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METABOLIC RESPONSES OF STEPPE FOREST TREES TO ALTIRUDE-ASSOCIATED LOCAL ENVIRONMENTAL CHANGES

SUMMARY

The effect of altitude-associated environmental gradient on leaves metabolic features of *Quercus robur* L. (an oak) and *Fraxinus excelsior* L. (an ash) was investigated in the natural coastal forest at Bellegarde' International Biosphere Reserve in Steppe zone, Ukraine. Decrease in relative humidity contrary to increase in temperature and lighting under the forest canopy were observed on the river steep bank with altitudinal elevation from lower (52 m a.s.l.) to middle (74 m.), and upper (96 m). Responses of tree leaves photosynthetic and antioxidant systems to the environmental local changes were studied by measuring chlorophyll (Chl) content, as well as catalase and peroxidase activities. Decrease in Chl amount in the ash leaves at middle and upper altitudes (17 and 38% compared with lower), along with increase (8% and 13%, respectively) in the oak leaves was found out. Chl content was determined to correlate with light, temperature, and humidity in both leaves of ash (respectively, $r = -0.94$, $r = -0.92$, $r = 0.90$) and oak ($r = 0.95$, $r = 0.93$, $r = -0.90$). Catalase activity grew with increasing altitude in leaves of ash (2 and 2.2 fold compared to lower altitude) and oak (1.2 and 1.4 fold) as well. Contribution of catalase to the total antioxidant enzymes activity enhanced in leaves of both species with increasing altitude. The results confirmed high sensitivity of steppe forest trees even to slight altitude-associated environmental deviations. Data obtained can be used to assess the adaptive potential of woody species to the climate changes aiming towards greater aridity traits and select tree species for planted forest creation as well.

Keywords: Quercus robur *L., Fraxinus* excelsior L., altitude, microclimate, enzymes.

INTRODUCTION

Ecological and economic importance of the forests worldwide necessitates their conservation under climatic changes tending to aridity features enhancement (Bussotti et al., 2015), and determines relevance of trees adaptive capabilities studying. Talbi et al. (2015) suggest the exacerbation of plants

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survival problem with increased drought in numerous areas throughout the world, especially in the arid regions. Bussotti et al. (2015) state that due to increasing temperature and drought southern genotypes are likely to replace forest species in Western and Central Europe. Mokria et al. (2015) agree that namely climatic changes, in particular heat increase, might have contributed to forest dieback in northern Ethiopia. It's noteworthy that El-Hajj et al. (2014) emphasize probable severe consequences of the climatic deviations for the forest ecosystems despite plodding effect and small range of changes.

Natural forest conservation and restoration appears as a significant problem for Ukraine. The percentage of forest land in our country reaches 16.0% (Tkach, 2012). This index is one of the lowest among European countries. It is known that Steppe zone occupies about 40% of Ukrainian territory. The forests distribution is complicated by unfavourable conditions of geographic mismatch for the forest ecosystems. Steppe climate is characterized by the seasonal drought periods accompanied by high temperature and dry winds. Average annual rainfall is 472 mm, while in dry years 250 mm only; the annual amount of evaporation exceeds the precipitation by 2–3 times. Existence of the plant forest communities in the Steppe zone is possible due to geomorphologic diversity which enables formation of special local environmental conditions in the steep rivers banks. The steppe forest ecosystems may be highly sensitive to any environmental deviations under such circumstances. According to Ramirez-Valiente et al. (2015), climate is a major selective force in nature.

Plant responses and adaptation to the environmental conditions are complex biological processes including physiological and biochemical changes (Harfouche et al., 2014; Granda et al., 2014; Parviz, 2016). The mechanism of tree metabolic adaptation to local environmental factors is an important aspect of the overall forest conservation problem. It has been studied intensively during recent years. Thus, Aranda et al. (2015) showed significant physiological differences among six *Fagus sylvatica* populations adapted to specific local water availability. Zadworny et al. (2015) established a strong seasonal variation in nitrogen concentration among roots of oak trees growing in two contrasting soil types. Sperlich et al. (2015) showed differences in photosynthetic potentials and drought-tolerance in sunlit and shaded leaves of four Mediterranean trees. The significant impact of microclimate on transition processes to the generative phase of plant development was described by Bahuguna and Jagadish (2015). Next point is the study of the altitude impact on tree taxa distribution. Rezende et al. (2015) concluded that altitude belongs to major environmental parameters which can be used for developing a forest conservation strategy. The aim of our study - to estimate abiotic factors (local temperature, relative humidity and lighting) impact on photosynthetic and antioxidant systems of coastal forest trees *Quercus robur* L. (an oak) and *Fraxinus excelsior* L. (an ash) associated with the different river slope altitudes.

