (*F_o*) were used in calculation of the maximum photochemical efficiency of PSII (*F_v/F_m*). Moreover, the steady-state levels of NPQ and qN (non-photochemical quenching) were evaluated as well as qP values (photochemical quenching).

The photochemical efficiency of the open reaction centres of PSII (*F_v'/F_m'*) with the qN values was measured in the presence of actinic light. These parameters were estimated as Rapid Light Curves (RLC) expressing the response to photosynthetic active radiation (PAR). RLC allow an insight into the physiological flexibility enabling the plant to adapt its photosynthetic apparatus in response to rapid changes of light intensity. Hence, RLC contain information on the induction as well as saturation characteristics of photosynthesis (WALZ, 1999). For measurement, there were set the following parameters: actinic intensity 3, length of the actinic-light-periods 0:15 min, saturation pulse intensity

Table 1. Characteristics of the studied beech provenances from Slovakia, Central Europe

	Provenance PV1	Provenance PV2
Location	Brezno (Central Slovakia)	Kriváň (Central Slovakia)
Climatic region	Medium wet climatic area	Medium wet climatic area
Altitude a.s.l. (m)	682 a.s.l.	525 a.s.l.
Longitude (°)	19°41'	19°37'
Latitude (°)	48°51'	48°28'
Annual mean rainfall (mm)	750 mm	750 mm

8, electronic signal damping 2, electronic signal gain 3 and intensity of measuring light 1.

Electron transport rate (ETR) was estimated as $ETR = Yield (Fv'/Fm') \times PAR \times 0.5 \times ETR\text{-factor}$ (0.84). The standard factor corresponds to the fraction of incident light absorbed by a leaf.

Growth parameters

Biometrics parameters were evaluated for all seedlings, and then there were calculated the average values for the individual variants. Stem height was measured as the distance from root collar to the top (cm). Stem diameter was measured as an average diameter from two measurements with a slide calliper (mm). Seedling growth, stem diameter and height, were measured at the beginning and at the end of experiment. We determined height and diameter increment as the difference between the values at the beginning and at the end of the experiment (Table 2).

Data analysis

The results are expressed as arithmetic means with corresponding standard deviations. The significance of differences in the parameters was analysed using two-way ANOVA with two independent variables: *treatment* and *provenance*. When a factor was declared statistically significant (Provenance, Treatment and Provenance ×

Treatment interactions), post-hoc Tukey tests were used to determine differences between the means. Statistical probabilities at levels of $p < 0.05$, $p < 0.01$ and $p < 0.001$ were considered significant. The statistical analyses were carried out using STATISTICA 8.0 (StatSoft).

Results

Water potential

The values of water potential (ψ), recorded regularly throughout the experiment, provide an insight into the impact of progressive water stress to seedlings (assimilative apparatus). The course of water potential values in the control seedlings and the treated seedlings are shown in Fig. 1. The control seedlings were not affected by drought, as these seedlings were watered regularly. Values of ψ in control seedlings ranged between -0.4 MPa to -0.5 MPa. A significant decrease in water potential (-1.5 MPa) was recorded in the middle of the experiment. At the end of the study, the leaf water potential for both provenances fell below the critical limit of -1.9 MPa for xylem embolism, and there was recorded a considerable decrease of water potential from -2.9 MPa to -3.0 MPa. There were not found statistically significant differences between the provenances in course of ψ values.

Table 2. Biometric characteristics of beech seedlings at the start and the end of experiment

	Diameter [mm]				Height [cm]			
	PV1		PV2		PV1		PV2	
	Drought	Control	Drought	Control	Drought	Control	Drought	Control
Start	7.49±0.44	7.69±0.87	6.85±0.36	6.97±1.37	71.80±5.19	71.55±5.59	38.63±5.63	44.88±5.02
End	7.78±0.44	8.46±1.02	7.25±0.31	8.33±1.05	72.90±5.53	73.95±5.24	40.5±5.73	50.38±6.56
Incr.	0.41±0.35c	0.77±0.35ab	0.40±0.32b	1.36±0.98c	1.80±1.49c	2.4±2.18b	1.88±1.25c	5.5±2.24a

Average values with their standard deviations.

