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VARIABILITY OF LEAF ANATOMICAL CHARACTERISTICS IN PEDUNCULATE OAK GENOTYPES (*Quercus robur* L.)

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ABSTRACT. The objective of this study was to determine genotype variability of leaf structural characteristics: leaf thickness, adaxial and abaxial epidermal cell dimensions (height and width), cuticle thickness of the adaxial leaf surface, the thickness of palisade and spongy tissues and dimensions of their cells, as well as height and width of main vein and its vascular bundle and vessel diameter. Leaves were sampled from seventeen pedunculate oak genotypes, originating from the clonal plantation Banov Brod (Srem, the Vojvodina Province). The results suggest that estimated variations of studied leaf characteristics were slight, but statistically significant. The highest variability was estimated for the main vein vascular bundle dimensions, and somewhat lower for the width of abaxial epidermis cells (14.52%) and the main vein (14.68%). The participation of epidermis in leaf lamina structure ranged between 14.0 and 19.2 %, of mesophyll from 80.8 to 86.0 %, and of palisade and spongy parenchma from 43.9 to 54.9 and from 30.5 to 41.8%, respectively. The smallest epidermal cells were found in genotype 25. Genotype 35 had the thickest, and genotype 22 the thinnest cuticle. The highest palisade tissue cells (57.3 μm) were found in genotype 16, and the shortest in genotype 6. The highest main vein was found in genotype 5, which was characterized also by the highest vascular bundle and the greatest vessel diameter. The lowest vessel dimensions were found in genotype 25. These quantitative differences between studied parameters are the consequence of interaction of certain genotype and common environmental conditions for all trees.

KEY WORDS: Leaf anatomy, *Quercus robur*

INTRODUCTION

Genus *Quercus*, represented by deciduous and evergreen trees and shrubs, belongs to the *Fagaceae* family. In our region, this genus is represented by several species. Among them, pedunculate oak (*Quercus robur* L.) is one of the most important forest species. Forests of pedunculate oak are the most valuable forests in Europe from the economic aspect (Orlović et al., 2000).

Leaf features, beside the influence of genetic information, greatly depend on environmental conditions. Leaves are highly sensitive organs of a tree, as they are continuously subject to ecological factors as well as to phonological cycles and growth rhythms (Bussoti et al., 2000), and these occurrences involve visual symptoms and physiological/ultrastructural changes. Numerous studies have shown that various water, light, temperature, and CO_2 regimes can influence leaf morphology, structure and physiology in various tree species. To our knowledge, a small number of authors studied the within-species variability of the leaf structure, without involvement of various ecological factors (Orlović et al., 1998; Mediavilla et al., 2001). According to Ceulemans et al. (1984) leaf anatomy and internal leaf organization have an important impact on gas exchange, especially on photosynthesis. Considering leaves as the main photosynthetic organs of trees, their structure is important from the aspect of biomass production.

In the present work, leaf anatomical characteristics were studied in seventeen pedunculate oak genotypes, grown under the same environmental conditions. The thickness of the leaf blade and its tissues (epidermis, spongy and palisade parenchyma), as well as the cells size, were determined to explore genotype variability.

The leaf structure characteristics could influence biomass production in tree species. For example, while studying poplar clones, Orlović et al. (1994) found the positive correlation between leaf anatomy and organic matter production. Also, Reich et al. (1997) found the connection between leaf photosynthetic capacity and plant potential primary production. Hence, parameters like this should be taken into consideration in the selection and breeding of tree species.

MATERIAL AND METHODS

Plant material

Leaf samples were taken from 20-year-old trees, originating from the clonal seed orchard Banov Brod, situated along the left bank of the river Sava (44°55′ N, 19° 23′ E). It was established by grafting, formed of 85 *Q. robur* genotypes. To obtain variability of leaf structure, seventeen genotypes were chosen: 4, 5, 6, 16, 18, 20, 21, 22, 25, 28, 29, 30, 33, 35, 38, 40, and 85. Aimed to reduce the within-tree variability, one branch was harvested from the middle of the crown of each genotype chosen. Branches were transported to the laboratory in closed plastic bags. For each genotype, five leaves were randomly chosen. Only fully expanded, undamaged leaves without signs of scarring, disease, or herbivory were used for examination.