MATERIAL AND METHODS

Study area

The study was conducted in the territory of Bellegarde' Prisamarsky International Biosphere Reserve located in in the northen part of steppe zone in Dnipropetrovs'k province (47°32'–49°11'N, 33°–33°56'E). Studied areas are located on the southern slope of the Samara river. Observed plots are disposed in right steep bank at lower (52 m a.s.l.), middle (74 m a.s.l.), and upper (96 m a.s.l.) altitude in an old-growth (more than 85 years old) mixed deciduous natural forest. The plant community of the coastal forest is represented by several trees and shrubs with an oak (*Quercus robur* L.) and an ash (*Fraxinus excelsior* L.) dominating along the slope. Both the *Q. robur* and *F. excelsior* are the autochthonous and edificatory species of natural forest. The list of co-dominant species includes a linden (*Tillia cordata Mill*.), two species from *Acer* genus (*Acer platanoides* L. and *Acer campestre* L.), an elm (*Ulmus laevis Pall.)*, and *Corylus avellana* (L.) H. Karst. The forest undergrowth is formed by *Acer platanoides* young trees, with shrub species *Sambucus nigra* L. and *Euonymus europaeus* L.

Data collection

Microclimate data within the studied area were collected during the period of trees vegetation (April–August 2015). Data on air temperature and relative humidity levels under the tree canopy were obtained with the help of a portable weather station equipped by Assmann psychrometer (model 225-5230, Germany). Lighting levels under canopy were measured with a luxmeter (model Y u16, Russia) at the height of 2 m which corresponds to the level of lowest trees branches. Simultaneously, lighting level in open areas at each studied altitude was measured. The leaves of *Quercus robur* and *Fraxinus excelsior* were collected in the mean of May 2015 from 3–5 same-age trees within three groups in each studied area, and frozen.

Data analysis

Chlorophyll content and antioxidant enzyme activities (catalase, guaiacol - peroxidase, and benzidine - peroxidase) were measured with spectrophotometric methods. Chlorophyll content (Chl a, Chl b, and total chlorophyll value) was measured according to the method of Wintermans and De Mots (1965) in the ethanol extracts of tree leaves, and expressed in mcg of chlorophyll per g fresh weight (mcg/g FW).

Antioxidant enzymes activities were determined in the supernatants obtained by centrifugation (15,000 g for 20 min and 4˚ C) of crude extracts (100 mg of fresh leaves homogenized with 0.2 M TRIS-HCl buffer, pH 7.0 contained 0.1% polyvinylpyrrolidone, 250 mM saccharose, and 1 mM $MgCl_2$). Catalase (CAT) activity was evaluated according to Goth (1991) by measurement at 410 nm of optical density of reactive mixture containing 0.2 ml sample, 0.1% H_2O_2, and 4% ammonium molibdate. The result was calculated through the calibration graph and expressed in mM H_2O_2/g FW. Activity of guaiacol-peroxidase (GPOD) was estimated in accordance with Ranieri *et al.* (2001) by detecting guaiacol oxidation at 470 nm in the reactive mixture containing acetate buffer (pH 6.0), 2 mM guaiacol, 0.2 ml sample, and 0.15% H_2O_2. The result was calculated with consideration of the guaiacol molar extinction coefficient, and expressed in mM guaiacol/g FW. Benzidine-peroxidase (BPOD) activity determination was based on the method proposed by Gregory *(*1966). Optical density change was registered for 1 min at 470 nm after adding 1% H_2O_2 to reactive mixture (acetate buffer, pH 6.0, 0.02 mM benzidine and 0.2 ml sample). The result was expressed in optical units/g FW.

All determinations of air temperature, relative humidity, and lighting (under tree canopy and in open areas) were performed in six replicates at each studied site. All determinations of the biochemical parameters characterizing the oak and ash leaves required five replicates. Data represent mean values and standard deviations (±SD).Differences significance was estimated using Student's t-test ($P<0.05$).

RESULTS AND DISCUSSION

The levels of temperature, relative humidity, and lighting under the forest canopy varied with the altitudinal elevation of the coastal slope, as shown in Table 1.

Table 1. Actinometrical and microclimatic conditions under the coastal forest canopy at the different altitudes of the Samara river bank.

