

## Anatomical analysis of red Juniper leaf (*Juniperus oxycedrus*) taken from Kopaonik Mountain, Serbia

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**Abstract:** In this study, the anatomical characteristics of *Juniperus oxycedrus* (Cupressaceae) leaves from Kopaonik Mountain (Serbia) are analysed. The level of distinction between leaves taken from various altitudes (420-1420 m) was determined by analysing anatomical characters. The clear correlation between increase in altitude and change in anatomical characters of the leaves was not established by descriptive analysis. The leaves collected from plants growing at 620 m had the most prominent xeromorphic characteristics (thick cuticles and thick epidermis walls). The discriminant canonical analysis also showed a clear distinction between red common juniper from 420 m and the samples taken from different altitudes.

**Key words:** *Juniperus oxycedrus*, leaf, anatomy, characters

### Introduction

Red common juniper, *Juniperus oxycedrus* L. (Cupressaceae), inhabits the Mediterranean area, reaching Crimea, the Caucasus, and northern Iran. In Serbia, it is widespread in the central and south-western areas, as well as in Kosovo.

In studying the anatomical characteristics of common juniper, cross sections of the leaves were measured. The epidermis of common juniper was mostly of one layer and consisted of epidermic cells covered with cuticle. Layers of the mesophyll consisted of palisade and spongy parenchyma. Palisade cells, which make palisade tissue, were present in a few layers, mostly on the adaxial epidermis of the leaf. Spongy parenchyma was made of round cells, and the intercellulars could be observed in between. The secretory canal was very well developed and

located on the abaxial epidermis of the leaf, as well as multiple-layer collenchyma cells. Stomas were located on the adaxial epidermis of the leaf. The xylem and phloem elements could be observed in the central part of the leaf.

The habitat of red common juniper is serpentinites in xerothermic oak forests and black pine forests (Jovanović, 1992). Ecological diversity in the regions and terrains that it inhabits, as well as its tertiary age, have conditioned its significant variability, which is the result of adaptation to specific environment conditions (Matović, 1997).

Most research done with red common juniper is related to the study of the essential oils in its leaves (Matović, 1997; Adams, 1998, 2000). There are not many papers that involve the study of morphological and anatomical characteristics of leaves (Vasić et

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al., 2008). Our research was rather difficult because a lack of adequate literature data on anatomical characteristics of red common juniper leaf from other localities made it difficult to compare our results.

In the current research, we focused on the study of variation in anatomical characters of leaves collected from various altitudes. The aim of the paper was to establish whether, and to what extent, anatomical characters of red common juniper leaf vary as a result of acclimatisation to the microclimate changes conditioned by an increase in altitude.

### Material and methods

The collected material was fixed in a formalin-alcohol (50:50) solution. Next, it was conducted through a series of alcohols of increasing concentrations due to dehydration. Unused conserved plant materials, as well as microscopic preparations, are located in the herbarium of the University of Pristina's Department of Biology at the Faculty of Science (head office temporarily located in Kosovska Mitrovica). Anatomical analyses of the needles were prepared by the standard method for light microscopy. Cross-sections of the needles were cut (up to 15 µm thick) on a manual microtome. Sections were then

coloured by safranin and light green, and all slides were mounted in Canada balsam after dehydration (Jensen, 1962; Blaženčić, 1990). Measuring of the dimensions of anatomical characters was performed by micrometre scale. For each anatomical character, 100 preparations and 16 anatomical characters were analysed.

Anatomical characteristics of *Juniperus oxycedrus* leaf were investigated in material collected from 2001 to 2004. The plant material used for comparative anatomical analysis was collected in the southwestern part of Kopaonik Mountain (Serbia) and came from altitudes of 420, 620, 820, 1020, 1220, and 1420 m (Figure 1).

The anatomical characters measured were leaf width, leaf thickness, front cuticle thickness, back side cuticle thickness, thickness of outer wall of front epidermis, thickness of inner wall of front epidermis, front epidermis width, back side epidermis width, front epidermis height, back side epidermis height, wider diameter of conductive vessel, thinner diameter of conductive vessel, thickness of inner wall of back side epidermis, thickness of outer wall of back side epidermis, resin canal width, and resin canal length. Morphological characters were leaf length, leaf width, and leaf thickness.



