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Research Article

Chemical Parameters of Oxidative Stress Adaptability in Beech

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The antioxidant activity, lipid peroxidation, and contents of free proline and soluble proteins were investigated on six-year-old beech plants. Provenance Avala, in Serbia, had the best adaptability to environmental factors on locality Fruska Gora due to low lipid peroxidation, high FRAP value, and free proline and soluble proteins contents. Provenances Scharnstein and Mitterndorf, in Austria, had the best adaptability to environmental factors on locality Debeli Lug due to high FRAP value and free proline and soluble proteins contents. FRAP values in majority of provenances from locality Debeli Lug were higher. Correlations parameters were much higher between provenances in locality Debeli Lug, situated at higher altitude, which is the consequence of better adaption to environmental factors influence.

1. Introduction

Biological combustion involved in the respiration process produces harmful intermediates called reactive oxygen species (ROS). Excess of ROS can lead to cumulative damage in proteins, lipids, and DNA, resulting in so-called oxidative stress. Oxidative stress, defined as the imbalance between oxidants and antioxidants in favor of the oxidants [1], has been suggested to be the cause of aging [2] and various diseases in humans [3].

There are many potential sources of ROS in plants. Some are reactions involved in normal metabolism, such as photosynthesis and respiration [4]. Other sources of ROS belong to pathways enhanced during abiotic stresses. These include drought stress [5–7] and desiccation, salt stress, chilling, heat shock, heavy metals, radiation [8], air pollutants such as ozone and SO_2, mechanical stress, nutrient deprivation, pathogen attack, and high light stress [9, 10]. Plants suffering increased oxidative stress generally respond with increases in antioxidative systems although this response appears not always to be sufficient to prevent injury and lipid peroxidation [11, 12]. Beside growth and biomass production, the survival of plants requires the ability to defend themselves against adverse biotic or abiotic environmental stresses [13, 14]. Unfavorable conditions such as drought or air pollutants may cause increased oxidative stress.

Antioxidants from natural sources have received much attention, and efforts have been made to identify new natural resources for health-promoting antioxidant agent in human diets with economical potential for the pharmaceutical industry [15]. In addition, these naturally occurring antioxidants can help to prevent oxidative damage caused by oxidative stress in plants. Hence, the balance between antioxidation and oxidation is believed to be a critical concept for maintaining a healthy biological system [16].

European beech (*Fagus sylvatica* L.), one of the principal tree species in Europe, has an invaluable role in many forest ecosystems. It is the most widely distributed forest tree species in Europe and is highly interesting for both economic and ecological reasons. Beech wood is excellent firewood, easily split and burning for many hours with bright but calm flames. Chips of beech wood are used in the brewing of Budweiser beer as a fining agent. Beech is also used to smoke some cheeses. Some drums are made from beech. Also, beech pulp is used as the basis for manufacturing a textile fiber known as Modal. Modal has been used alone or with other fibers in household linens such as towels, bathrobes, and bed sheets, and the fabric has increased in popularity in the early 21st century. The wood is also used to make the pigment known as bistre favored by such artists as Rembrandt.

As a result of this historic bottleneck, northern populations of beech are genetically quite uniform while higher diversity is found near the southern limit of their range [17]. Nevertheless, the latter are more sensitive to stressful environmental conditions since they are living under ecological conditions just barely within the limit of their requirements. This situation could become even worse if global air temperature increases, which would favor periods of drought in the Mediterranean and east European regions as well as increasing the risk of spring frost [18]. The reaction of beech forests to potential climate change is one of the key issues in forestry today [19]. Climate changes will negatively affect beech ecosystems, causing reduction of the beech range. Given to particular sensitivity to drought, it is projected that beech will face severe problems under global rising of temperature [20]. The most endangered will be stands in the southern and south-eastern parts of present distribution range, while the conditions in the northern parts of beech distribution range may become more favorable for beech and its ecosystems. Less than 38% of *Fagus sylvatica* trees in Western Europe are healthy today. The health and growth of forest trees, however, are determined by a variety of natural and anthropogenic site factors and antioxidants [21]. One of the main indicators of tree decline and air pollution is accelerated leaf ageing, and this process is characterized in beech leaves by antioxidants and pigment destruction. Antioxidants decreased with beech decline [18]. As the beech is less tolerant to water shortage, in the southern parts of its range its competitiveness will largely be limited by increased water stress [22]. Declining of the beech vitality, caused by droughts, may weaken physiological condition of populations, leading to insect and disease outbreaks [23]. Furthermore, declines in the vitality of beech could result in the disappearance of beech from some habitats as a consequence of the loss of space by the competing species [24]. Due to the complex influence of climate and soil variables on plants, it is not simple and straightforward to assess the adaptability of beech plants in the provenance trial. The most frequently used variables for assessing adaptability in trials with young plants are survival and height [25].

