European Beech (*Fagus sylvatica* L.) from Serbian Mountains – Capacity to Resist Ecological and Oxidative Stress

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Abstract

European beech (*Fagus sylvatica* L.) is the most abundant tree species in Serbia and is highly interesting for economic and ecological reasons. Today, forests are subject to various environmental stresses that lead to the overproduction of reactive oxygen species in plants. Existing beech forests in Serbia are exposed to stressful environmental conditions since they are living under ecological conditions barely within the limit of their requirements, and with the predicted climate change it will become endangered. The present study was designed to define and compare antioxidant capacity of beech populations from three localities (Kopaonik, Tara and Stara Planina) in order to assess their adaptability to oxidative stress caused by specific ecological conditions during investigated period. Total phenols, tannins, flavonoids and proanthocyanidins contents, proline, total protein, soluble protein, and pigment content were analyzed. Lipid peroxidation and protein oxidation intensity were also determined as indicators for oxidative damages. The antioxidant activity was investigated by using ferric reducing power, permanganate reducing antioxidant capacity and $^{\bullet}OH$, $^{\bullet}NO$, $O_2^{\bullet-}$ and $DPPH^{\bullet}$ scavenger activity. The extract from Kopaonik contained the highest amount of phenols, flavonoids, tannins and proanthocyanidins and had lowest malondialdehyde (MDA) and protein carbonyl accumulation. The highest accumulation of proline and high accumulation of MDA were observed in the extract from Tara, the locality with the highest temperature. In the present study, extract from Kopaonik exhibited the highest reducing ability and $DPPH^{\bullet}$ scavenger activity. The relatively high antioxidant capacity may be a determinant for beech acclimation processes, what could be an important tool for the improvement of breeding strategies and reforestation programmes for European beech.

Key words: antioxidant activity, *Fagus sylvatica* L., lipid peroxidation, oxidative stress.

Introduction

Beech (*Fagus*) is a genus of deciduous trees from Fagaceae family, native to the Northern Hemisphere (Wagner et al. 2010). European beech (*Fagus sylvatica* L.) is the most commonly cultivated throughout western and central Europe. Beech is present in regions with moist and relatively cool climate like mountainous regions of the Mediterranean (García-Plazaola and Becerril 2000). In Serbia, pure beech forests cover about 660,400 ha or 29.3% of total forest coverage area (Banković et al. 2009), while mixed forests of beech and other deciduous and coniferous tree species occupy an area of 379,302 ha, or 16.4% of forest coverage area (Vučićević 2004).

Recently, European beech received intensive attention in the light of global warming (Fotelli et al. 2009). Von Wuehlisch (2004) estimated that natural range of beech is going to decrease, and that the most threatened habitats will be those at lower elevations in southern and southeastern parts of its range, where Serbia is located. Serbian beech forests are more exposed to stressful environmental conditions since they are living under ecological conditions barely within the limit of their requirements. This situation could become even worse if global air temperature increases, which would favour periods of drought or spring frost. In favour of this prediction there are the findings of Stojanovic et al. (2013), suggesting that in 90 years about 90% of the current beech stands in Serbia will be lo-

cated outside of their niche. Over the last decade, climatic conditions in Serbia on investigated localities have characteristics of drought. We have chosen to examine beech populations located at protected areas in Serbia after the extensive drought period during the vegetation season of 2011 (Anon. http://www.hidmet.gov.rs/podaci/download/RHMZSrbije_Godisnjak_2011.pdf). Monitoring of physiological and biochemical adaptations gives clear indications how drought and global warming affect the distribution and survival of the dominant and the indigenous population of beech in this area. Research of metabolic response of woody plant species to adverse changes in global climate is topical and of special strategic and national importance, because the mountain populations of indigenous tree species is an important element in planning for sustainable development of forest ecosystems. Current predictions of climate change-induced reductions of water availability require a comprehensive investigation of the future drought stress risk potential with respect to stability of beech forest in Serbia.

Forest plants are subjected to various environmental stresses that lead to the overproduction of reactive oxygen species (ROS), such as H_2O_2, $O_2^{\cdot-}$, $^1O_2$ or $^{\cdot}OH$, which are responsible for oxidative damages on lipids, proteins as well as gene mutations under chronic oxidative stress. Under present climate conditions in Serbia, light becomes excessive and the overexcitation of the photosynthetic apparatus leads to oxidative stress. Oxidative stress is a condition, in which ROS or free radicals exert toxic effects on cells. ROS may affect cell membrane properties and cause oxidative damage to nucleic acids, lipids and proteins that may make them nonfunctional (Gil and Tuteja 2010). It is important to note that ROS can act as damaging, but also as protective agents or signaling factors. This depends on the delicate equilibrium between ROS production and scavenging at the proper site and time (Gratão et al. 2005).

During evolution, trees have developed a number of molecular, anatomical, morphological and physiological adaptations that enhance the probability of survival in harsh environment and oxidative stress conditions caused by the production of ROS. To prevent ROS accumulation, plants possess several protective enzymes and antioxidant molecules such as glutathione, carotenoids and the wide group of phenolic compounds, including flavonoids that quench ROS.