Leaf structure

Five mature leaves of each genotype were used for lamina thickness and structure investigations. Till use, leaves were fixed in 50 % ethyl alcohol. For each leaf, three cross sections of the lamina middle part were made, using freezing microtome. For each of them, measurements of leaf thickness, adaxial (upper) and abaxial (lower) epidermal cell dimensions (height and width), cuticle thickness of the adaxial leaf surface, the thickness of palisade and spongy tissues and dimensions of their cells, as well as height and width of main vein and its vascular bundle and vessel diameter, were made. Microscopic measurements of temporary preparations were performed using a micrometric ocular inserted into an "Olympus" light microscope. Also, the percentage of individual tissues in leaf total thickness was calculated.

Statistical analyses

The data were subjected to various statistical analyses including calculation of parameter means, LSD test, and coefficients of variation. The comparison of genotypes was done by Duncan's test at $\alpha=0.05$ significance level. The mean values of the parameters were ranked and marked with letters. Values with the same letter did not differ significantly.

RESULTS

Leaf structure

Anatomically, pedunculate oak leaves are dorsiventral. Their epidermis is single-layered, and adaxial has a relatively thick cuticle. In all genotypes, only solitary eglandular trichomes were observed on the adaxial leaf surface, while both solitary eglandular and uniseriate glandular hairs were present on the abaxial surface (Nikolić et al., 2003). The mesophyll is differentiated into palisade and spongy tissues. The palisade parenchyma consists of elongated cells, at the right angle below the adaxial epidermis, arranged in 1-3 layers. The spongy tissue is composed of 2-4 layers of cells, positioned at the right angle with the epidermis. The main vein is almost round, abaxially more conspicuous, while adaxial smaller conical prominence could be seen. Colenchyma is arranged subepidermally, followed by a few layers of parenchyma cells. In the vascular bundle, continuous (or almost continuous) ring of xylem is surrounded by phloem. A few layers of sclerenchyma fibers occur along the phloem.

Leaf anatomy variables (the thickness of leaf blade, epidermis, mesophyll, palisade and spongy parenchyma) are shown in Table 1. Leaf thickness, measured among veins, ranged between 132.1 and 188.7 µm. The thickness of individual tissues (expressed as % of leaf thickness) varied between genotypes. The participation of epidermis in leaf lamina structure ranged between 14.0 and 19.2 %, of mesophyll from 80.8 to 86.0 %, and of palisade and spongy parenchma from 43.9 to 54.9 and from 30.5 to 41.8%, respectively.

Leaf cells size

The dimensions of leaf cells are summarized in Table 2. The adaxial epidermis cells had larger width than height. Their width varied between 21.8 (genotype 25) to 27.3 µm (genotype 22), and height from 18.3 (genotype 25) to 23.3 µm (genotype 30). The highest palisade tissue cells (57.3 µm) were found in genotype 16, and the shortest in genotype 6. Cell width was between 21.8 and 27.3 µm. The spongy tissue cells height varied from 12.6 to 17.0 µm; the largest width was recorded in genotypes 6, 29, 30, 35, and 40.

Parameters of the main vein

Main vein anatomical characteristics are shown in Table 3. On average, the main vein width (910.2 µm) was greater than height (879.4 µm). But, in some individual genotypes, the oposite relation was found. Values estimated for main vein height and width varied in a wide range: from 728 to 1011 µm, and from 728 to 1040 µm, respectively.

The results obtained for the main vein vascular bundle dimensions showed that their width was greater than height in all genotypes studied. Values varied between 501.8 and 692.9 µm for height, and 566.8 and 806.0 µm for width. In contrast to the main vein and its vascular bundle dimensions, vessel height was greater than width in all genotypes. Vessel height ranged from 23.5 to 36.6 µm, and width from 17.4 do 27.7 µm. The lowest vessel dimensions were found in genotype 25.