The impacts of specific variables on beech phenology, such as changes in the temperature, water availability, or an increase in atmospheric CO_2 concentrations, have been tested on different provenances with experiments in controlled environments [26–28]. However, in case of European beech, those experiments are limited to young plants (aged up to a few years).

The aim of this research was to propose efficient method for establishing the adaptability of different European beech provenances (aged up to a six years) to oxidative stress by evaluating the antioxidant properties and free proline and soluble proteins contents grown under different environmental conditions.

Table 1: Investigated beach provenances, country of origin, and appropriate altitude.

No.	Provenance	Country	Altitude (m)
1	Sjeverni Dilj Čaglinski	Croatia	350
2	Vrani Kamen	Croatia	600
3	Tajan, Zepce	Bosnia	700
4	Grmec, Bosanska Krupa	Bosnia	650
5	Fruška Gora	Serbia	370
6	Kopaonik	Serbia	510
7	Valkonya	Hungary	300
8	Schelklingen	Germany	650
9	Höllenbach	Germany	755
10	Hasbruch	Germany	35
11	Scharnstein, Mitterndorf	Austria	480
12	Vranica-Bistrica	Bosnia	750
13	Crni Vrh	Bosnia	500
14	Alesd	Romania	490
15	Alba-Iulia	Romania	860
16	Sihlwald	Swiss	1050
17	Avala	Serbia	475
18	Boranja	Serbia	410
19	Fruška Gora	Serbia	370
20	Cer	Serbia	745

2. Material and Methods

2.1. Plant Material. The experiment is the part of international provenance trial which includes 20 different beech provenances from Croatia, Bosnia, Serbia, Hungary, Germany, Austria, Romania, and Swiss (Table 1).

Samples were taken from six-years-old beech. 20 leaves were sampled from each of five trees from the same locality in order to obtain an average sample. Plants were transplanted in a complete randomized block design in three blocks (repetitions). The trial was located within two localities: Fruška Gora (altitude 370 m) and Debeli Lug (altitude 742 m). Fresh expanded leaves from the top were used to determine all antioxidant parameters, lipid peroxidation, and free proline and soluble proteins contents.

2.2. Measurements

2.2.1. Determination of Total Antioxidant Capacity. Total antioxidant capacity was estimated according to the FRAP (Ferric Reducing Antioxidant Power) assay [29]. Total reducing power is expressed as FRAP units. FRAP unit is equal to 100 μmol/dm^3 Fe^{2+}. FRAP value was calculated using the following formula:

$$\text{FRAP value} = \frac{\Delta A_{\text{sample}}}{\Delta A_{\text{standard}}}. \quad (1)$$