Figure 1. Geographic position of Kopaonik Mountain (Serbia), where materials were collected.

The obtained measured values of anatomical characters were processed with the programme package Statistics 6.0 (StatSoft, 2001). Descriptive statistical analysis was made for all samples, by which mean values and range were calculated. In order to determine variability, as well as the degree of statistical difference between mean values of anatomical characters of red common juniper leaf from various altitudes, discriminative canonical analysis (DCA) was carried out. On the basis of calculated values of Mahalanobis distances, unweighted pair-group average (UPGMA) cluster analysis was also performed. With this method, the distance between 2 clusters is calculated as the average distance between all pairs of objects in the 2 different clusters. This method is very efficient when the objects form natural, distinct clumps; however, it performs equally well with elongated, chain-type clusters. Note that in their book, Sneath & Sokal (1973) introduced the abbreviation UPGMA to refer to this method as unweighted pair-group method using arithmetic averages.

## Results and discussion

In the cross sections of red common juniper leaf collected from altitudes of 620, 1020, and 1420 m, 3 main layers were observed: adaxial epidermis, mesophyll, and abaxial epidermis (Figure 2). The adaxial epidermis and the abaxial epidermis of the leaf were well developed and formed the outer and inner wall. The adaxial and abaxial epidermis sides were covered with cuticle. Inside the epidermis are stomata that are retracted in comparison with the surface of the epidermis. Layers of mesophyll are also well developed and are made of spongy and palisade tissue. Palisade parenchyma cells are of cylindrical shape. Spongy parenchyma is made of ball-shaped cells with intercellulars between them. The resin canal is well developed and is located on the leaf abaxial side. On leaf edges, the collenchyma cells, which are distributed in several layers, can be observed. The main vessel consists of one conductive vessel of collateral type with clearly differentiated xylem cells and phloem cells (Figure 2).

