

## Assessment of the impact of drought stress on particular biochemical and physiological characteristics of beech saplings leaves from different provenances

Eva Pšidová, Ľubica Ditmarová, Gabriela Jamnická, Jozef Váľka, Dušan Gömöry

Institute of Forest Ecology of the Slovak Academy of Sciences, Štúrova 2, Zvolen, Slovak Republic,  
e-mail: psidova@savzv.sk

### Abstract

PŠIDOVÁ, E., DITMAROVÁ, Ľ., JAMNICKÁ, G., VÁĽKA, J., GÖMÖRY, D. 2013. Assessment of the impact of drought stress on particular biochemical and physiological characteristics of beech saplings leaves from different provenances. *Folia oecol.*, 40: 97–106.

There were studied particular biochemical and physiological characteristics of European beech (*Fagus sylvatica* L.) saplings of different origin, responding to different water supply. Three different provenances (PV1, PV2, and PV3) representing the lower margin, the upper margin of beech distribution range and the optimal growth and environmental conditions for this species were investigated in a control (well-watered) and a drought treatment. For each provenance, the water potential values ( $\Psi_w$ ) towards the end of the experiment were found low, indicating a severe water stress. However, there were not observed significant differences in decreasing  $\Psi_w$  values between the studied provenances. There were also recorded significant decreasing osmotic potential values ( $\Psi_s$ ) influenced by drought. In the saplings of the PV2 provenance, the plants deprived of watering (drought treatment) showed the largest decrease of osmotic potential values. All provenances showed significant impacts of drought on the content of assimilatory pigments (*Chl a*, *Chl b*, *Chl a + b*, *Chl a/b*, *Chl/Car*). Nevertheless, the observed changes in individual provenances did not differ significantly. The analysis of the response to the controlled water deficit confirmed that the proline content increased significantly for all provenances. The largest increase in the proline concentration was observed in PV2 provenance. These results imply that the most sensitive to water deficit was the provenance PV2 from the optimal ecological beech area.

### Key words

assimilation pigments, drought stress, *Fagus sylvatica* L., proline, provenance, water and osmotic potentials

### Introduction

Among the factors that determine plant distribution and life is important shortage of water – drought stress in the recent years. The phenomenon of drought and dry periods is linked to the ongoing climate change. In some parts of Central Europe, an increase in rainfall in the winter is expected, and, on the other hand, declining rainfall totals during the summer months are supposed just because of the global warming (SCHÄR et al., 2004). At present research of the impact of climate change on forest ecosystems and their management is much

needed from many points of view (ČABOUN, 2008). The lack of water can disturb physiological activity and biomass production in trees. The weather conditions and climate change significantly affect the water availability. The same applies to the tolerance and resistance of plants to drought. In order to achieve high quality of production, cognition of critical effect of water deficit and identification of response mechanisms in forest trees is necessary. It is known that physiological and biochemical processes precede visible manifestations of primary damage. Therefore, methods for diagnosing changes in forest trees at the physiological level are es-

entially important. Our research plan is based on the knowledge presented in the papers CZAJKOWSKI et al., 2006; CZAJKOWSKI and BOLTE, 2006; ROSE et al., 2009. These papers consider forest tree provenances (species originated) from Central and Eastern Europe as a perspective source of ecotypes of forest trees resistant to both drought and frost.

Water deficit has a strong influence on physiological processes in plants. The assessment of physiological processes can be used in stress bio-indication at various levels in plants. Tree responses to drought are complex and varied, and they involve the entire tree (RYAN, 2011). Water deficit in plants reduces metabolic activity and stomatal conductance, and causes a decrease in the photosynthetic rate (ESCÓS et al., 2000). The reduction of photosynthetic rate under drought stress can be ascribed to both, stomatal and non-stomatal factors. From a physiological perspective, leaf chlorophyll concentration is a parameter of significant interest in its own right (ANJUM et al., 2011). The dynamic of pigment concentrations has a diagnostic value for a range of plant physiological properties and processes (BLACKBURN, 2007). Assimilation pigments rank among the important conditions for photosynthesis. As available water is necessary for biosynthesis of assimilation pigments, we monitored changes in their concentration – indicating water stress before visible symptoms. Chlorophylls have the dominant control over the amount of irradiance absorbed by leaves; therefore, foliar concentrations of chlorophylls control the photosynthetic potential and, consequently, the primary production in plants (BLACKBURN, 2007).

Among the many responses of plants to drought stress, we focused our study on the observation of increased proline accumulation in beech leaves. One of the essential tasks of increased proline accumulation is to maintain turgor in cytoplasm (BLUM, 1999). Under water deficit and as a result of solute accumulation, the

osmotic potential of the cell is lowered, which attracts water into the cell and helps with the maintenance of turgor (FAROOQ et al., 2009). Accumulation of proline is regarded as a means of providing biochemical adaptation during drought (KANDPAL et al., 1981). In the studies RIAZI et al. (1985); KRIVOSUDSKÁ and BRESTIČ (2010) are reported correlations between the osmotic potential and proline amino acids. Proline is a good marker of osmotic adjustment (OA). One of proline functions under stress is the role of mediating osmotic adjustment (MOLINARI et al., 2007).

In the present study we characterize selected provenances of European beech (*Fagus sylvatica* L.) in terms of their tolerance to drought. We describe the biochemical and physiological response, of beech seedlings at the level (i) changes in content of assimilation pigments and (ii) increased proline accumulation. At the end we propose one of the provenances as a suitable source of drought resistant beech ecotype.