2.2.2. Determination of Lipid Peroxidation. Lipid peroxidation (LP) was determined by the thiobarbituric acid (TBA)

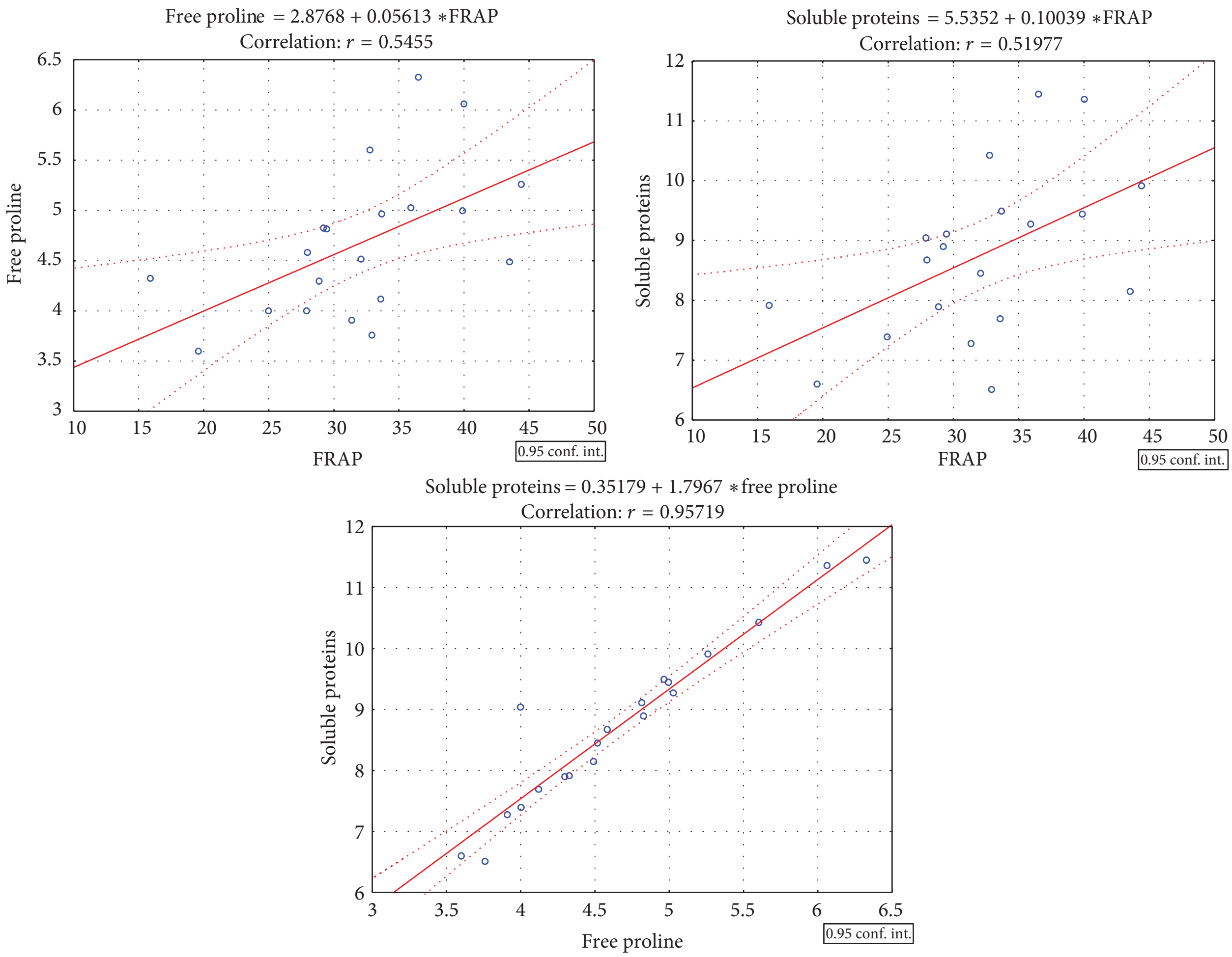

Figure 1: Significant and positive correlations between different parameters (free proline, FRAP, and soluble proteins) in beech provenances in forest Fruška Gora. *Free proline was expressed as μmol/mg protein, FRAP as FRAP units (1FRAP unit = 100 μmol/dm^3 Fe^{2+}), and soluble proteins were expressed as mg/g.

method. Values were given as equivalent amounts of malonyldialdehyde (MDA). The calibration curve was prepared with malonyldialdehyde bis-diacetal [30, 31].

2.2.3. Determination of Proline Accumulation. Proline accumulation was determined by the method as described by Paquin and Lechasseur [32]. Proline was determined after extracion with sulphosalicylic acid and reaction with ninhydrin. A standard curve of proline was used for calibration [33].

2.2.4. Soluble Protein Content Determination. Soluble protein content was determined by the method of Bradford [34].

All determinations were performed in triplicate.

2.3. Statistical Analysis. Results were expressed as mean ± standard error. Statistical comparisons between samples were performed with Student's t-test for independent observations. Differences were considered significant at $P < 0.05$. Correlations between FRAP values, LP, and free proline and soluble proteins contents were established by regression analysis.