DISCUSSION

The results presented above suggest that estimated intraspecific variations of studied pedunculate oak leaf structural characteristics were slight, but statistically significant. The highest variability among genotypes was estimated for the main vein vascular bundle dimensions (Table 3). Somewhat lower variability was obtained for the width of abaxial epidermis cells (14.52%) and the main vein (14.68%).

Genotype divergence in leaf blade thickness (CV=11.49%) arrised from variations in individual tissues thickness. Genotype 30 developed the thickest, while genotype 4 the thinnest leaves. The values of leaf lamina thickness in our investigation were higher then Castro-Diez et al. (2000) obtained for pedunculate oak and other *Quercus* species. The thickest mesophyll was found in genotypes 5, 16, and 18, while the thinnest in genotypes 4, 21, and 38. On average, in the total leaf blade thickness mesophyll participated with 84%, while palisade and spongy parenchyma with 49 and 34 %, respectively (Table 1). Our results for palisade parenchyma were higher than those published by Valladares et al. (2002). These authors found no significant differences in palisade layer thickness in *Q. robur* seedlings exposed to different light environments. Previous studies of leaf thickness and structure also showed the variability between genotypes (clones) in other tree species (Orlović et al., 1998). The palisade parenchyma is the most important tissue from the aspect of organic matter productivity. The positive correlation between spongy and palisade tissues structure and biomass production has been reported previously by Orlović (1993). Mediavilla et al. (2001) investigated the internal leaf anatomy in 6 woody deciduous and evergreen species with different leaf life spans. They found that oak species with a deciduous habit were characterized by high percentage of palisade mesophyll. Also, our results obtained for mesophyll and spongy tissue are comparable with other deciduous oak species (Mediavilla et al., 2001).

The size of photosynthetic tissue cells is also important for biomass production, because the inner photosynthetically active area size increases with the cells size decrease. Considering all genotypes, the dimensions of palisade and spongy cells were 45.2 x 25.1 and 14.9 x 11.9 µm, respectively. The palisade cells of the genotypes investigated were more variable in height than in width. This is in accordance with previous studies (Orlović et al., 2004) where variability of anatomical and physiological traits were studied.

The participation of epidermis in leaf blade total thickness was the lowest in genotype 16, and the highest in genotype 38. Our results for epidermis thickness are comparable with previous investigations (Valladares et al., 2002), where intraspecific variability of pedunculate oak leaf anatomy in different light conditions was studied. Some differences between genotypes were observed in adaxial cuticle thickness and in abaxial epidermis cells size. The smallest epidermal cells were found in genotype 25. On average, abaxial epidermis cells were smaller than adaxial. Genotype 35 had the thickest, and genotype 22 the thinnest cuticle. Considering all genotypes, the adaxial cuticle thickness was greater than Uzunova et al. (1997) reported for pedunculate oak. Also, the cuticle thickness of studied genotypes was higher than Mediavilla et al. (2001) found in other deciduous oak species. According to Koike (1988) who studied leaf structure of deciduous broad-leaved trees, species with a long leaf life span have a high percentage of cuticle.

Among all parameters studied, the greatest genotype divergence was obtained for vascular bundle dimensions (Table 3). The highest main vein was found in genotype 5, which was characterized also by the highest vascular bundle and the greatest vessel diameter. Also, in our previous research on acorn morphological traits (Nikolić, Orlović, 2002), it was found that genotype 5 produced acorns with the highest length and weight among all studied genotypes. These findings are consistent with investigations of Kebede et al. (1992), who found the significance of structural and functional organization of the vascular tissue from the aspect of assimilate transport and final yield.

Results presented above suggest that *Q. robur* genotypes did not exhibit large divergence regarding the leaf traits studied. These quantitative differences, illustrating intraspecies variability of parameters studied, are the consequence of interaction of certain genotype and common environmental conditions for all trees. According to Castro-Diez et al. (1997), the within-species variability of leaf morphology and structure may improve plant performance, allowing species to maintain their fitness in a wide range of environmental conditions and resource availability. Additionally, considering common environmental conditions for all studied plants, these results could provide information on the degree of genetic control of these parameters.