## Material and methods

### Design of the experiment

A pot experiment for investigating the issue was established in the Arboretum Borová hora in Zvolen. We tested 4-year-old saplings of European beech (*Fagus sylvatica* L.) from three different beech provenances, by 30 ex. from each provenance. The characteristics of the studied beech provenances (PV1, PV2 and PV3) are in Table 1. These 30 samples were divided in two variants: control (15 samplings) and drought (15 samplings). The basic scheme of the experiment is in Fig. 1, the technical realization of the experiment is in Fig. 2. The saplings were planted into pots, each containing 7 litres of universal soil substrate KERA (Table 2). At the same time, the soil substrate of drought variants was supple-

Table 1. Characteristics of the studied beech provenances (PV1, PV2 and PV3)

	PV1	PV2	PV3
Location	Čierňany (region Ružomberok)	Banská Štiavnica	Divín
Altitude a.s.l. [m]	1116	710	400
Climatic region	Wet climatic area	Medium wet climatic area	Dry climatic area
Latitude [°]			48°33' N
Longitude [°]	48°58' N 19°10' E	48°28' N 18°58' E	19°36' E
Annual mean temperature [°C]	4.3 (Štrbské pleso)	7.7	8.2
Annual mean rainfall [mm]	1089 (Partizánska Ľupča)	610	627

\*30-year averages for the years 1951 to 1980, data calculated from surrounding places.

mented with a soil adjuvant called Perlit, in a ratio of 10:3 (10 litres of soil and 3 litres of Perlit). Perlit is generally used as a controlling agent of soil moisture. A controlled regime of humidity, air temperature, air

circulation and light conditions was provided over the whole experiment, and the saplings of drought variants were exposed to simulated drought conditions for 55 days. The saplings of control variants were irrigated

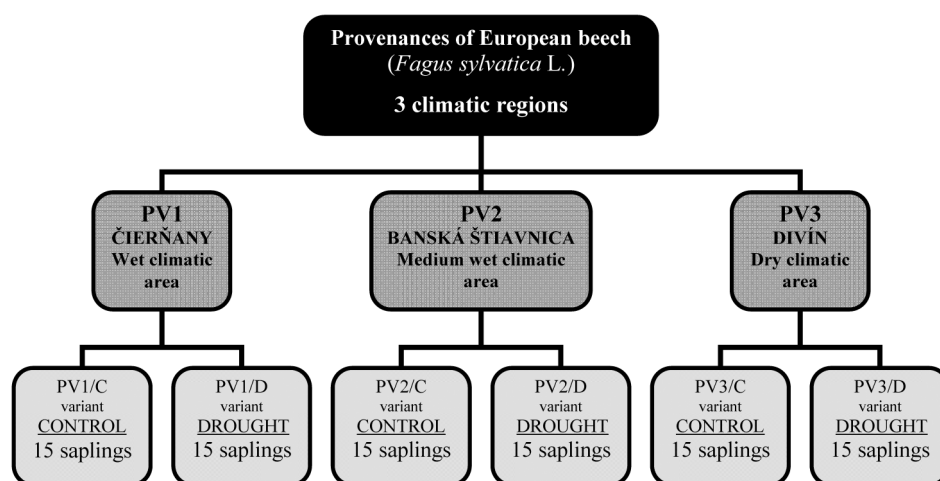


Fig. 1. Design scheme of experiment (PV1, provenance location Čierňany; PV2, provenance location Banská Štiavnica; PV3, provenance location Divín; C, variant of control; D, variant of drought).



Fig. 2. Technical realization of experiment (under the film – variants of drought, without film – variants of control).

Table 2. Chemical and physical properties of soil substrate KERA Universal

Property	Value
The content of total nitrogen as N in dry matter [%]	0.3–1.2
The content of total phosphorus as $P_2O_5$ [ $mg\ kg^{-1}$ ]	100
The content of total potassium as $K_2O$ [ $mg\ kg^{-1}$ ]	200
Humidity [%], max.	65.0
Combustible substances in dry mater [%], min.	25.0
pH (aqueous extract)	5.0–6.5
Electrical conductivity mS/cm max. in aqueous extract 1 : 25	1.2
The content of particles larger than 20 mm [%], max.	5.0

following the requirements of the automatic irrigation system used.

### Measurement methodology

The measurements of leaf water potential ( $\Psi_w$ ) and leaf osmotic potential ( $\Psi_s$ ) were carried out by psychrometric method, with a device PSYPRO (Wescor, USA). Leaf discs we put in psychrometrics chambers C-52 with thermocouples, components of the device PSYPRO. The measurements of leaf water potential ( $\Psi_w$ ) were performed regularly on a weekly basis. In order to determine leaf osmotic potential ( $\Psi_s$ ), leaf samples were taken, wrapped into an aluminium foil and stored in liquid nitrogen until the measurement. We determined  $\Psi_s$  in three points: start of the experiment, middle of the experiment (day 28 of experiment) and ending of the experiment (day 55 of experiment).

### Pigment analyses and determination of proline concentration

In two points, start and termination of the experiment, determination of the concentration of assimilation pigments and free proline was performed by spectrophotometric method.

The concentration of assimilation pigments was determined by measuring the absorbance of the mixture of pigments at different wavelengths, corresponding to the absorption maxima of the individual components. We obtained extracts from assimilation organs of beech saplings. These extracts were analysed by means of spectrophotometer UV VIS Cintra 6.5 GBS (Australia). The absorbance values were defined at different wavelengths: 470.0 nm – carotenoids, 663.2 nm – chlorophyll *a*, 646.8 nm – chlorophyll *b*, 750.0 nm – reference values. We used the formulas modified by LICHTENTHALER (1987) to calculate the concentration of pigments.

Using the method according to BATES et al. (1973), we determined spectrophotometrically the free proline content in leaves of beech saplings. The principle of this method is the evaluation of a color reaction of proline with ninhydrin.

### Data analysis

The initial statistical analysis of the data included basic statistical characteristics. We observed the effects of drought on concentrations of assimilation pigments and on free proline content. Statistically significant differences in leaf water potential ( $\Psi_w$ ), leaf osmotic potential ( $\Psi_s$ ), pigments content and proline content between provenances and treatments were revealed using multi-factor analysis of covariance (MANCOVA) in the program SAS 6.03.