Literature reports the occurrence of many polyphenols species, flavonoids, carotenes, proline, glutathione and other antioxidant compounds in beech (Richter et al. 2007, Štajner et al. 2013). Antioxidants from natural sources have enormous values in plant adaptation to oxidative stresses. Naturally occurring antioxidants can help to prevent oxidative damage caused by oxidative stress induced by unfavourable conditions for beech forests grow.

In view of all these, added to the high abundance of this raw material, this work aims to study the antioxidant capacity of Serbian beech leaves in order to point to naturally occurring antioxidants that can help to prevent oxidative damage caused by oxidative stress imposed by drought conditions in 2011. By selecting the most important indicators of oxidative stress and the most active antioxidants, we can get the diagnostic criteria for beech resistance to oxidative stress in various ecological conditions from different investigated locations in Serbia.

Materials and Methods

Collection regions

The study was made on beeches growing in natural conditions, in mixed forests at three locations in Serbia: Kopaonik Mt (the location is in the creek valley between two slopes on altitude 1,447 m), Tara Mt (the location is on the plain section on altitude 1077 m) and Stara Planina Mt (the location is at the slope of the mountain on altitude 1097 m). Average temperatures during June, August and September of 2011 according to the report of Hydrological Service of Serbia (Anon. http://www.hidmet.gov.rs/podaci/download/RHMZSrbije_Godisnjak_2011.pdf) are presented in Table 1.

Table 1. Average temperatures during June, August and September of 2011

Location \ Month	June	August	September
Stara Planina	12.2	16.5	18.9
Kopaonik	9.5*	10.9	18.5
Tara	15.5	17.6	18.1

*Increased cloudiness, reduced light intensity, increased air humidity

Plant material and extraction procedure

Plant material was collected during September 2011. Leaves were collected from 20 trees, from lower and medium region and the average sample was made. The leaves were dried at open air in the dark and ground to a fine powder in a mill. Powdered samples were extracted with water (w:V = 1:2.5) for 24 h at ambient temperature, followed by filtration. These extracts were used for lipid peroxidation, soluble protein and permanganate reducing antioxidant capacity determination. Pigments were extracted with acetone with pestle and mortar and filtered over a cotton pad. The

extracts were made up to 50 mL with acetone. For determination of total flavonoids, plant material (1 g) was extracted with solvent methanol-water-acetic acid (V:V:V = 140:50:10). For determination of total phenolic and tannin contents, acidic ethanol (0.1 mol L^{-1} HCl in EtOH) was used for extraction. The ethanol extracts were obtained by subjecting the powdered leaves at maceration in 80 % ethanol (in water) at ambient temperature for 24 h in the dark. These extracts were evaporated to dryness and the dry residues were re-dissolved again in 80 % EtOH (in water) to obtain mass concentration 25 mg mL^{-1} and were used for determination DPPH$^{\bullet}$, $^{\bullet}NO$, $^{\bullet}OH$ and $O_2^{\bullet-}$ antiradical power (ARP) and for Ferric Reducing Antioxidant Power (FRAP) methods.

Lipid peroxidation, proline, soluble protein, nitrogen content, total proteins, protein oxidation and pigment contents

Lipid peroxidation (LP) was estimated based on thiobarbituric acid (TBA) reactivity by the method of Dhindsa et al. (1981) with minor modification. A 0.5 mL aliquot of appropriately prepared sample was added to a test tube with 4.5 mL of TBA reagents comprised of 0.5% (w/v) TBA in 20% (w/v) TCA. Then, samples were mixed vigorously, heated for 25 min at 95 °C in a water bath, quickly cooled in an ice bath, and centrifuged at 3,000 g for 10 min. The absorbance was read spectrophotometrically at 532 nm with Thermo Scientific Evolution 220 UV-Visible Spectrophotometer, and MDA quantity is expressed as nmol of MDA mg^{-1} protein.

Proline accumulation was determined by the method as described by Bates et al. (1973). Proline was determined after extraction with sulphosalicylic acid, and reaction with ninhydrin. A standard curve of proline was used for calibration.

Soluble protein content was determined by the method of Bradford (1976).

The nitrogen content was determined using Kjeldahl method, in which plant samples are prepared by dissolving organic compounds with $_{cc}H_2SO_4$ (Nelson and Sommers 1973). Total protein content can be calculated by multiplying nitrogen content of the sample with a conversion factor (5.71) (Sosulski and Imafidon 1990).

Protein oxidation intensity was described using the content of carbonyl groups in proteins (Lenz et al. 1989). It was determined as amount of 2,4-dinitrophenylhydrazone formed upon reaction with 2,4-dinitrophenylhydrazine (DNPH). Samples (> 1.5 mg protein) were treated with 10 mmol L^{-1} DNPH in 2 mol L^{-1} HCl at room temperature for 60 min. Blanks contained 2 mol L^{-1} HCl without DNPH. Proteins were precipitated by addition of TCA up to final concentration 10%, centrifuged at 4,000 g for 10 min at 4 °C, and washed three times with 1 mL solution of ethanol: ethyl acetate (1:1). The final pellets were dissolved in 6 mol L^{-1} guanidine hydrochloride in 20 mmol L^{-1} phosphate buffer, pH 2.3. Carbonyl content was calculated using the absorbance maximum of DNPH measured at 370 nm. The results were expressed as nanomoles carbonyls per milligram protein.