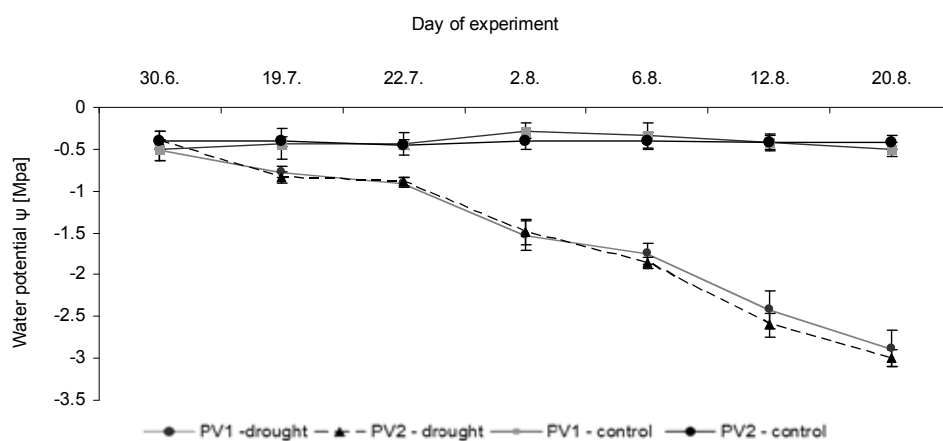


Fig. 1. Water potential values in PV1 and PV2 provenances during dehydration process.

Effect of drought on chlorophyll pigment and proline contents

The drought significantly decreased the contents of Chl *a*, Chl *b*, and the total chlorophyll content in both populations, at probability levels of $P = 0.01$ and $P = 0.001$ (Fig. 2a, b, c). The Chl *a* content decreased on average by 20%. A marked decrease was observed in content of Chl *b*: by 23% in PV1 and by 36% in PV2, and increased Chl *a/b* ratio in both provenances. There were recorded statistically significant differences in Car $x + c$

content (Fig. 2 d); however the Chl/Car ratio was significantly affected only in PV2 in interaction with drought stress. A more decreasing trend in Chl *a* and Chl *b* – in comparison with the content of carotenoids, resulted in significant changes in Chl/Car ratio in PV2. A parallel decreasing trend in chlorophyll content and carotenoid content resulted in an insignificant change in Chl/Car ratio in PV1 (Fig. 3). There was revealed a significant interaction effect between the treatment and provenance in Chl *b*, Chl *a + b* and Chl/Car ratio.