We considered three factors: 1. provenance (discrete factor), 2. drought (discrete factor) and 3. initial height of saplings (continuous covariate). The means were compared by using Tukey-Kramer test at significantly level  $P < 0.05$ .

### Results

#### Water and osmotic potential

The values of  $\Psi_w$  for the control saplings (PV1, PV2 and PV3) during the whole study period were optimal and ranged from  $-0.2$  MPa to  $-0.5$  MPa. The saplings under the drought treatment responded to the controlled process of dehydration by decreasing their values of  $\Psi_w$ . The course of  $\Psi_w$  values in the control and drought treatment is presented in Fig. 3. On the day 14, the values were significantly reduced due to drought in the range from  $-0.53$  MPa to  $-0.65$  MPa. In the middle of experiment (day 28), there were recorded values below  $-1.5$

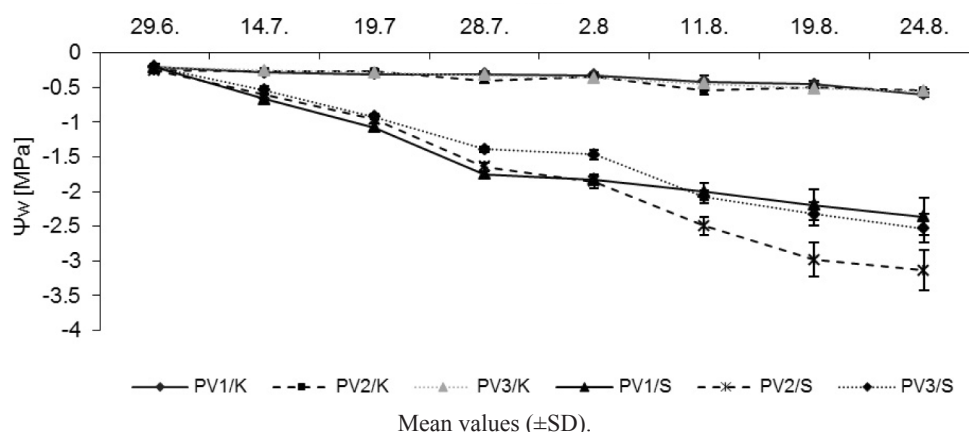


Fig. 3. Values of water potential ( $\Psi_w$ ) during progressive water deficit (K, variant of control; S, variant of drought).

MPa, which is generally considered as the threshold value for severe stress for plants. On day 55, at the end of the experiment, we found  $\Psi_w$  values well below the optimum conditions for plant growth and development. The values of  $\Psi_w$  -2.4 MPa (PV1), -2.5 MPa (PV3), -3.1 MPa (PV2), indicated very severe stress causing adverse changes at the level of physiological parameters. Differences in  $\Psi_w$  were not statistically among the monitored provenances.

### Osmotic potential

The osmotic potential was significantly reduced under drought stress conditions. There were found no significant differences between provenances (PV1, PV2 and PV3) under drought treatment in  $\Psi_s$  in the middle of the experiment. The  $\Psi_s$  of the stressed saplings in this term (day 28) decreased to the range from -1.8 MPa (PV3) to -2.0 MPa (PV1). Simulation of drought conditions for 55 days resulted in a large reduction of  $\Psi_s$  values in all studied provenances. Nevertheless, provenance PV2 responded to water deficit the most sensitively (Fig. 4)

compared with the other two provenances (PV1 and PV3). At the end of dehydration period (day 55), PV2 achieved reduction of  $\Psi_s$  value to -2.0 MPa. The impact of drought to the values of  $\Psi_w$  and  $\Psi_s$  was proved to be significant. The interaction between drought and selected provenances was significant in  $\Psi_s$ , but not in  $\Psi_w$  values.

### Effect of drought on assimilation pigments

All three tested provenances (PV1, PV2 and PV3) showed similar contents of assimilatory pigments. The chlorophyll content (*Chl a*, *Chl b*, *Chl a + b*) was significantly reduced in dry conditions. On the other hand, the drought stress treatment did not cause significant changes in the content of carotenoids. At the end of the experiment, we observed relevant decrease of *Chl a*, *Chl b* and *Chl a + b* content in sapling leaves due to water deficit (Table 3). Nevertheless, comparing the provenances (after 55 days drought treatment) did not show significant differences between them. The carotenoids content did not significantly decrease under the drought conditions

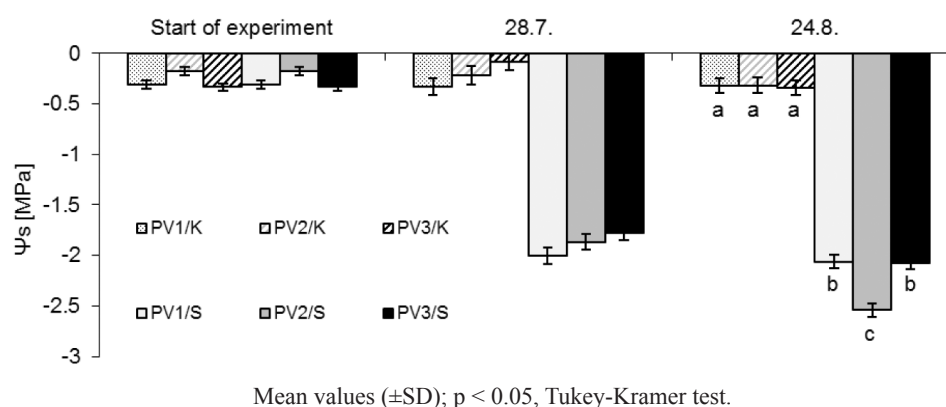


Fig. 4. Values of osmotic potential ( $\Psi_s$ ) during progressive water deficit (K, variant of control; S, variant of drought).