3. Results and Discussion

Results concerning beech provenances transplanted on locality Fruška Gora are presented in Tables 2 and 3 and Figures 1 and 2. The highest level of total antioxidant capacity was observed in provenance number 16 (44.410 FRAP units) followed by provenances number 10 (43.513 FRAP units) and number 17 (40.013 FRAP units) (Table 2). The lowest LP was observed in provenance number 17 (20.701 nmol MDA/mg protein), provenances 15 (21.535 nmol MDA/mg protein) and 9 and 19 (22.232 and 22.253 nmol MDA/mg protein). Highest accumulation of free proline was in provenances 20 (6.329 μmol/mg protein) and 17 (6.061 μmol/mg protein) which is a benefit because proline may protect protein structures by maintaining their structural stability [35]. It is also known that drought stress significantly increases proline accumulation [36]. Content of soluble proteins was also highest in provenances number 20 (11.446 mg/g) and number

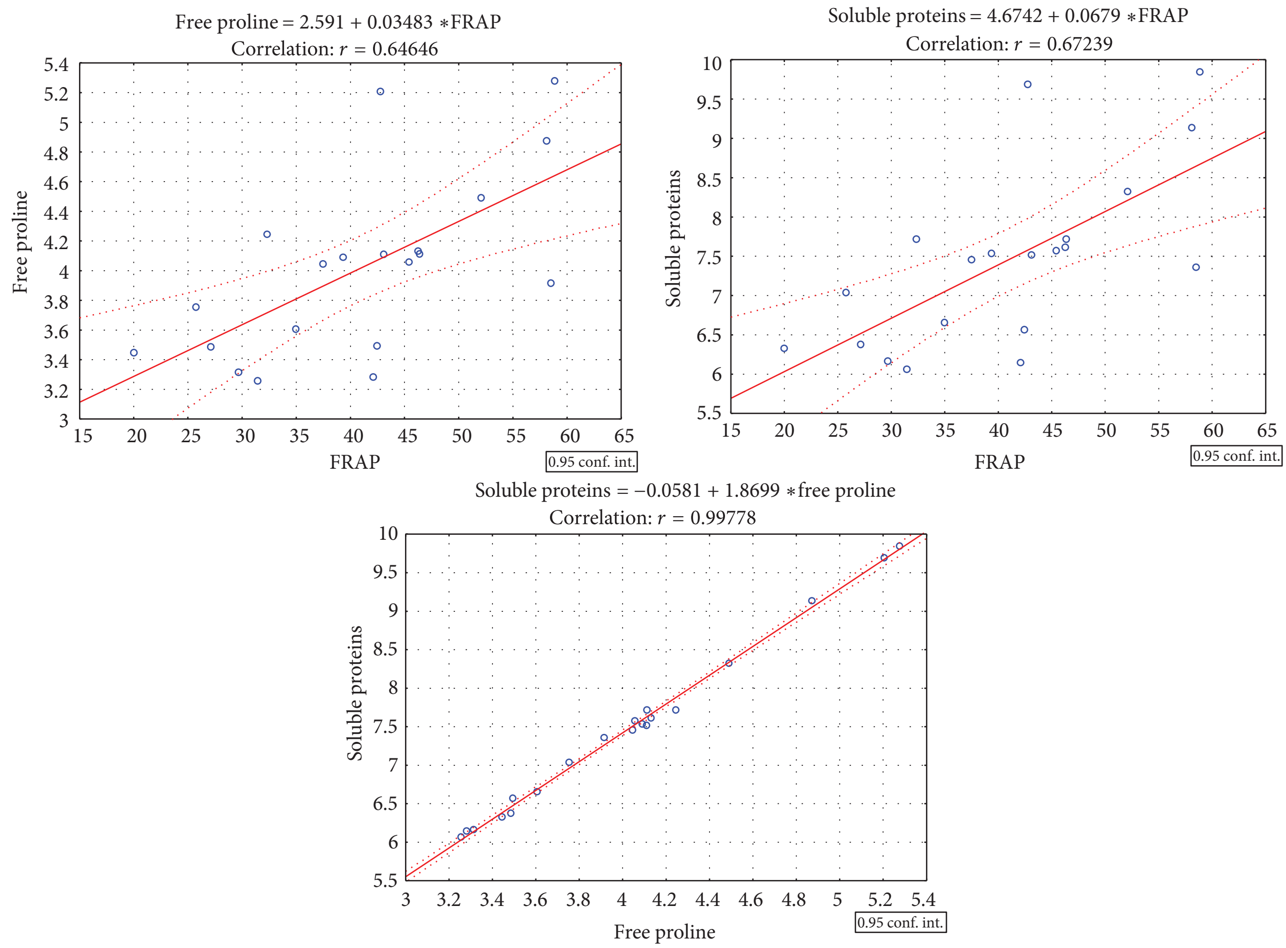

Figure 2: Significant and positive correlations between different parameters (free proline, FRAP, and soluble proteins) in beech provenances in meadow Debeli Lug. *Free proline was expressed as μmol/mg protein, FRAP as FRAP units (1FRAP unit = 100 μmol/dm^3 Fe^{2+}), and soluble proteins were expressed as mg/g.

17 (11.360 mg/g) (Table 2). Proteins rich in proline have particular roles in the development, structure, and function of the cell walls [37]. According to our results provenance number 17, Avala in Serbia, had the best adaptability to environmental factors in forest Fruška Gora due to low LP, high FRAP value, and contents of free proline and soluble proteins.

Significant positive correlations ($P < 0.05$) were observed between numbers of examined parameters.

FRAP values were significantly positively correlated with free proline ($r = 0.545504$) and soluble proteins content ($r = 0.519771$). Free proline was significantly high positively correlated with soluble proteins content ($r = 0.957191$). Results obtained by correlation analysis supported our previously presented results (Figure 1).