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Table 1. *Leaf anatomy variables for seventeen Q. robur genotypes: leaf blade thickness (μm), and epidermis, mesophyll, palisade and spongy parenchyma thickness (% of leaf thickness). Letters after the mean values denote differences among genotypes (Duncan's test, p<0.05).*

Genotype	Thickness among veins	Percentage in leaf total thickness (%)			
		Epidermis	Mesophyll		
				Palisade tissue	Spongy parenchyma
4	139 [e]	18.6	81.4	46.7	34.7
5	175 [abcd]	14.2	85.8	53.6	32.2
6	153 [de]	15.7	84.3	45.0	39.3
16	186 [ab]	14.0	86.0	54.9	31.1
18	163 [bcde]	14.3	85.7	43.9	41.8
20	150 [de]	17.8	82.2	50.0	32.2
21	154 [cde]	18.6	81.4	44.4	37.0
22	162 [bcde]	15.6	84.4	48.3	36.1
25	155 [cde]	15.9	84.1	52.4	31.7
28	171 [bcd]	18.3	81.7	48.1	33.6
29	164 [bcde]	16.3	83.7	46.4	37.3
30	199 [a]	15.1	84.9	53.5	31.4
33	168 [bcde]	14.9	85.1	54.6	30.5
35	169 [bcd]	16.6	83.4	50.7	32.7
38	160 [bcde]	19.2	80.8	48.3	32.5
40	181 [abc]	15.3	84.7	52.9	31.8
85	147 [de]	18.1	81.9	48.2	33.7
Average	*165*	*16.4*	*83.6*	*49.5*	*34.1*

Table 2. *Leaf cells size (μm) in seventeen Q. robur genotypes. Letters after the mean values denote differences among genotypes (Duncan's test, p<0.05).*

Genotype	Palisade cells		Spongy cells		Adaxial epidermis cells		Abaxial epidermis cells		Cuticle thickness
	Height	Width	Height	Width	Height	Width	Height	Width	(adaxial)
4	40.7^{def}	24.2^{abc}	15.2^{bc}	11.0^{bc}	18.5^{d}	24.2^{abc}	13.0^{cd}	18.8^{abc}	3.8^{bcd}
5	49.6^{bc}	22.7^{bc}	17.0^{a}	11.4^{abc}	20.1^{abcd}	22.7^{bc}	13.7^{cd}	20.8^{a}	3.4^{cde}
6	38.3^{f}	25.4^{abc}	15.3^{abc}	12.5^{a}	18.7^{d}	25.4^{abc}	14.1^{bcd}	17.7^{abc}	3.2^{de}
16	57.3^{a}	26.8 ^{a}	14.8^{bc}	11.0^{bc}	20.7^{abcd}	26.8^{a}	14.8^{bc}	19.0^{abc}	3.9^{bc}
18	42.5^{cdef}	23.5^{abc}	15.5^{ab}	11.7^{abc}	18.8^{cd}	23.5^{abc}	14.5^{bcd}	19.4^{abc}	3.4^{cde}
20	48.8^{bcd}	26.2^{ab}	15.9^{ab}	12.1^{abc}	20.0^{bcd}	26.2^{ab}	14.5^{bcd}	17.1^{abcd}	4.0^{bc}
21	40.6 ^{def}	25.4^{abc}	15.9^{ab}	12.0^{abc}	20.0^{bcd}	25.4^{abc}	14.1^{bcd}	19.7^{ab}	3.8^{bcd}
22	46.4^{bcdef}	27.3^{a}	15.0^{bc}	12.1^{abc}	21.0^{abcd}	27.3^{a}	13.8^{cd}	17.9^{abc}	3.1^{e}
25	47.3^{bcde}	21.8 ^{c}	13.5^{cd}	11.3^{abc}	18.3^{d}	21.8^{c}	12.7^{d}	13.5^{d}	3.8^{bcd}
28	45.4^{bcdef}	25.7^{ab}	14.6^{bc}	12.0^{abc}	21.4^{abcd}	25.7^{ab}	17.2^{a}	18.2^{abc}	4.2^{ab}
29	39.1^{ef}	23.6^{abc}	12.6^{d}	12.6^{a}	20.3^{abcd}	23.6^{abc}	12.9^{cd}	16.5^{bcd}	3.8^{bcd}
30	52.1^{ab}	26.0^{ab}	14.7^{bc}	12.6^{a}	23.3^{a}	26.0^{ab}	16.1^{ab}	19.1^{abc}	4.5^{a}
33	41.7^{cdef}	24.7^{abc}	14.9^{bc}	12.4^{abc}	20.0^{bcd}	24.7^{abc}	14.1^{bcd}	16.9^{abcd}	3.4^{cde}
35	43.5^{cdef}	27.2^{a}	14.6^{bc}	12.5^{a}	22.3^{ab}	27.2^{a}	13.3^{cd}	18.7^{abc}	3.8^{bcd}
38	43.4^{cdef}	24.8^{abc}	14.6^{bc}	11.8^{abc}	22.1^{abc}	24.8^{abc}	14.2^{bcd}	16.7^{bcd}	3.8^{bcd}
40	49.4^{bc}	25.7^{ab}	14.9^{bc}	12.6^{a}	20.3^{abcd}	25.7^{ab}	13.6^{cd}	15.7^{cd}	3.8^{bcd}
85	42.3^{cdef}	25.6^{ab}	14.7^{bc}	10.9^{c}	18.5^{d}	25.6^{ab}	13.9^{cd}	16.0^{bcd}	3.8^{bcd}
Average	*45.2*	*25.1*	*14.9*	*11.9*	*20.2*	*25.1*	*14.2*	*17.7*	*3.7*
CV%	12.13	7.73	8.18	8.31	10.76	10.03	9.83	14.52	10.89