Table 3. Pigment contents of European beech saplings exposed to drought stress for 55 days

Treatments	Chl <i>a</i> content [mg g <sup>-1</sup> ]	Chl <i>b</i> content [mg g <sup>-1</sup> ]	Chl <i>a + b</i> content [mg g <sup>-1</sup> ]	Chl <i>a/b</i>	Car ( <i>x + c</i> ) content [mg g <sup>-1</sup> ]	Chl/Car
0 day	2.83 ± 0.10	1.12 ± 0.05	3.85 ± 0.15	2.81 ± 0.09	0.82 ± 0.05	4.70 ± 0.12
PV1 55 days control	2.81 ± 0.21	1.05 ± 0.08	3.86 ± 0.28	2.70 ± 0.13	0.76 ± 0.07	5.23 ± 0.30
55 days drought	1.89 ± 0.21***	0.67 ± 0.07***	2.56 ± 0.27***	2.90 ± 0.13	0.72 ± 0.07	3.56 ± 0.29***
0 day	2.46 ± 0.1	1.02 ± 0.05	3.48 ± 0.15	2.41 ± 0.81	0.72 ± 0.05	4.86 ± 0.12
PV2 55 days control	2.68 ± 0.22	1.03 ± 0.08	3.71 ± 0.28	2.59 ± 0.13	0.68 ± 0.07	5.46 ± 0.30
55 days drought	1.79 ± 0.20***	0.82 ± 0.07***	2.61 ± 0.26***	2.23 ± 0.12	0.68 ± 0.07	3.89 ± 0.28***
0 day	2.72 ± 0.09	0.97 ± 0.05	3.69 ± 0.13	2.80 ± 0.07	0.80 ± 0.04	4.58 ± 0.11
PV3 55 days control	3.10 ± 0.19	1.13 ± 0.07	4.23 ± 0.25	2.71 ± 0.12	0.79 ± 0.06	5.35 ± 0.27
55 days drought	1.59 ± 0.19***	0.60 ± 0.07***	2.19 ± 0.25***	2.70 ± 0.12	0.52 ± 0.06	4.18 ± 0.27***

Each value represents the mean of five replicates (n = 5) and its standard deviation (±SD); \*\*\* significant effect of drought.

compared to the control saplings. The *Chl/Car* ratio followed the trends of its constituents. The drought affected this parameter, but provenances responded in the same manner. In summary, statistically significant differences at the level of assimilation pigments content among the monitored provenances responding to adverse humidity conditions were not recorded.

### Proline content and osmotic adjustment

During the drought period, proline levels in leaves of beech saplings increased to a great extent. The changes in the content of amino acid proline at the end of the experiment are in Fig. 5. The initial proline concentrations ranged from 0.97  $\mu\text{mol g}^{-1}$  (PV1) to 1.17  $\mu\text{mol g}^{-1}$  (PV2). The Tukey-Kramer test informs about significant provenance-dependent differences in response to water deficit at the end of the experiment (Fig. 5). The most substantial and statistically significant increase

in proline accumulation was observed in provenance PV2: from 1.174  $\mu\text{mol g}^{-1}$  to 14.94  $\mu\text{mol g}^{-1}$ , representing an 12.7-fold increase in proline content in the variant drought compared with the initial measurement. The analysis of covariance for the day 55 confirmed a significant impact of drought, provenance and their interaction (provenance  $\times$  drought) to proline content in assimilation organs of beech saplings.

### Osmotic adjustment

Accumulation of proline in plants is generally considered as their response to stress. Moreover, the increased accumulation of proline is one of the signals of osmotic adjustment (*OA*). Figure 6 shows the correlation values of the  $\Psi_s$  with the proline contents in beech saplings from provenances PV1, PV2 and PV3 under the drought treatment. With  $\Psi_s$  -0.5 MPa, there was no considerable accumulation of proline (0.97  $\mu\text{mol g}^{-1}$

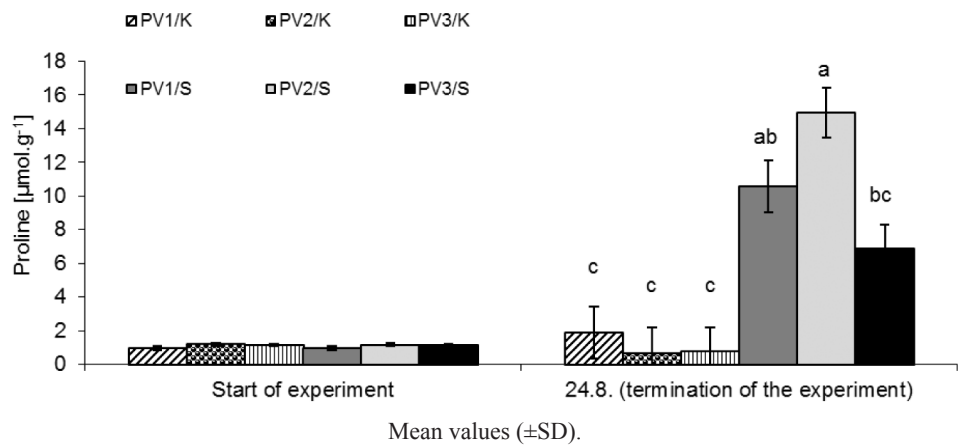


Fig. 5. Changes in proline content in beech saplings during progressive water deficit (K, variant of control; S, variant of drought).