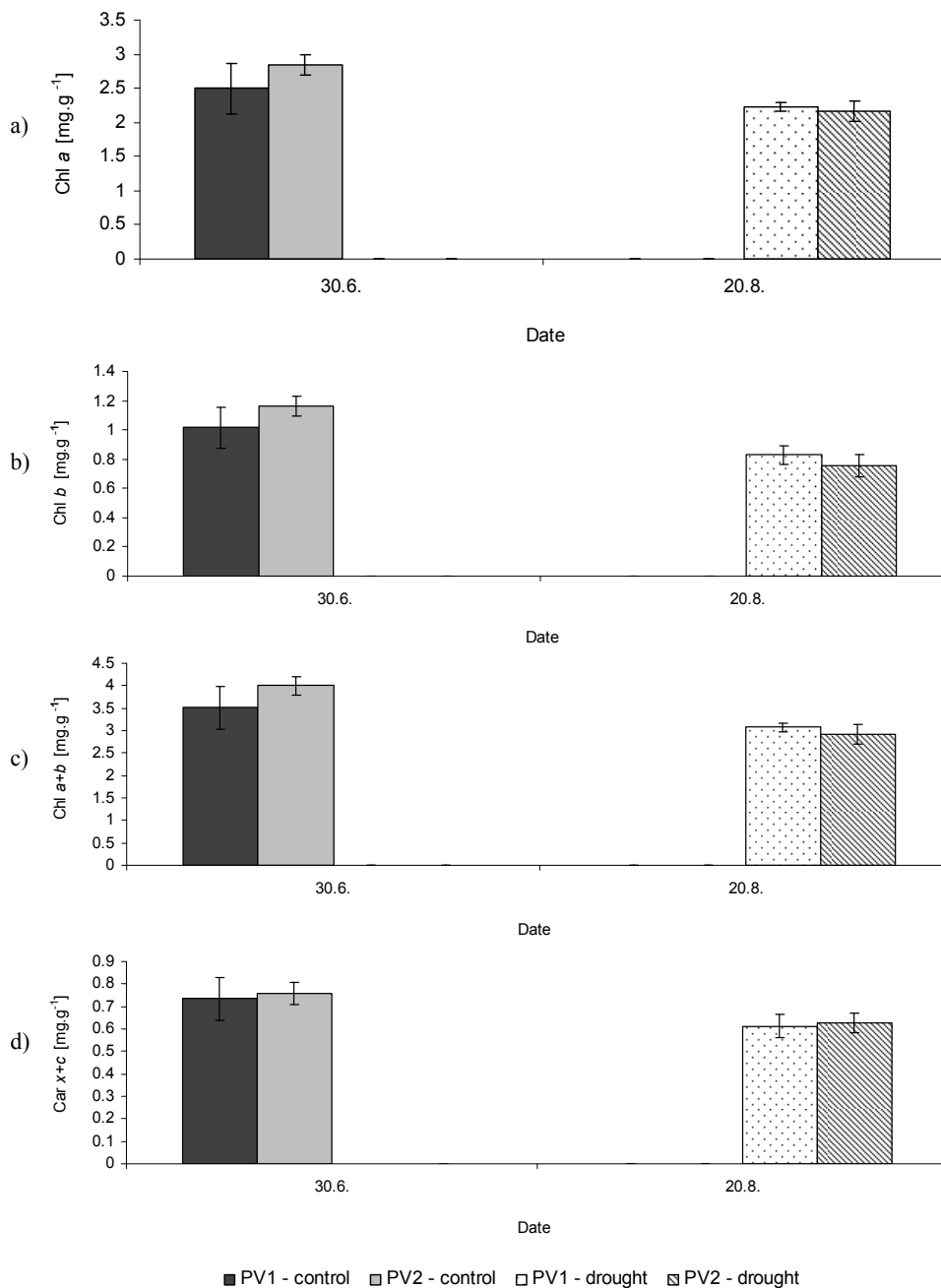


Fig. 2. Changes in pigments content in two beech provenances in response to water-stress, a) Chl *a*, b) Chl *b*, c) Chl *a + b*, d) Car $x + c$.