Table 3. *Main vein anatomical characteristics (μm) for seventeen Q. robur genotypes: the dimensions of the main vein and its vascular bundle, and vessel diameter (μm). Letters after the mean values denote differences among genotypes (Duncan's test, p<0.05).*

Genotype	Main vein dimensions		Vascular bundle		Vessel diameter	
	Height	Width	Height	Width	Height	Width
4	728^{e}	728^{c}	502^{c}	614^{b}	29.4^{b}	22.0^{bcd}
5	1011^{a}	971^{ab}	693^{a}	745^{ab}	33.1^{a}	27.7^{a}
6	881^{abcde}	868^{abc}	568^{abc}	628^{b}	27.7^{b}	21.5^{bcde}
16	779de	881^{abc}	511^{bc}	575^{b}	25.4^{bc}	19.0^{def}
18	728^{e}	826^{bc}	506^{c}	567^{b}	26.7^{bc}	18.6^{ef}
20	818^{cde}	894^{abc}	521^{bc}	594^{b}	28.4^{b}	22.0^{bcd}
21	941^{abcd}	962^{ab}	619^{abc}	655^{ab}	36.6^{a}	23.2^{bc}
22	876^{abcde}	978^{ab}	582^{abc}	733^{ab}	28.8^{b}	23.6^{b}
25	842^{bcde}	827^{bc}	543^{bc}	608^{b}	23.5^{c}	17.4^{f}
28	840^{bcde}	894^{abc}	556^{abc}	676^{ab}	25.7^{bc}	21.6^{bcde}
29	972^{abc}	1040^{a}	650^{ab}	806^{a}	28.2^{b}	21.7^{bcde}
30	949^{abc}	952^{ab}	608^{abc}	684^{ab}	28.3^{b}	19.6^{def}
33	840^{bcde}	920^{abc}	567^{abc}	671^{ab}	26.5^{bc}	20.5^{cde}
35	936^{abcd}	980^{ab}	601^{abc}	712^{ab}	26.8^{bc}	20.7^{bcde}
38	887^{abcde}	946^{ab}	624^{abc}	718^{ab}	25.8^{bc}	20.1^{cdef}
40	998^{ab}	946^{ab}	614^{abc}	658^{ab}	27.4^{bc}	19.9^{def}
85	923^{abcd}	858^{abc}	582^{abc}	624^{b}	27.5^{bc}	19.4^{def}
Average	879	910	579	663	28.0	21.1
CV%	12.78	14.68	16.07	17.56	10.00	9.99