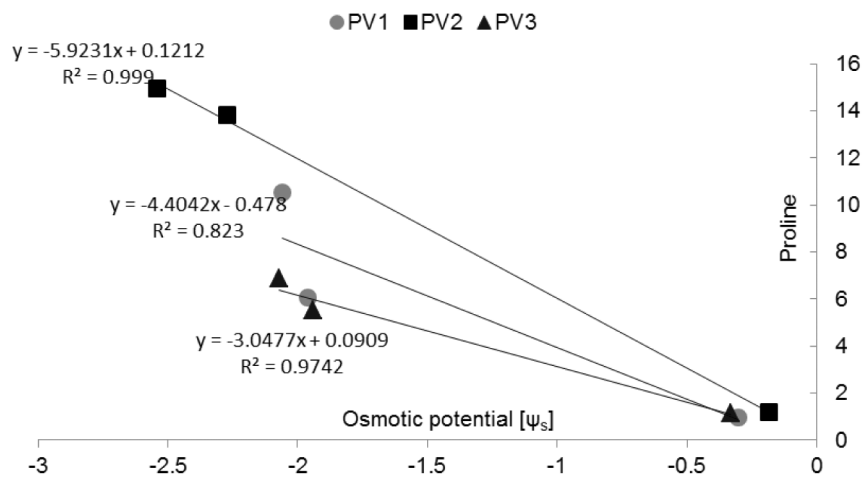


Fig. 6. Correlation between osmotic potential and proline content in leaves of European beech saplings under drought treatment (55 days).



(PV1) to  $1.17 \mu\text{mol g}^{-1}$  (PV2)). The highest capacity to accumulate proline was in provenance PV2 (710 m a.s.l., medium wet climatic area). Among the observed provenances, the lowest value of  $\Psi_s$  recorded ( $-2.54$  MPa) was linked with the most increased proline accumulation (up to 12.7-fold), which gives evidence for osmotic adjustment. Compared with provenance PV1 (1,116 m a.s.l., wet climatic area) the proline accumulation in PV2 was 30% higher, and compared with provenance PV3 (400 m a.s.l., dry climatic area) 54% higher.

## Discussion

In the recent years, severe drought has become the leading environmental stress factor painstaking equally in cultivation of agricultural crops and in forestry. The stress resistance of plants depends, to a considerable extent, on how these can maintain their water balance by absorbing water with their roots and stalks and distributing it to the leaves (TZVETKOVA and ANEV, 2008). Adverse effects of water deficit in plant organisms are present at all levels (root, shoot, leaf). In our experiment we focussed on study of physiological changes occurring in leaves. Under water deficit, the parameter  $\Psi_w$  commonly drops significantly, and thus it is a proper parameter for monitoring the water condition in plants. The decrease of  $\Psi_w$  values with increasing dehydration has also been reported by ROSE et al. (2009), with no significant differences in  $\Psi_w$  values confirmed between the provenance from the margin of the native range of beech (Poland) and the central provenance for the native distribution area of this species (Germany). Similarly, we did not confirm significant differences at the  $\Psi_w$  level among the Slovak provenances of beech with origin in the following areas: PV1 – wet climate of Slovakia, altitude 1,116 m a.s.l.; PV2 – medium wet climate of Slovakia, altitude 710 m a.s.l. and PV3 – dry climate of Slovakia, altitude 400 m a.s.l. The progressive impact of drought on 4-year-old seedlings was also investigated by GALLE and FELLER (2007), recording after 36 days of dehydration the minimum  $\Psi_w$  values about  $-2.7$  MPa. In our experiments, parallel with  $\Psi_w$  decreasing with dehydration during the study period, we recorded also a decreasing trend in the osmotic potential of leaves ( $\Psi_s$ ). The lowered values of  $\Psi_s$  in plants ensure to keep up the turgor and, consequently, the sufficient stomatal conductivity in leaves under low water potential (NGUYEN-QUEYRENS and BOUCHET-LANNAT 2003). TSCHAPLINSKI et al. (1998) monitored the response of several forest woody plants (*Quercus prinus* L., *Quercus alba* L., *Acer rubrum* L., *Cornus florida* L., *Nyssa sylvatica* Marsh.) to three different regimes of rainfall penetration into the soil (drought, control, wet) in June–September. During this experiment, in the variant with reduced rainfall penetration (drought),  $\Psi_w$

and  $\Psi_s$  decreased. Towards the end of the experiment, the  $\Psi_s$  values were below  $-1.5$  MPa in all the monitored woody plants; the most remarkable drop  $\Psi_s$  due to the stress influence was recorded in *Quercus prinus* L., with  $\Psi_s$   $-1.94$  MPa (in the understorey) to  $-1.96$  MPa (in the main stand layer). The corresponding  $\Psi_w$  values ranged from  $-2.89$  MPa (in the main stand layer) to  $-3.02$  MPa (understorey). To measure osmotic potential is also important for assessment of osmotic adjustment. In our experiment we focussed on assessment of osmotic adjustment through enhanced accumulation of proline under significantly reduced osmotic potential of leaves. In the water regimen of plants, osmotic potential is exactly the component closely connected with proline accumulation in these plants. This correlation between proline and osmotic potential has also been found by HANDA et al. (1986). These authors observed enhanced proline accumulation in a tomato cell suspension with a relatively low osmotic potential ( $-1.06$  MPa to  $-1.5$  MPa).

The amino-acid proline is a compatible solute generally recognised to perform in plant protection against stress in various effects: by contributing to osmotic adjustment, detoxifying reactive oxygen forms, stabilising membranes and natural structures of enzymes and proteins (FAROOQ et al., 2009). Proline is generally accumulated in plants stressed by osmosis, and it plays the key role in the control of osmosis and in the anti-oxidative protective mechanisms (CHA-UM and KIRDMANEE, 2009). The accumulation of free proline under stress conditions is enormously important for plant adaptation to stress (MOLINARI et al., 2007). An instant raise of proline in assimilation organs of wheat stressed by drought was observed by TATAR and GEVREK (2008), who recorded proline accumulation more than 13.7-fold compared to the normal conditions. A similar response – increased proline accumulation in chickpeas seedlings stressed by drought, ensuring apart from maintaining the cell turgor also preserving the structural integrity of membranes has also been confirmed by NAJAPHY et al. (2010). Proline accumulation in leaves of beech and spruce under drought stress was observed by SLUGENOVÁ et al. (2011) – beech and spruce. SLUGENOVÁ (2010) investigated the seedlings' response to the process of controlled dehydration. Under significantly reduced water potential (below  $-2.0$  MPa), she recorded enhanced proline accumulation in the two woody plants: 5.21-fold in beech and 5.7-fold in spruce. The presented facts give evidence that the capacity of accumulation of free proline in plants under stress is species-specific.