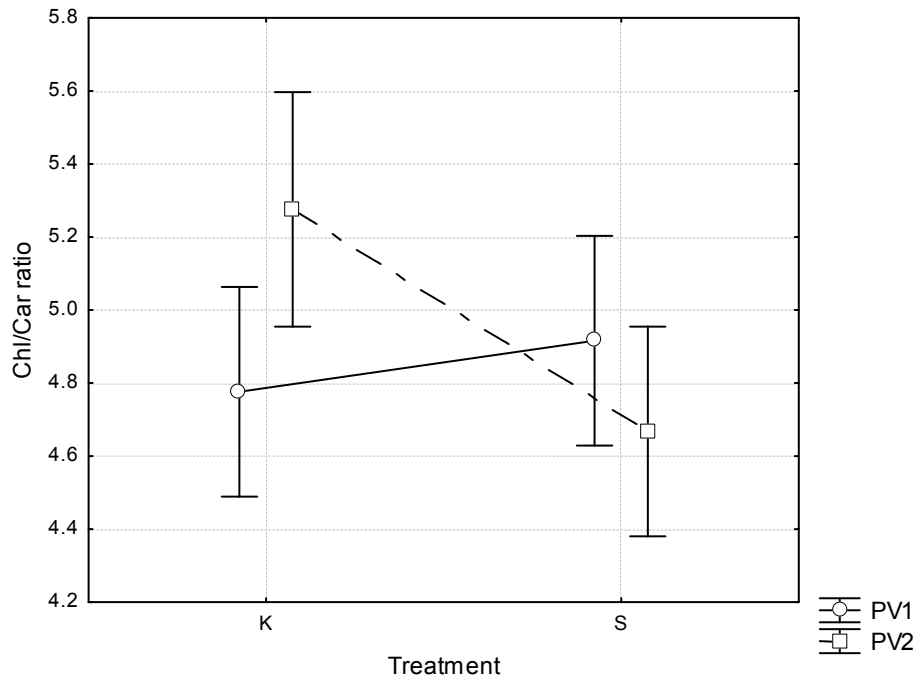


Fig. 3. Influence of progressive drought-stress on Chl/Car ratio in two beech provenances. K, control; S, stress.

The drought significantly increased the accumulation of free proline ($P = 0.001$) in both provenances, with more increasing PV2 towards the end of the experiment (Fig. 4).

Drought-affected chlorophyll fluorescence parameters

The drought-stress led to a decrease in F_v/F_m (a dark-adapted measurement), approximately from 0.8 to 0.7,

but these changes were not statistically significant. Significant differences were recorded in case of ETR (from 5 to 2) and fluorescence quenching, non-photochemical as well as photochemical, at a significance level of $P = 0.01$ (Table 3). The only difference between the provenances was recorded in q_N parameter. The RLC curves demonstrate (Fig. 5) more energy dissipation in PV1, while the photochemical efficiency of the open reaction centres of PSII was similar in both provenances.

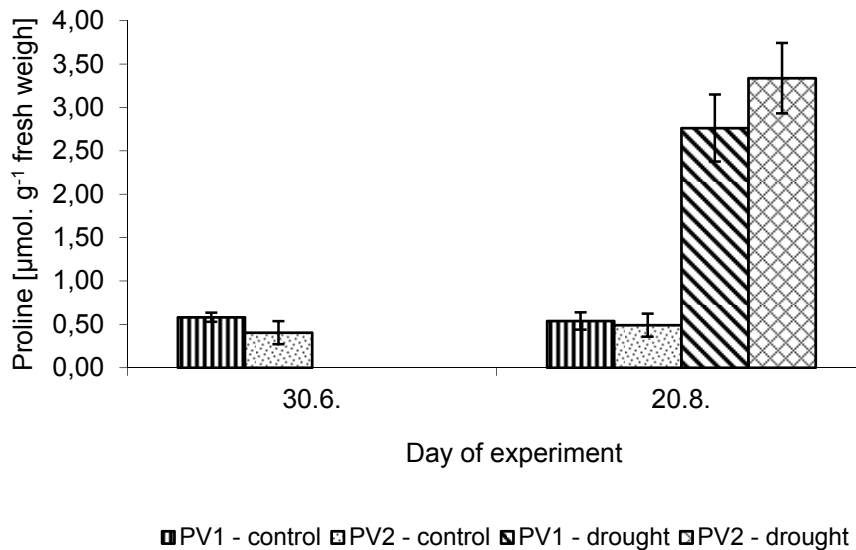


Fig. 4. Changes in proline content in two beech provenances (PV1, PV2) during the experiment.

Table 3. Changes in fluorescence parameters in response to drought stress

	Fv/Fm	NPQ	qN	qP	ETR
Provenance	0.224	0.756	0.044*	0.465	0.262
Treatment	0.051	0.001**	0.002**	0.001**	0.002**
Prov x Treat	0.713	0.282	0.681	0.145	0.304

Significant differences at * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, two-way ANOVA.