Pigments were determined spectrophotometrically according to von Wettstein (1957). Absorbance of prepared acetone extracts was recorded at 662, 644 and 440 nm using acetone as blank and pigment content was calculated using molar absorption coefficients following equations determined by von Wettstein (1957).

Total phenol, tannin, flavonoids and proanthocyanidin contents

The total content of phenol was determined by the Folin-Ciocalteu method (Hagerman et al. 2000), using catechin as a standard. The amount of total phenolic content was calculated as a catechin equivalent from the calibration curve of catechin standard solutions (covering the concentration range between 0.1 and 1.0 mg mL^{-1}), and expressed as mg catechin g^{-1} dry plant material.

Determination of total tannin content was done by Folin–Ciocalteu procedure as above, after removal of tannins by their adsorption on insoluble matrix (polyvinylpolypyrrolidone, PVPP). Calculated values were subtracted from total polyphenol contents and total tannin contents expressed as mg catechin g^{-1} dry plant material.

Total flavonoids were determined according to Markham (1989). The number of flavonoids was calculated as a rutin equivalent from the calibration curve of rutin standard solutions, and expressed as mg rutin 100 g^{-1} of dry plant material.

Proanthocyanidins content were determined by butanol – HCl assay (Hagerman et al. 2000) and were expressed as mg leucoanthocyanidin 100 g^{-1} of dry plant material, assuming that the specific absorbance of leucoanthocyanidin was 460.

Antioxidant capacity determinations

Total antioxidant capacity was estimated according to the FRAP assay (Benzie and Strain 1999) and details were as described by Popović et al. (2013). Permanganate reducing antioxidant capacity (PRAC) determination was done using procedure by Cacig et al. (2006) and details were done as described by Popović et al. (2013).

Radical scavenging (RSC) determinations

Determination of DPPH•-RSC of extracts was done using procedure proposed by Brand-Williams et al. (1995) and details were done as described by Popović et al. (2013). •NO-RSC was done according to the Griess reaction (Green et al. 1982) and details were as described by Popović et al. (2013). $O_2^{\bullet-}$-RSC was done using assay based on the capacity of crude extracts to inhibit the photochemical reduction of nitroblue tetrazolium (NBT) in the riboflavin-light-NBT system (Dasgupta and De 2004). The increase in absorbance at 560 nm was monitored. The scavenging capacity was expressed as reduction percentage of NBT absorbance induced by sample. Determination of HO-RSC of extracts was done by the inhibition of 2-deoxyribose degradation (Cheesman et al. 1988).

The radical (DPPH•, •NO, $O_2^{\bullet-}$, OH) scavenging capacities of the extracts in assays were expressed as antiradical power (ARP) and it was defined as:

$$ARP = \frac{1}{IC_{50}} \cdot 100$$

All measurements were done in triplicate.

Statistical analysis

Statistical comparisons between samples were performed with Duncan *t*–test for independent observations using a software package STATISTICA 9.1. Differences were considered significant at $P < 0.05$. Correlations between different parameters were considered significant at $r > 0.95$ ($P < 0.05$). Principal component analysis (PCA) implemented in the Statistica for Windows version 13, StatSoft Inc. (Dell Statistica) was the chemometric method used to analyze the results.

Results

Results concerning contents of total phenols, flavonoids, tannins and proanthocyanids in leaves of beech from Kopaonik, Tara and Stara Planina were provided in Figure 1. In general, results demonstrate that extract from Kopaonik has the highest content of polyphenol compound and significant differences ($P < 0.05$) were observed between almost all investigated locations. Contents of flavonoids was statistically significantly positively correlated ($P < 0.05$) with content of tannins ($r = 0.998998$) and negatively correlated with soluble proteins ($r = -0.998091$), total nitrogen and total proteins. Also, highly negative correlations were observed between total tannins with soluble proteins and total nitrogen and total proanthocyanidins with soluble proteins, total proteins and total nitrogen.