Under water deficit and enhanced accumulation of solutes, the osmotic potential of plant cells is reduced. This allows the water to enter the cells, and to ensure their appropriate turgor. This phenomenon is known as osmotic adjustment (OA) (FAROOQ et al., 2009). OA is an effective mechanism for retaining the turgor of tissues (BREŠTIČ and OLŠOVSKÁ, 2001) and also a mechanism

enabling the plants to avoid adverse effects of drought. The leaves able to perform *OA* are able to keep up their turgor under lower water potential than the leaves in which no *OA* has occurred. For that reason, the plants can also use water fixed in soil with rather strong bonds (SLOVÁKOVÁ and MISTRÍK, 2007). This fact has also been supported with the soil water potential values measured in our experiment (Fig. 6), with especially low values recorded in the provenance PV2/S (710 m a.s.l., medium wet climate of Slovakia). The appropriately maintained turgor also helps to keep up the stomata open, enhancing in such a way the exchange process of  $\text{CO}_2$ . Despite the significantly enhanced proline accumulation observed with aggravating drought, the lowered  $\Psi_s$  values and significantly lower soil water potential values give evidence for *OA*. Nevertheless, this is only a hypothesis, as the parameters of gas exchange in leaves were not found sufficiently unchanged in the end of our experiment.

The slow synthesis of photosynthetic pigments and fast decomposition of these pigments also belong to the numerous effects of water deficit on plant organisms. The photosynthetic pigments are used as reliable markers for assessment of metabolic imbalance in photosynthetic and growth processes under drought conditions. Reduction of chlorophyll content – considered as a typical symptom of oxidation stress, may be the result of photo-oxidation of pigments and degradation of chlorophyll. The chlorophyll loss due to drought has been recognised to be the main cause of inactivation of photosynthesis (ANJUM et al., 2011). The reduction of chlorophyll content due to drought is primarily caused by the damage to chloroplasts by active forms of oxygen (MAFAKHERI et al., 2010). The adverse effect of water deficit in beech seedlings subjected to the 55-day dehydration was manifested through the decrease in contents of *Chl a*, *Chl b* and the total chlorophyll *a + b* in comparison with the control seedlings. The drought, however, did not cause significant differences in contents of photosynthetic pigments between the provenances in the final phase of the experiment. SLUGEŇOVÁ (2010) published that the drought had a strong influence on contents of assimilation pigments – their reduction in beech and spruce seedlings. On the other hand, there was not detected significant influence of drought on the *Chl a/b* ratio in either of the studied woody plants (beech and spruce). DITMAROVÁ et al. (2010) studying spruce exposed to strong water deficit (36-day dehydration) observed that the *Chl a/b* ratio was unchanged, while the particular *Chl a* and *Chl b* contents were noticeably reduced. The preserved *Chl a/b* ratio in dehydrated individuals of *Arbutus unedo* L. has been documented by MUNNÉ-BOSCH and PENUELAS (2004). The authors observed the total chlorophyll *a + b* reduction under severe drought representing 63% compared to moderate drought stress. No comparable reduction of *Chl a + b* was reached in our three provenances (PV1 – 26%, PV2 – 25%, PV3 – 41%). GALLÉ

and FELLER (2007) testing the influence of drought on 4-year-old beech seedlings obtained a reverse result – the ratio of chlorophylls *a* and *b* was raised due to the drought stress.

The water stress due to the 55 days of dehydration resulted in a decrease of chlorophyll *a + b* content in all seedlings. We also recorded lowered content of carotenoids (*Car x + c*) in both beech provenances. Similar findings under drought stress were obtained by EFEÖĞLU et al. (2009) with three cultivars of maize and by MUNNÉ-BOSCH and PENUELAS (2004) with individuals of *Arbutus unedo* L.

The study of stress physiology and competition ecology is essential for evaluation of the climatic and site limitations of European beech (BOLTE et al., 2007). The provenance study focused on beech, its distribution and survival in drought has become truly needed in European context in the recent years. This is also evident based on the large number of works dealing with this issue (FOTELLI et al., 2009; ROSE et al., 2009; ROBSON et al., 2012). All the studies focussed on beech ecosystems suffering from water deficit agree in the finding that the provenances (ecotypes) of beech from southern or south-eastern parts of the beech native distribution range are tolerant to drought.

## Conclusions

The physiological response to prolonged water deficit (as negative drought effect) was found expressive in most of the parameters (parameters of water deficit, ratio *Chl/Car*, proline accumulation) in the plants representing the provenance. This provenance is native to the optimum growth and ecological conditions of beech. Despite the PV2's origin, our results showed a very sensitive response to drought only in this provenance. The provenances representing the marginal areas of beech distribution range (PV1 – wet climate of Slovakia, altitude 1,116 m a.s.l.; PV3 – dry climate of Slovakia, altitude 400 m a.s.l.) responded to adverse water regimen much less sensitively than PV2. Quantification of the differences among the studied provenances has revealed that the provenance PV3 was the most resistant against the drought because is originated from dry climate of Slovakia.

## Acknowledgements

This publication is the result of the project implementation: Extension of the Centre of Excellence “Adaptive Forest Ecosystems”, ITMS: 26220120049, supported by the Research & Development Operational Programme funded by the ERDF (50%).