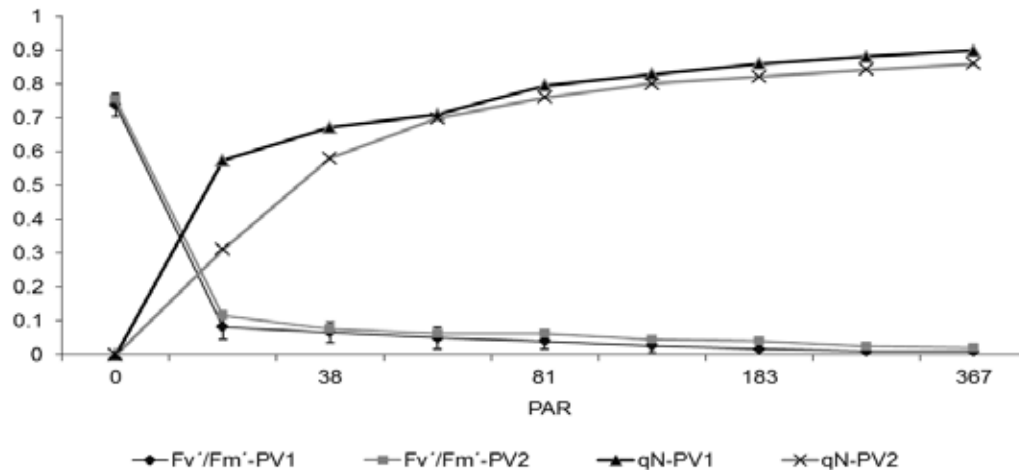


Fig. 5. Fluorescence parameters as Rapid Light Curves in response to photosynthetic active radiation at the end of experiment.

Changing growing parameters

The drought significantly decreased the height and the stem diameter of the seedlings (Table 2). The high increments were significantly affected by all the discussed factors, and also by the *Provenance* × *Treatment* interaction, while the diameter increments were affected only by the *Treatment*. In the *Treatment K*, the differences in height increments between the two provenances were statistically significant, while in the *Treatment S* the decrease rates in height increments were almost the same.

Discussion

Drought is one of the most important factors adversely affecting production and development of seedlings as well as adult trees, by restricting their nutrients uptake and growth. The water deficit persisting for 51 days resulted in a progressive limitation of water availability, which was reflected in the decline of water potential in the stressed beech seedlings. The leaf water potential ψ of the stressed plants decreased with increasing water stress (PESOLI et al., 2003). Moreover, all water-stressed plants slowed the tempo of their growth. In our case, the drought significantly reduced the height and the stem diameter of the studied seedlings. CZAJKOWSKI and BOLTE (2006) observed minimum differences in mean

dimensions (leaf area, plant shoot length, root collar diameter) and growth between a drought-exposed and control group of beech seedlings from eleven different provenances.

We observed an evident decrease in contents of pigments and carotenoids in both populations, while the drought-stressed plants exhibited less reduced carotenoid content than chlorophyll content – in accordance with observations of other authors (BAQUEDANO and CASTILLO, 2006; DUAN et al., 2005). The possible underlying cause of decrease of photosynthetic pigment contents is photo-oxidation. On the other hand, the photosynthetic machinery in green plants is protected by effects of carotenoid-mediated dissipation of excess radiation of and by xanthophyll cycle (VERHOEVEN et al., 1996). GALLÉ and FELLER (2007) report that under a drought lasting 36 days, the content of chlorophylls (Chl *a* + *b*) per leaf area unit was lower in the stressed than in the control plants at the drought period (day 36). Progressively decreasing pigment contents were found by DITMAROVÁ et al. (2009). These authors confirmed significant differences in Chl *a* + *b* content between the group of severely stressed seedlings and the other groups (seedlings subjected to mild drought stress and non-stressed seedlings) after 36 days of treatment.

At the end of the experiment, significant differences between the provenance were recorded in Chl *a/b* ratio per provenances, resulting from non-parallel decreasing contents of Chl *a* and Chl *b*. On the contrary,

LEI et al. (2006) and DITMAROVÁ et al. (2009) observed parallel decreasing trend in Chl *a* and Chl *b*, resulting in absence of significant changes in Chl *a/b*.