Results concerning beech provenances transplanted on locality Debeli Lug are presented in Tables 4 and 5 and Figure 2. The highest level of total antioxidant capacity was observed in provenance number 11, (58.872 FRAP units), followed by provenances number 15 (58.513 FRAP units) and number 12 (58.128 FRAP units) (Table 4). Presented FRAP values were generally higher in provenances from meadow Debeli Lug. It has been proved that efficient antioxidative characteristics can provide better protection against oxidative stress [38], promote the growth of plants, and improve their productivity [39]. It is known that antioxidant varied provenances probably due to different adaptation ability of each provenance [40]. The lowest LP was observed in provenance number 20 (32.288 nmol MDA/mg protein), provenances number 1 (33.557 nmol MDA/mg protein) and number 14 (35.869 nmol MDA/mg protein). Increased MDA content suggested to oxidative damages in examined provenances, similarly as detected in olive trees. On the contrary low, accumulation of MDA is indicator of drought stress tolerance [35]. Highest accumulation of free proline was detected in provenances No 11 (5.277 μmol/mg protein), number 9

Table 2: Total antioxidant capacity (FRAP), lipid peroxidation (LP), and free proline and soluble proteins contents in beech provenances transplanted in forest Fruška Gora.

	Locality—Fruška Gora			
Provenance No.[a]	FRAP (FRAP units)[b]	LP[c] (mmol MDA/mg protein)	Free proline (μmol/mg protein)	Soluble proteins (mg/g)
1	32.910 ± 0.202	43.423 ± 0.291	3.760 ± 0.131	6.515 ± 0.121
2	32.090 ± 0.158	37.420 ± 0.891	4.5170 ± 0.401	8.452 ± 0.116
3	33.590 ± 0.315	35.195 ± 0.426	4.126 ± 0.192	7.692 ± 0.021
4	24.949 ± 0.078	30.157 ± 0.662	4.004 ± 0.298	7.395 ± 0.023
5	28.872 ± 0.114	30.653 ± 0.191	4.297 ± 0.086	7.898 ± 0.115
6	27.974 ± 0.056	23.157 ± 0.351	4.582 ± 0.158	8.675 ± 0.124
7	19.565 ± 0.068	29.779 ± 0.456	3.601 ± 0.205	6.601 ± 0.061
8	33.679 ± 0.243	26.232 ± 0.399	4.965 ± 0.18	9.494 ± 0.128
9	39.885 ± 0.222	22.232 ± 0.483	4.996 ± 0.216	9.446 ± 0.173
10	43.513 ± 0.253	29.913 ± 0.465	4.489 ± 0.209	8.151 ± 0.166
11	31.346 ± 0.451	28.364 ± 0.466	3.912 ± 0.211	7.281 ± 0.134
12	27.910 ± 0.092	29.271 ± 0.808	4.838 ± 0.364	9.042 ± 0.146
13	29.205 ± 0.105	30.117 ± 0.224	4.826 ± 0.101	8.903 ± 0.143
14	29.462 ± 0.081	30.002 ± 0.413	4.815 ± 0.186	9.111 ± 0.081
15	15.897 ± 0.046	21.535 ± 0.251	4.325 ± 0.113	7.918 ± 0.072
16	44.410 ± 0.046	51.814 ± 0.267	5.264 ± 0.121	9.914 ± 0.088
17	40.013 ± 0.114	20.701 ± 0.101	6.061 ± 0.045	11.36 ± 0.085
18	35.910 ± 0.091	24.228 ± 0.447	5.026 ± 0.201	9.276 ± 0.086
19	32.782 ± 0.110	22.253 ± 0.253	5.603 ± 0.114	10.431 ± 0.115
20	36.487 ± 0.078	24.828 ± 0.331	6.329 ± 0.149	11.446 ± 0.130