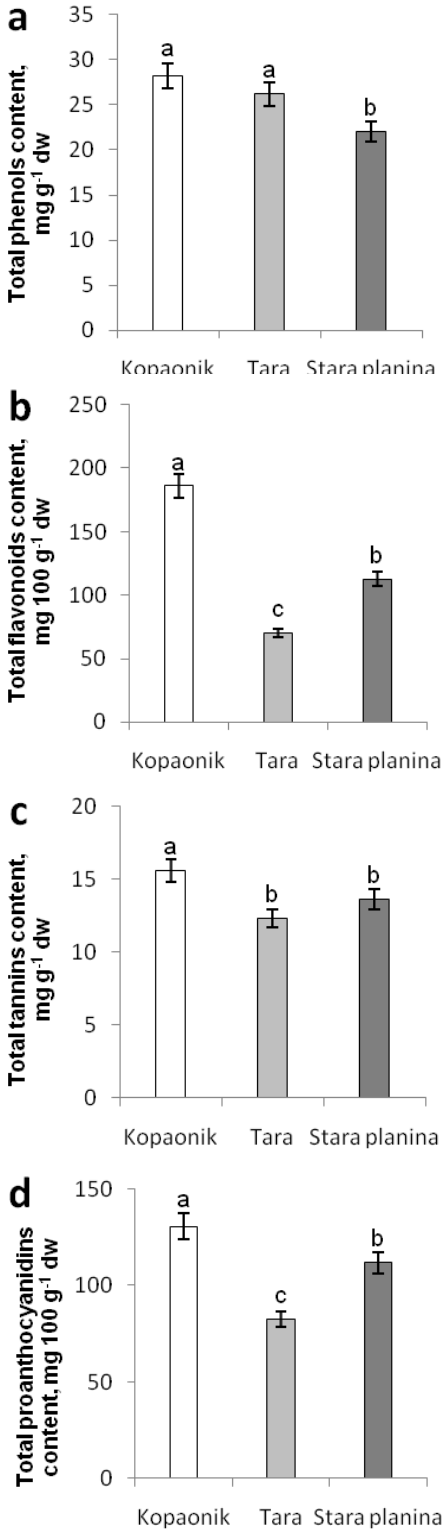

Figure 1. Contents of total phenols (**a**), flavonoids (**b**), tannins (**c**) and sproanthocyanidins (**d**) in leaves of beech from Kopaonik, Tara, and Stara Planina. Values presented mean ± *SE* of three replicates. Different characters on each bar represent significant difference according to Duncan test at the p <0.05 level

The pigment and proline contents of leaves of each locality is shown in Figure 2. The highest contents of chlorophyll *a*, chlorophyll *b* and carotenoids, but the lowest content of proline were in extract from Stara Planina (2.67 mg g^{-1}, 2.09 mg g^{-1}, 0.91 mg g^{-1}, and 0.46 µg g^{-1}, respectively). The lowest content of chl *a* and chl *b* was in extract from Kopaonik (2.31 and 1.34 mg g^{-1}, respectively), while the lowest content of carotenoids was in extract from Tara (0.47 mg g^{-1}). The content of chl *b* was significantly positively correlated with chl *a* and carotenoids.

Stara Planina had statistically higher contents (30.47 mg g^{-1} dw and 0.34 nmol carbonyl mg^{-1} protein). Contents of soluble proteins were significantly positively correlated ($P < 0.05$) with nitrogen content and total proteins ($r = 0.998976$).

The intensity of LP expressed as nmol MDA mg^{-1} protein are presented in Figure 4. Malondialdehyde quantity ranged from 8.12 nmol mg^{-1} protein (Kopaonik) to 31.66 nmol mg^{-1} protein (Stara Planina). The best ARP values had shown beech extract from Stara Planina; ARP toward $^{\bullet}OH$ was 28.39, ARP toward $^{\bullet}NO$ was 0.76 and ARP toward $O_2^{\bullet-}$ was 3.03; but there was no statistically significant difference in the ability of extracts to neutralize $^{\bullet}OH$, $^{\bullet}NO$ and $O_2^{\bullet-}$.