This research was also supported by the Slovak Research and Development Agency, APVV – No. 0436/10 and from Grant Agency VEGA – No. 2/0006/11.



## References

- ANJUM, S.A., FAROOQ, M., WANG, L.C., XUE, L.L., WANG, S.G., WANG, L., ZHANG, S., CHEN, M. 2011. Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions. *Pl. Soil Environ.*, 57, 2011, (7): 326–331.
- BATES, L.S., WALDREN, R.P., TEARE, I.D. 1973. Rapid determination of free proline for water-stress studies. *Pl. and Soil*, 39: 205–207.
- BLACKBURN, G.A. 2007. Hyperspectral remote sensing of plant pigments. *J. exp. Bot.*, 58: 855–867.
- BLUM, A., ZHANG, J.X., NGUYEN, H.T. 1999. Consistent differences among wheat cultivars in osmotic adjustment and their relationship to plant production. *Field Crops Res.*, 64: 287–291.
- BOLTE, A., CZAJKOWSKI, T., KOMPA, T. 2007. The north-eastern distribution range of European beech – a review. *Forestry*, 80: 413–429.
- BRESTIČ, M., OLŠOVSKÁ, K. 2001. *Vodný stres rastlín: príčiny, dôsledky, perspektívy* [Plant water stress: causes, consequences, perspective]. Nitra: Slovenská poľnohospodárska univerzita. 149 p.
- CHA-UM, S., KIRDMANEE, CH. 2009. Proline accumulation, photosynthetic abilities and growth characters of Sugarcane (*Saccharum officinarum* L.) plantlets in response to iso-osmotic salt and water-deficit stress. *Agric. Sci. China* 8: 51–58.
- CZAJKOWSKI, T., BOLTE, A. 2006. Unterschiedliche Reaktion deutscher und polnischer Herkunft der Buche (*Fagus sylvatica* L.) auf Trockenheit. *Allg. Forst-Jagdztg.*, 177: 30–40.
- CZAJKOWSKI, T., KOMPA, T., BOLTE, A. 2006. Zur Verbreitungsgrenze der Buche (*Fagus sylvatica* L.) im nordöstlichen Mitteleuropa. *Forstarchiv*, 77: 203–216.
- ČABOUN, V. 2008. *Vplyv globálnej klimateckej zmeny na lesy Slovenska. Správa pre záverečnú oponentúru úlohy výskumu a vývoja* [Impact of global climate change on forests in Slovakia I/III. Message to the final opponency role of research and development]. Zvolen: National Forest Centre. 305 p.
- DITMAROVÁ, L., KURJAK, D., PALMROTH, S., KMEŤ, J., STŘELCOVÁ, K. 2010. Physiological responses of Norway spruce (*Picea abies*) seedlings to drought stress. *Tree Physiol.*, 30: 205–213.
- EFEÖĞLU, B., EKMEKÇI, Y., ÇİÇEK, N. 2009. Physiological responses of three maize cultivars to drought stress and recovery. *S. Afr. J. Bot.*, 75: 34–42.
- ESCÓS, J., ALADOS, C.L., PUNGNAIRE, F.I., PUIGDEFÁBREGAS, J., ELMEN, J. 2000. Stress resistance strategy in an arid land shrub: interaction between development instability and fractal dimension. *J. arid Environ.*, 45: 325–336.
- FAROOQ, M., WAHID, A., KOBAYASHI, N., FUJITA, D., BASRA, S.M.A. 2009. Plant drought stress: effects, mechanisms and management. *Argon. Sustain. Dev.*, 29: 185–212.
- FOTELLI, M.N., NAHM, M., RADOGLU, K., RENNEBERG, H., HALYVOPOULUS, G., MATZARAKIS, A. 2009. Seasonal and interannual ecophysiological responses of beech (*Fagus sylvatica*) at its south-eastern distribution limit in Europe. *Forest Ecol. Mgmt.*, 257: 1157–1164.
- GALLÉ, A., FELLER, U. 2007. Changes of photosynthetic traits in beech saplings (*Fagus sylvatica*) under severe drought stress and during recovery. *Physiol. Plant.*, 131: 412–421.
- HANDA, S., HANDA, A.K., HASEGAWA, P.M., BRESSAN, R.A. 1986. Proline accumulation and the adaptation of cultured plant cells to water stress. *Pl. Physiol.*, 80: 938–945.
- KANDPAL, R.P., VAIDYANATHAN, C.S., UDAYA KUMAR, M., KRISHNA SASTRY, K.S., APPAJI RAO, N. 1981. Alterations in the activities of the enzymes of proline metabolism in Ragi (*Eleusine coracana*) leaves during water stress. *J. Biosci.*, 3: 361–370.
- KRIVOSUDSKÁ, E., BRESTIČ, M. 2010. Osmotické prispôsobenie vybraných genotypov cícerá baranieho (*Cicer arietinum* L.) počas prehlbujúceho sa sucha [Osmotic adjustment of selected genotypes of chickpea (*Cicer arietinum* L.) during increasing drought conditions]. *Acta fytotechn. zootechn.*, 4: 99–102.
- LICHTENTHALER, H.K. 1987. Chlorophylls and carotenoids: pigments of photosynthetic biomembranes. *Meth. Enzymol.*, 148: 350–382.
- MAFAKHERI, A., SIOSEMARDEH, A., BAHRAMNEJAD, B., STRUIK, P.C., SOHRABI, E. 2010. Effect of drought stress on yield, proline and chlorophyll contents in three chickpea cultivars. *Austral. J. Crop Sci.*, 4: 580–585.
- MOLINARI, H.B.C., MARUR, C.J., DAROS, E., FREITAS DE CAMPOS, M.K., PORTELA DE CARVALHO, J.F.R., FILHO, J.C.B., PEREIRA, L.F.P., VIEIRA, L.G.E. 2007. Evaluation of stress-inducible production of proline in transgenic sugarcane (*Saccharum* spp.): osmotic adjustment, chlorophyll fluorescence and oxidative stress. *Physiol. Plant.*, 130: 218–229.
- MUNNÉ-BOSCH, S., PENUELAS, J. 2004. Drought-induced oxidative stress in strawberry tree (*Arbutus unedo* L.) growing in Mediterranean field conditions. *Pl. Sci.*, 166: 1105–1110.
- NAJAPHY, A., NIARI KHAMSII, N., MOSTAFAIE, A., MIRZAEI, H. 2010. Effect of progressive water deficit stress on proline accumulation and protein profiles of leaves in chickpea. *Afr. J. Biotechnol.*, 9 (42): 7033–7036.
- NGUYEN-QUEYRENS, A., BOUCHET-LANNAT, F. 2003. Osmotic adjustment in three-year-old seedlings of five provenances of maritime pine (*Pinus pinaster*) in response to drought. *Tree Physiol.*, 23: 397–404.
- RIAZI, A., MATSUDA, K., ARSLAN, A. 1985. Water-stress induces changes in concentrations of proline and other solutes in growing regions of young barley leaves. *J. exp. Bot.*, 36 (172): 1716–1725.