[a] Names of investigated beach provenances, country of origin, and appropriate altitude are presented in Table 1.
[b] 1FRAP unit = 100 μmol/dm^3 Fe^{2+}.
[c] LP: lipid peroxidation.

Table 3: Correlations between total antioxidant capacity (FRAP), lipid peroxidation (LP), and free proline and soluble proteins contents in beech provenances transplanted in forest Fruška Gora.

Variable	FRAP	LP	Free proline	Soluble proteins
FRAP	1.000000	0.377605	**0.545504**	**0.519771**
LP	0.377605	1.000000	−0.059480	−0.102208
Free proline	**0.545504**	−0.059480	1.000000	**0.957191**
Soluble proteins	**0.519771**	−0.102208	**0.957191**	1.000000

[*] Bolded correlations are significant at $P < 0.05$.

(5.205 μmol/mg protein), and number 12 (4.873 μmol/mg protein). Content of soluble proteins was highest in provenances number 11 (9.842 mg/g) and number 8 (9.689 mg/g). According to our results, provenance number 11, Scharnstein, Mitterndorf, in Austria, had the best adaptability to environmental factors in meadow Debeli Lug due to high FRAP value and free proline and soluble proteins contents.

Significant positive correlations ($P < 0.05$) were observed between examined parameters.

FRAP values were significantly high positively correlated with LP ($r = 0.593202$), free proline ($r = 0.646462$), and soluble proteins contents ($r = 0.672390$). Free proline was significantly high positively correlated with soluble proteins content ($r = 0.997778$). Results obtained by correlation analysis (Table 5) supported our previously presented results (Figure 2). It is also obvious that correlations parameters are much higher in provenances in meadow Debeli Lug from higher altitude, which is the consequence of their better adaption to environmental factors influence. Similar results were obtained by other plant species [41].

Antioxidant capacities of examined beech provenances not only depend on plant but also on the drought adaptation which are closely related to the environmental factors of their natural habitats which was in agreement with statements that physiological and biochemical processes of plants depend on the rapidity, severity, and duration of the drought event [42]. Furthermore, no single method is sufficient because more than one type of antioxidant capacity measurement needs to be performed to take into account the various modes of action of antioxidants [43]. The results suggest that beech provenances originating from the higher altitude (Debeli Lug) have a better drought tolerance due to higher FRAP values and higher correlations between oxidative stress parameters than provenances originating from the low altitude, which is in agreement with results obtained by investigation conducted on two poplar species [44]. It is well known that beech originating from the higher altitude possesses a better drought tolerance and stronger drought adaptation than those beech originating from the low altitude, which can be explained by high and abundant precipitations due to much rainfall at high altitude; the trees at high altitudes may be water-stressed due to wind and ice blasting in the winter time, and colder soils reduce the water uptake of the root system,

Table 4: Total antioxidant capacity (FRAP), lipid peroxidation (LP), and free proline and soluble proteins contents in beech provenances transplanted in meadow Debeli Lug.