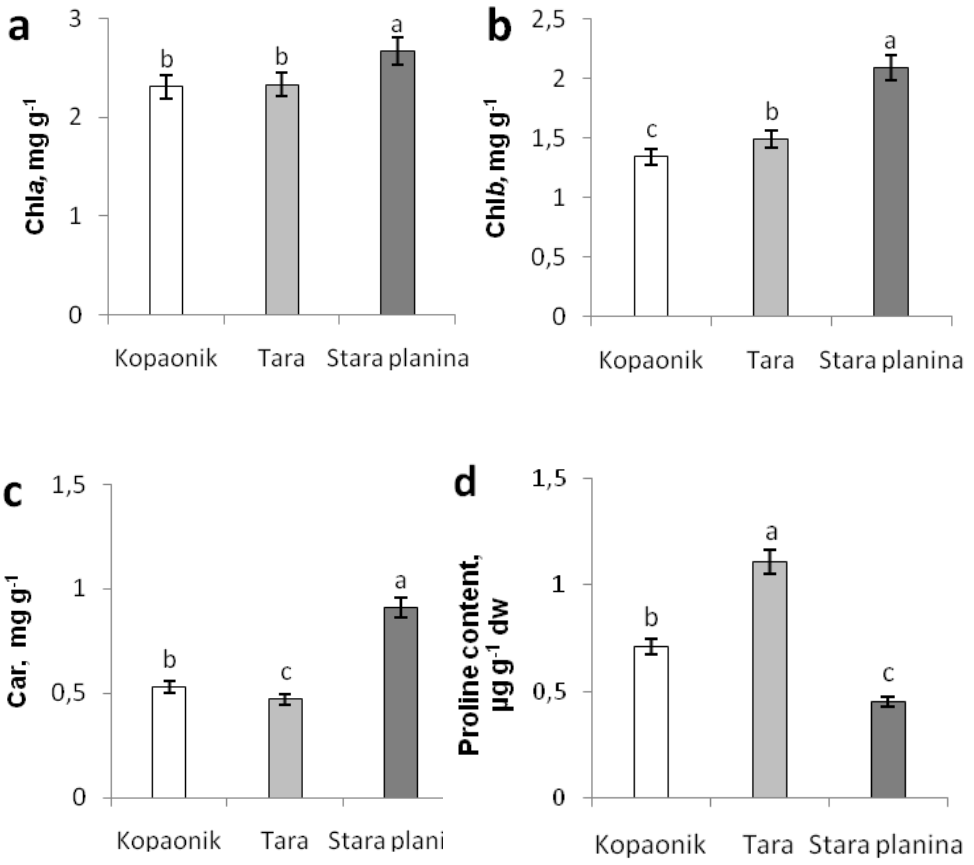

Figure 2. Contents of chlorophyll *a* (**a**), chlorophyll *b* (**b**), carotenoids (**c**) and proline (**d**) in beech leaves from Kopaonik, Tara and Stara Planina. Values presented mean ± *SE* of three replicates. Different characters on each bar represent significant difference according to Duncan test at the $p < 0.05$ level

The results concerning content of total proteins and nitrogen, soluble proteins and protein oxidation intensity in leaves of investigated plants are presented (Figure 3). Foliar content of total nitrogen in beech trees from Kopaonik, Tara and Stara Planina averaged 1.98, 2.61, and 2.32 %, respectively. The highest total and soluble protein contents was detected in beech leaves from Tara (149.03 and 36.18 mg g^{-1} dw, respectively). The smallest soluble protein contents and lowest intensity of protein oxidation were observed in extract from Kopaonik (22.61 mg g^{-1} dw, 0.02 nmol carbonyl mg^{-1} protein, respectively), while extract from

The ability of extracts to scavenge DPPH radicals was increased in the following order: Stara Planina < Tara < Kopaonik, indicating high antioxidant activity of examined beech extract from Kopaonik, what is also confirmed with assays of antioxidant capacity determination (Figure 5). PRAC values were statistically positively correlated with FRAP ($r = 0.998694$). Also, PRAC values were positively correlated with TPC and DPPH$^{\bullet}$-ARP, but it was not statistically significant.

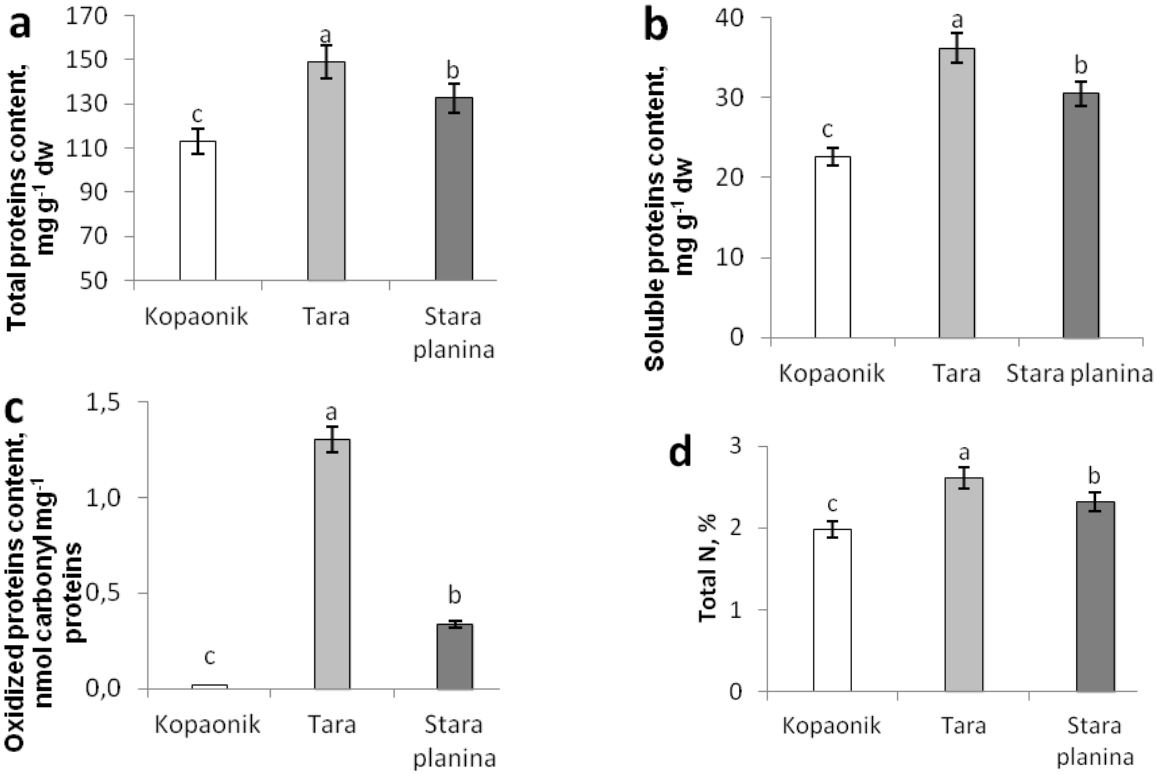

Figure 3. Contents of total proteins (**a**), soluble proteins (**b**) oxidized proteins (**c**) and total nitrogen (**d**) in leaves of beech from Kopaonik, Tara and Stara Planina. Values presented mean ± *SE* of three replicates. Different characters on each bar represent significant difference according to Duncan test at $p < 0.05$ level