Indices, units	Lower slope altitude	Middle slope altitude	Upper slope altitude
Lighting, (Lx)	1745.0±95.3	2190.0±115.4	4154.0±204.7
Lighting under canopy	3.5±0.2	4.3±0.3	8.2±0.7
Lighting in open area, (%)	3.5±0.2	4.3±0.3	8.2±0.7
Air average temperature, (ºC)	25.5±0.4	26.8±0.4	27.7±0.3
Air average relative humidity, (%)	63.60±1.14	59.90±1.48	55.94±1.49

Increase in lighting reached 1.3 fold at the middle altitude and 2.4 fold at upper altitude compared to the lower ($P<0.05$). Air temperature increase as well as relative humidity reduction under the canopy occurred to have gradient character in the course of moving upwards on the slope. The given study defines these local changes of microclimate and lighting as a conventional enhancing aridity trait with increasing altitude. It is possible to see the plants physiological and biochemical responses to the environmental changes.

Total chlorophyll content and ratio of chlorophyll forms (Chl a/Chl b) varied depending on the altitude-associated microclimatic conditions both in the oak and ash leaves (Table 2). We observed that an increase in temperature and lighting along with decrease in relative humidity upward the slope accompanied by the growth of total chlorophyll content in *Q. robur* leaves at the middle and upper altitudes compared to the lower (respectively, 8% and 13%, $P<0.05$).

Table 2. Effect of altitude associated environmental gradient on chlorophyll content in leaves of coastal forest trees.

Species	Chl a content (mcg/g FW)	Chl b content (mcg/g FW)	Total Chl content (mcg/g FW)	Ratio Chla/Chlb
Lower slope altitude				
Quercus robur L.	1.95±0.06	0.78±0.02	2.73±0.06	2.50
Fraxinus excelsior L.	2.30±0.08	1.29±0.04	3.59±0.08	1.77
Middle slope altitude				
Quercus robur L.	2.03±0.06	0.92±0.05	2.95±0.06	2.21
Fraxinus excelsior L.	2.15±0.08	0.91±0.05	3.06±0.08	2.38
Upper slope altitude				
Quercus robur L.	2.11±0.07	0.97±0.03	3.08±0.07	2.18
Fraxinus excelsior L.	1.85±0.07	0.75±0.03	2.60±0.07	2.46

At the same time, reduction in chlorophyll amount (17% and 38% at the middle and upper altitudes compared to the lower, $P<0.05$) was found in leaves of F. excelsior. The results obtained are consistent with the notion (Ramirez-Valiente et al, 2015) that photosynthesis is one of most sensitive process to the environmental stresses. In particular, intensity of photosynthetic pigments biosynthetic pathway depends on lighting and moisture. In oak leaves, accumulation both of chlorophyll a and chlorophyll b contributed to the increase in total chlorophyll amount with altitudinal elevation. However, decrease in the ratio Chl a/Chl b indicates the heightened Chl b accumulation. In contrast, more significant reduction of chlorophyll b content was observed in ash leaves at the middle and upper altitudes, thereby increasing the ratio Chl a/Chl b. According to Caudle et al. (2014), high Chl a/Chl b ratio is an index of plant adaptation to drought. Thus, we assumed that the Quercus robur trees showed higher adaptability to relative humidity reduction, as well as complex environmental changes tending to enhance the aridity traits uphill the slope. Correlation analysis revealed strong positive coefficients between changes of Q. robur leaves total Chl content and temperature ($r = 0.92$) together with lighting ($r = 0.86$), whereas the interaction with relative humidity changes was negative ($r = –0.86$). On the contrary, leaves of F. excelsior showed a positive relationship between humidity and Chl content ($r = 0.92$), while correlation was getting negative in the course of temperature ($r = –0.89$) and lighting ($r = –0.90$) changes uphill. Hereof, the rate of Chl biosynthesis in oak leaves was stimulated by an increase in temperature and light despite the decrease in relative humidity; at the same time, Chl accumulation in ash leaves was oppressed due to environmental changes. These conclusions resonate with data by Caudle et al. (2014), according to which drought-tolerant species are able to maintain high intensity of photosynthesis and protect photosystem II during dry periods. In addition, our results coincide with data by Rajsnerova et al. (2015) on significant growth of the total Chl content in the lower leaves of Fagus sylvatica canopy just at the upper altitude and the highest light intensity.

The different changes in catalase and peroxidase activity followed the altitude- associated modifications of temperature, lighting, and relative humidity levels both in the leaves of *Q. robur and F. excelsior* (Table 3).