		Locality—Debeli Lug		
Provenance No.[a]	FRAP (FRAP units)[b]	LP[c] (mmol MDA/mg protein)	Free proline (μmol/mg protein)	Soluble proteins (mg/g)
1	25.769 ± 0.256	33.557 ± 0.327	3.754 ± 0.402	7.034 ± 0.065
2	27.154 ± 0.146	39.733 ± 0.297	3.485 ± 0.365	6.377 ± 0.103
3	39.372 ± 0.126	35.879 ± 0.45	4.090 ± 0.554	7.534 ± 0.105
4	42.487 ± 0.248	46.982 ± 0.751	3.494 ± 0.924	6.566 ± 0.092
5	52.090 ± 0.238	47.335 ± 0.188	4.490 ± 0.231	8.323 ± 0.151
6	42.128 ± 0.173	67.590 ± 0.612	3.283 ± 0.753	6.143 ± 0.075
7	37.501 ± 0.133	44.697 ± 0.202	4.045 ± 0.248	7.455 ± 0.101
8	43.103 ± 0.189	36.781 ± 0.252	4.111 ± 0.312	7.514 ± 0.564
9	42.769 ± 0.102	47.107 ± 0.119	5.205 ± 0.146	9.689 ± 0.075
10	31.449 ± 0.101	44.156 ± 0.727	3.256 ± 0.894	6.062 ± 0.099
11	58.872 ± 0.114	48.358 ± 0.159	5.277 ± 0.196	9.842 ± 0.218
12	58.128 ± 0.167	58.179 ± 0.289	4.873 ± 0.355	9.135 ± 0.144
13	46.269 ± 0.111	38.868 ± 0.151	4.132 ± 0.186	7.613 ± 0.092
14	34.987 ± 0.071	35.869 ± 0.346	3.606 ± 0.426	6.653 ± 0.053
15	58.513 ± 0.122	61.393 ± 0.369	3.915 ± 0.454	7.357 ± 0.084
16	45.436 ± 0.078	43.943 ± 0.207	4.056 ± 0.255	7.573 ± 0.087
17	20.010 ± 0.182	38.341 ± 0.774	3.446 ± 0.952	6.326 ± 0.073
18	29.705 ± 0.11	45.139 ± 0.187	3.315 ± 0.23	6.161 ± 0.129
19	46.385 ± 0.146	45.601 ± 0.441	4.113 ± 0.541	7.716 ± 0.179
20	32.346 ± 0.135	32.288 ± 0.537	4.245 ± 0.661	7.713 ± 0.073

[a] Names of investigated beach provenances, country of origin, and appropriate altitude are presented in Table 1.
[b] 1FRAP unit = 100 μmol/dm^3 Fe^{2+}.
[c] LP: lipid peroxidation.

Table 5: Correlations between total antioxidant capacity (FRAP), lipid peroxidation (LP), and $Typeequationhere$.free proline and soluble proteins contents in beech provenances transplanted in meadow Debeli Lug.

Variable	FRAP	LP	Free proline	Soluble proteins
FRAP	1.000000	**0.593202**	**0.646462**	**0.672390**
LP	**0.593202**	1.000000	0.082635	0.125785
Free proline	**0.646462**	0.082635	1.000000	**0.997778**
Soluble proteins	**0.672390**	0.125785	**0.997778**	1.000000

[*] Bolded correlations are significant at $P < 0.05$.

and then they possessed better acclimation to drought stress than ones at low altitude [45]. It was shown that although antioxidant protection was important in plants, there were significant differences among the plant species. In addition to antioxidant protection, the higher soluble protein and proline contents have a very important role in the stress resistance of the woody plants [46]. The results also showed that drought adaptations of beech provenances are closely related to the environmental factors. Similar conclusions were obtained by investigations of adaptability of birch (*Betula pendula* Roth) and aspen (*Populus tremula* L.) genotypes to different soil moisture conditions [47].

4. Conclusion

Presented results can contribute to explain differences of beech provenances in response to oxidative stress due to duration of the drought event and their individual altitude. It was proved that efficient antioxidative characteristics and proline accumulation can provide better protection against oxidative stress in leaves under drought stress. It is also established that beech originating from the higher altitude expresses a better drought tolerance than those beech originating from the low altitude. Information on the patterns of biochemical response to environmental stress provides an important tool for the improvement of environmental bioengineering strategies and reforestation programs for European beech.

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