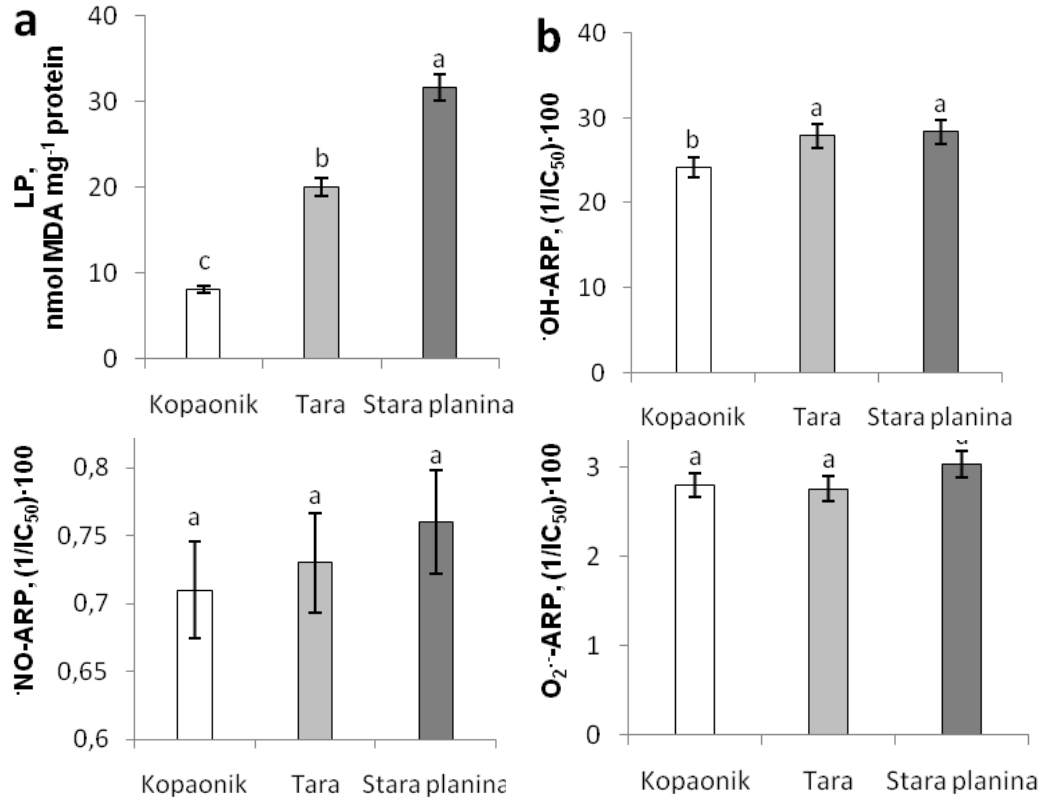

Figure 4. Lipid peroxidation (**a**),·OH (**b**), ·NO (**c**), and $O_2^{\cdot-}$ (**d**) antiradical powers in leaves of beech from Kopaonik, Tara and Stara Planina. ARP-Antiradical power; ARP = $((1/IC_{50}) \cdot 100)$; IC_{50} – The concentration of a sample, at which 50% inhibition of free radical activity is observed. Values presented mean ± *SE* of three replicates. Different characters on each bar represent significant difference according to Duncan test at $p < 0.05$ level

DPPH· ARP value was statistically significantly negatively correlated with LP (r = -0.997387) and with ·NO ARP value (r = -0.998822).

Chemometric evaluation (PCA analysis) of interrelations between parameters is presented by graph of loading plot for all investigated parameters (Figure 6). Chemometric evaluation showed close interdependence between FRAP, PRAC, DPPH ARP values and TPC, as well as between pigments and SOA ARP, and between total proteins, nitrogen, soluble proteins content and protein oxidation intensity.

Discussion and Conclusions

According to Štajner et al. (2013) and Isajev et al. (2009), the stability of forest ecosystems depends on population's ability to adapt to changing environmental conditions. Therefore, starting from the fact that plant with the ability to scavenge and control the level of cellular ROS may be useful to withstand changes of environmental conditions, the focus of this study was to evaluate the antioxidant potential and determine total phenols, total tannins, flavonoids and proanthocy-