- ROBSON, T.M., SÁNCHEZ-GÓMEZ, D., CANO, F.J., ARANDA, I. 2012. Variation in functional leaf traits among beech provenances during a Spanish summer reflects the differences in their origin. *Tree Genet. Genomes*, 8: 1111–1121.
- ROSE, L., LEUSCHNER, C.H., KÖCKEMANN, B., BUSCHMANN, H. 2009. Are marginal beech (*Fagus sylvatica* L.) provenances a source for drought tolerant ecotypes? *Eur. J. Forest Res.*, 128: 335–343.
- RYAN, M. G., 2011. Tree responses to drought. *Tree Physiol.*, 31: 237–239.
- SCHÄR, C., VIDAŁE, P.L., LUTHI, D., FREI, C., HABERLI, C., LINIGER, M.A., APPENZELLER, C. 2004. The role of increasing temperature variability in European summer heatwaves. *Nature*, 427: 332–336.
- SLOVÁKOVÁ, L., MISTRÍK, I. 2007. *Fyziologické procesy rastlín v podmienkach stresu* [Physiological processes of plants under stress]. Bratislava: Univerzita Komenského v Bratislave, 2007. 240 p.
- SLUGEŇOVÁ, K. 2010. *Fyziologická odozva vybraných druhov drevín na abiotické stresové vplyvy* [The physiological response of selected tree species to abiotic stress factors]. Dissertation thesis. Zvolen: Technical University in Zvolen, Faculty of Ecology and Environmental Sciences. 104 p.
- SLUGEŇOVÁ, K., DITMAROVÁ, L., KURJAK, D., VÁEKA, J. 2011. Drought and aluminium as stress factors in Norway spruce (*Picea abies* [L.] Karst.) seedlings. *J. Forest Sci.*, 57: 547–554.
- TATAR, Ö., GEVREK, M.N. 2008. Influence of water stress on proline accumulation, lipid peroxidation and water content of wheat. *Asian J. Pl. Sciences*, 7: 409–412.
- TSCHAPLINSKI, T.J., GEBRE, G.M., SHIRSHAC, T.L. 1998. Osmotic potential of several hardwood species as affected by manipulation of throughfall precipitation in an upland oak forest during a dry year. *Tree Physiol.*, 18: 291–298.
- TZVETKOVA, N., ANEV, S. 2008. Water regime of common beech (*Fagus sylvatica* L.) in drained terrains. In *International conference of water observation and information system for decision support BALWOIS 2008. 23–26 May, Ochrid, Paper 183: 1–6.*

## Posúdenie vplyvu stresu zo sucha na vybrané biochemické a fyziologické charakteristiky listov bukových sadeníc rôznej proveniencie

### Súhrn

V príspevku sú predložené výsledky štúdie reakcie sadeníc buka lesného (*Fagus sylvatica* L.) rozdielneho pôvodu na riadený proces dehydratácie. V rámci experimentu sme otestovali proveniencie PV1 (1 116 m n. m., vlhká klíma Slovenska), PV2 (710 m n. m., stredne vlhká klíma Slovenska) a PV3 (400 m n. m., suchá klíma Slovenska) na vybrané fyziologické a biochemické parametre. Jednotlivé proveniencie boli prezentované sadenicami vo veku 4 roky, pričom každá proveniencia obsahovala dva varianty: *kontrola* a *sucho*. Sadenice variant *sucho* boli po dobu 55 dní vyradené zo zálievky a miera ich dehydratácie bola monitorovaná prostredníctvom vodného potenciálu listov ( $\Psi_w$ ) a osmotického potenciálu listov ( $\Psi_s$ ). V závere experimentu sme zaznamenali zníženie hodnôt  $\Psi_w$  a  $\Psi_s$  zodpovedajúce veľmi silnému stresu.  $\Psi_w$  poklesol na hodnoty –2,37 MPa (PV1), –3,14 MPa (PV2) a –2,53 MPa (PV3). Hodnoty  $\Psi_s$  sa najvýraznejšie znížili vplyvom vodného deficitu u proveniencii PV2 (–2,54 MPa). Sucho na jednej strane spôsobilo významnú degradáciu asimilačných pigmentov (*Chl a*, *Chl b*, *Chl a + b*, *Chl/Car*) a na druhej strane zapríčinilo významne zvýšenú akumuláciu prolínu v listoch stresovaných sadeníc. Na základe pozorovaných zistení a reakcií sadeníc vystavených nepriaznivým vlhkostným podmienkam, možno spomedzi monitorovaných proveniencií považovať provenienciu PV2 za najcitlivejšiu na pretrvávajúci vodný deficit.

Received March 14, 2013

Accepted April 23, 2013