The most sensitive response to water deficit was found in parameters Chl *b* and proline content. Except to many other functions, proline serves an important role in osmosis protection. The protection effects of proline mean stabilization of membranes, proteins, and enzymes and reduction of free radicals (OKUMA et al., 2004). In our case, the drought was associated with a large increase in proline content in leaves of both provenances. The similar results obtained PEUKE et al., 2002, founding out a significant increase in proline content of different beech ecotypes under drought stress.

The drought did not have significant effects on Fv/Fm – which suggests a considerable resistance of PSII to water deficit (DUAN et al., 2005; LAWLOR and CORNIC, 2002). However, significant differences between the provenances were found in qN under drought stress, indicating differences in capacity for non-radiative dissipation of excitation energy in form of heat. Moreover, among the fluorescence parameters, qN may be supposed to be the best indicator. It seems that the provenance PV1 from an altitude of 682 m a.s.l. has a higher photoprotective capacity than PV2 from 525 m a.s.l. However, these findings cannot affect the conclusions derived based on a comprehensive assessment of physiological responses to the drought stress, according to which there have been confirmed only insignificant differences between the two provenances.

GALLÉ and FELLER (2007) ask a question about whether such physiological changes can provide an improved drought tolerance for following drought periods. Answering this question is a great challenge requiring more research aimed also to adult beech trees of contrasting provenances.

Conclusions

This study has shown that a progressive drought stress caused significant changes in photochemical processes, photosynthetic pigments and proline contents in beech seedlings. The difference in altitude (157 m) between the localities of the origin of the studied provenances did not cause significant differences between the two ecotypes coming from the same climatic region, representing optimum conditions for *Fagus sylvatica* L. (4th forest vegetation degree, medium wet climatic area, annual mean rainfall 750 mm).

Future research will focus on more contrasting beech provenances, originated not only from different altitudes, but also different climatic regions of Central Europe.

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Predbežná štúdia fyziologických zmien stredoeurópskych proveniencií buka lesného v reakcii na postupujúci stres zo sucha

Súhrn

V roku 2010 bol počas mesiacov jún až august realizovaný riadený experiment, v rámci ktorého sme otestovali dve proveniencie buka lesného (*Fagus sylvatica* L.) na základe vybraných fyziologických reakcií na postupujúci stres zo sucha. Rastlinný materiál reprezentovali 4-ročné sadenice buka pochádzajúce z rovnakého klimatického regiónu, ale z rozdielnych nadmorských výšok. V rámci každej proveniencie boli 2 varianty experimentu – sucho a kontrola. Po dobu 51 dní sme pravidelne v týždenných intervaloch sledovali hodnoty vodného potenciálu (ψ) listov, na základe ktorého bol sledovaný i priebeh postupujúcej dehydratácie. Klesajúca tendencia a záverečný pokles hodnôt ψ na $-2,9$ MPa až $-3,0$ MPa bol zaznamenaný u oboch proveniencií (varianty so suchom) spolu s prehlbujúcim sa suchom. Sucho výrazným spôsobom ovplyvnilo aj koncentrácie chlorofylov (Chl *a*, Chl *b*, Chl *a* + *b*) a karotenoidov (Car *x* + *c*). V rámci sledovaných parametrov fluorescencie chlorofylu *a* (F_v/F_m , NPQ, q_N , q_P , ETR) bolo pozorované pôsobenie vplyvu sucha ako stresového faktora, vplyvom ktorého hodnoty jednotlivých parametrov k záveru experimentu reagovali poklesom. Napriek tomu, že v priebehu pokusu boli potvrdené významné zmeny vo fotochemických procesoch fotosyntézy, v obsahu fotosyntetických pigmentov a obsahu voľného prolínu u oboch proveniencií, nemožno z nich jednoznačne určiť ekotyp tolerantnejší voči suchu.

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