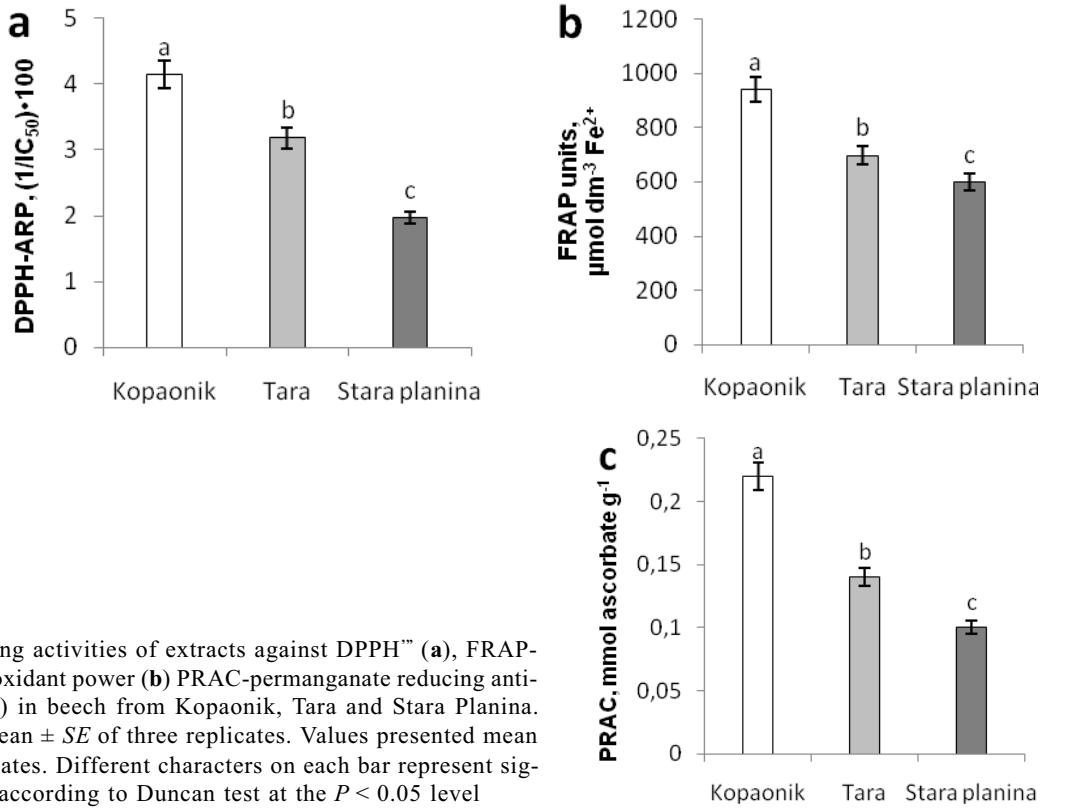

Figure 5. Scavenging activities of extracts against DPPH˙ (**a**), FRAP-ferric reducing antioxidant power (**b**) PRAC-permanganate reducing antioxidant capacity (**c**) in beech from Kopaonik, Tara and Stara Planina. Values presented mean ± *SE* of three replicates. Values presented mean ± *SE* of three replicates. Different characters on each bar represent significant difference according to Duncan test at the $P < 0.05$ level

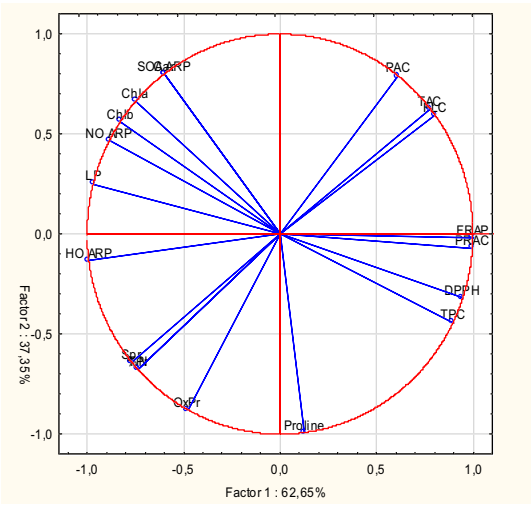

Figure 6. PCA analysis of investigated parameters: graph of loading plot.* Abbreviations present investigated parameters: Total phenolic content (TPC); proanthocyanidines (PAC); total tannin content (TAC); total flavonoids content (FLC); lipid peroxidation (LP); proline (Pro); soluble protein content (Spr); nitrogen content (N); total protein content (TPr); protein oxidation intensity (OxPr); carotenoids (Car); chlorophyll *a* (Chla); chlorophyll *b* (Chlb); DPPH; FRAP; PRAC; NO ARP; HO ARP; SOA ARP

anidins of the beech leaves from three different locations in Serbia. In this study, beech leaves extract from Kopaonik had the highest total phenols, flavonoids, tannin and proanthocyanidins content, as well as significantly higher level of DPPH·-ARP, FRAP and PRAC values than other extracts (Figure 5). PRAC values were statistically positively correlated with FRAP values and DPPH· ARP. Strong positive correlation between mentioned parameters could be easily explained by the same mechanism of antioxidant action by single electron transfer (Popović et al. 2012). Also, mentioned antioxidant parameters were positively correlated with phenols content what is in accordance with the results of other authors (Clarke et al. 2013). Radical-scavenging activity towards reactive oxygen species such as ·OH, ·NO and $O_2^{\cdot-}$ radicals generally proceeds by multiple different mechanisms and could be less selective, especially for ·OH because of its high reactivity (Popović et al. 2012). DPPH· ARP value was statistically significantly negatively correlated with LP. Similar results were reported by Kumar et al. (2013), who found that plant extracts reach in polyphenols showed concentration-dependent free radical scavenging activities and lipid peroxidation inhibition. Other authors also investigated antioxidant metabolism in plants, trees (including beech leaves) and fungi during stress conditions and found that antioxidants such as ascorbate, polyphenols and glutathione were accumulated during stress and that induction of antioxidants was ubiquitous strategy of plants defense under oxidative stress (Luwe 1996, Vamanu and Nita 2013).