Table 3. Effect of altitude-associated environmental gradient on the antioxidant enzymes activity in leaves of coastal forest trees.

Species	GPOD activity	BPOD activity	CAT activity
	(mM guaiacol/g FW)	(optical units/g FW)	(mM H_2O_2/ g FW)
Lower slope altitude			
Quercus robur L.	0.19±0.02	6.07±0.17	13.22±0.61
Fraxinus excelsior L.	9.91±0.14	1.52±0.06	1.42±0.29
Middle slope altitude			
Quercus robur L.	0.26±0.02	4.36±0.20	15.53±1.72
Fraxinus excelsior L.	9.61±0.11	0.99±0.06	2.90±0.19
Upper slope altitude			
Quercus robur L.	0.09±0.02	1.64±0.11	17.91±1.44
Fraxinus excelsior L.	3.41±0.09	1.15±0.04	3.11±0.29

Significant decrease in BPOD activity was observed in oak leaves at the middle and upper altitudes compared to lower (1.4 and 3.7 folds respectively, $P<0.05$), while activity of GPOD tended to enhance at the middle altitude and decrease at the upper (1.3 and 2.2 folds respectively, $P<0.05$). In ash leaves decrease in BPOD activity was insignificant, whereas GPOD activity declined 2.9 folds ($P<0.05$) at the upper altitude compared to lower. Halliwell (2006) proclaims plant peroxidase functions to be associated with eliminating the excessive hydrogen peroxide accumulated during various physiological processes. Therefore, the results obtained indicate significant metabolic adjustment in oak and ash leaves in the course of slope altitude elevation and enhancing aridity. Probably, changes may affect the accumulation of phenols and sugars, since Allison and Schultz (2004) emphasized the peroxidases appear to play the important role in these metabolic pathways.

The catalase activity at the middle and upper altitudes exceeded the lower level both in the oak (1.2 and 1.4 folds respectively, $P<0.05$), and ash of leaves (respectively 2 and 2.2 folds, $P<0.05$). Together, the results showed increase in catalase proportion in the total antioxidant activity of tree leaves with growing altitude. This proportion in oak leaves varied from 68% at lower altitude to 77% at the middle and 91% at the upper. The catalase contribution in the leaves of ash was 11%, 22%, and 41% of total antioxidant activity respectively at the lower, middle and upper altitudes. Strong positive correlation between the catalase activity and lighting together with temperature was revealed in the leaves of oak ($r = 0.83$ and $r = 0.93$ respectively) and ash ($r = 0.78$ and $r = 0.82$). Thus, our data are consistent with the conclusion of Mhamdi et al. (2010) about high plant catalases sensitivity to light. In the whole, the above data showed a substantial growing catalase involvement in the antioxidant processes in leaves of .Q. robur and F. excelsior with increasing altitude and enhancing aridity traits. This conclusion is consistent with Queval's et al. (2007) opinion on the important plant catalase role in hydrogen peroxide elimination during photosynthesis as well as photorespiration stimulated by solar radiation and high temperature. Besides, the results obtained coincide with data by Mhamdi et al. (2010) that catalases are highly activated enzymes. It is an integral part of plant defense system. So, enchanting antioxidant protective capacity associated with catalase activation was revealed both in oak and ash leaves with increasing altitude of the slope and enhancing aridity. Comparing results of the our study with data by Rajsnerova et al. (2015) on the beech leaves metabolic changes due to significant altitude difference (400–1100 m), it is reasonable to mention high sensitivity of Quercus robur and Fraxinus excelsior leaves to the environmental changes, associated with smallest altitudinal elevation.

CONCLUSIONS

Results of present study confirmed the hypothesis of a high sensitivity of Quercus robur L. and Fraxinus excelsior L. metabolic processes to even small environmental differences with increasing altitude. Decrease in relative humidity along with increase in temperature and lighting under the forest canopy were assessed as a conventional increase in aridity up the slope. Significant catalase activation together with increasing enzyme contribution to the total antioxidant capacity was common trait both in oak and ash leaves. Species - determined differences in the metabolic changes were manifested in the variations of photosynthetic process. Accumulation of chlorophyll in oak leaves was stimulated by an increase in temperature and light in spite of the decrease in relative humidity; and it declined in ash leaves due to environmental changes. The results of study may be useful for assessing adaptive capacity of woody species to increased aridity, and species selection for further creation of forest plantations in the Steppe zone.

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