Plant pigments are of key importance for the photosynthetic processes and for preventing photo-oxidation and photo-inhibition (García-Plazaola and Becerril 2000). Earlier trials have shown that drought has a negative impact on the photosynthetic reaction centers (loss of chlorophyll *a*), which can also lead to a reduction of carotenoids content (Efeoglu et al. 2009). As reported Lei et al. (2006) reduced carotenoids content is indicator of oxidative stress and in this study beech from Tara has the lowest content of carotenoids. Numerous studies have shown that the proline content in higher plants increases under different environmental stresses such as drought, high salinity, high light and UV irradiation, heavy metals, oxidative stress and in response to biotic stress (Szabados and Savoure 2010). In Figure 2 the results concerning proline accumulation in leaves of investigated plants are presented. The highest free proline, as well as protein content, was detected in beech leaves from Tara, where the highest average temperatures during June-August 2011 were recorded. Accumulation of free proline under stress conditions is connected with its numerous functions in plants. Free proline acts as osmolyte, and have role in stabilization of proteins, membranes and subcellular structures (Szabados and Savoure 2010). Also, it may be acting as a storage compound for both carbon and nitrogen during water stress when both starch and protein syntheses are inhibited (Hsiao 1973). Szabados and Savoure (2010) have reported on the antioxidant feature of proline, indicating its role as a singlet oxygen quencher, H_2O_2 scavenger and capability to buffer cellular redox potential.

Results obtained for nitrogen and protein content were in accordance with the study published by Peuke and Rennenberg (2004). Extract from Tara exhibited high nitrogen and total protein content, what can be explained by the accumulation of soluble proteins in these plants (Figure 3). Negative correlation between tannin content and soluble proteins could be attributed to the tannin preference to precipitate with soluble proteins.

Lipid peroxidation is well-established mechanism of cellular injury in both plants and animals, and is used as an indicator of oxidative stress in cells and tissues (Dalle-Donne et al. 2003). Results for MDA production in investigated extract showed that extract from Tara had high accumulation of MDA, and interestingly there was observed the highest free proline, what indicated that beech from Tara were exposed to stress and also had reaction to the stress, which was not observed in extract from Stara Planina, where was the highest LP but low proline accumulation. This result was also confirmed with high abundance of oxidized proteins in this extract. Along with lipid peroxidation, protein oxidation intensity appears as specific method for detection of oxidative stress and could be useful indicators of it. Dębska et al. (2013) also showed that increase of oxidative stress manifested by accumulation of ROS including hydrogen peroxide also provoked protein carbonylation.

Principal component analysis (PCA) applied to the renormalized data by an autoscaling transformation (data not shown) explained 100% of the total variance (PC1 = 62.65% and PC2 = 37.35%). The loads characterize the trends between variables (Figure 6). The first component (PC1) explains 62.65% of all information, and separates horizontally parameters. Along the *x* axis (horizontally), it can be seen that the variables, which most influenced this component, are the most important antioxidant parameters and indicators of antioxidant activity, FRAP, PRAC, DPPH ARP, TPC, FLC and TAC and with negative values it is the lipid peroxidation. Along *y* axis (vertically) the levels of carotenoids, SOA ARP and chlorophylls (with positive values) and the carbonyled proteins and proline (with negative values) influenced the most the second component

(PC2). As reported in the loading plots (Figure 6) investigated parameters, which are closely positioned to each other, are in significant positive correlation, such as cluster of FRAP, PRAC, DPPH and TPC.

In this study, beech leaf extracts from different locations in Serbia were assayed after period of drought to explore their antioxidant activities and possibility to adapt to oxidative stress. Obtained results showed that the same species (beech) had different alterations after drought period at three different localities (Kopaonik, Tara and Stara Planina). On Kopaonik, drought could not affect these plants in such significant extent, what is supported by the facts that this extract had the lowest content of carbonyled proteins and MDA. Also, it is possibly that drought was not so evident here because of characteristics of locality (valley) and observed cloudiness and humidity (Table 1). But on the other two localities, drought with observed high temperatures caused significant disturbances in oxidative balance, what is obvious from the data obtained for LP and oxidized proteins content. PCA analysis proved causal link among content of phenols, antiradical power and LP. LP was the highest in the extract from Stara Planina, the locality on the slope of mountain, where water drained quickly. In terms of protein oxidation, extract from Tara locality was highlighted. Our results suggest that negative impact of drought will be more evident in beech populations located at higher areas of mountain slopes, where water runoff is faster (Stara Planina). On this locality, we observed highest LP, high content of oxidized proteins, the lowest content of proline, and the lowest DPPH ARP, FRAP and PRAC values. It also may be noted differences in adaptations in leaves from Kopaonik and Tara to drought conditions. Observed adaptations consist primarily of increased proline content and higher polyphenol content.

This investigation shows that beeches at localities Tara and Stara Planina are undoubtedly affected by a drought, while on Kopaonik that was not case. The present results indicate a higher antioxidant capacity in leaves from Kopaonik, but also good oxidative adaptation in beech from Tara. Obtained results may help to explain differences in the biochemical response to environmental stress of beech trees from different locations, which could be an important tool for the improvement of breeding strategies and reforestation programmes for European beech.

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