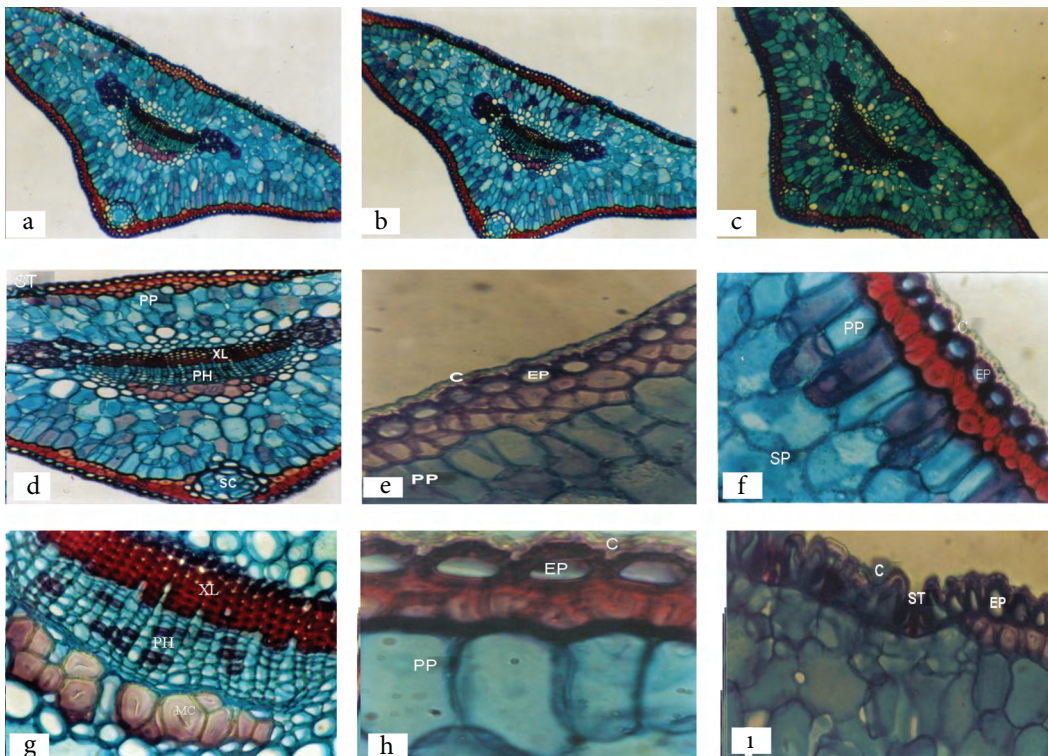


Figure 2. Cross sections of common juniper (*Juniperus oxycedrus*) leaf ( $\mu\text{m}$ ) from altitudes of 620 m (a, d, g), 1020 m (b, e, h), and 1420 m (c, f, i). C: cuticle; EP: epidermis; MC: mechanical cells; PP: palisade parenchyma; XL: xylem; PF: phloem; ST: stomas; SC: secretory canal; SP: spongy parenchyma.



Comparing *J. oxycedrus* from different altitudes, it can be determined that the epidermis and cuticle at lower altitudes (Figure 2) are thin and that the thickness of the epidermis and cuticle increase with increasing altitude (Figure 2). Stomata at an altitude of 1020 m are closer to the surface, while at 1420 m they are shaped and covered with a thick cuticle (Figure 2). At lower altitudes (620 m), there are few mechanical cells around the vascular bundle, and with increasing altitude the mechanical cells become thicker and multilayered (Figure 2).

The results of descriptive statistics of anatomical characters of red common juniper leaf have shown that mean values change with a change in altitude; however, there is no obvious regularity related to these changes. Leaves at 620 m (2.80  $\mu\text{m}$ ) have the thickest cuticle on the leaf front, while the cuticle is thinnest on both leaf sides in leaves collected at 1020 m (1.18-1.24  $\mu\text{m}$ ).

Regarding the epidermis characters, descriptive statistics have shown that the thickness of the outer and inner walls of the epidermis on the adaxial and abaxial side of the leaf gradually increases with an increase in altitude. Leaves at 620 m (2.15-2.31  $\mu\text{m}$ ) have the thickest outer epidermis wall on the adaxial and abaxial side of the leaf. Mean values for wider diameters of conductive vessels and thinner diameters of conductive vessels decrease with an increase in altitude; however, at the maximal height (1420 m), their values increase rapidly, reaching its maximum at (-48.90)-125.45  $\mu\text{m}$ .

In analysing the results of the descriptive statistics, it can be concluded that the xeromorphic properties of the leaf (thick cuticle and thick epidermis walls) are the most prominent on plants at 620 m. The red common juniper, which inhabits lower and higher altitudes, is also acclimatised to the ecological conditions of the habitat, but in a different manner from the common juniper at 620 m. As an evergreen sclerophyte, red common juniper is exposed to difficulty in accessing water in the leaves during winter, as well as in spring, due to frozen ground. To overcome this problem, a thick cuticle and thick epidermis wall are of major importance. At higher altitudes it is more difficult for plants to subsist, and they do not manage to develop sufficiently strong defensive mechanisms; for this reason, they need thinner walls in their epidermis

cells and a thinner cuticle (Güvenç et al., 2011). Therefore, the xeromorphic properties that exist in this type of common juniper are properties typical not only for other types of common juniper (Vasić et al., 2008), but for other coniferous trees and some ligneous plants, as well (Carr & Carr, 1977; Burrows, 1987; Castro et al., 1997; Mastroberti & Jorge, 2003; Kishchenko & Vantenkova, 2007; Şen et al., 2011).

Several studies have pointed out that water deficit and high light intensity are the main factors causing a lower ratio of leaf surface to volume (Field & Mooney, 1986; Castro et al., 1997), and reduction of leaf area exposed to the external environment leads to primary structural modifications of the leaf anatomy, such as decrease in cell size, thicker walls and cuticles, and a strongly developed palisade at the expense of spongy mesophylls (Shields, 1950; Eliçin, 1977; Güvenç & Kendir 2012).

The more significant anatomical features are the trichomes that cover the abaxial surfaces of leaves. Such structures are able to regulate the water budget of plants both by influencing the diffusion boundary layer of the leaf surface and by regulating leaf optical parameters and, hence, leaf temperature (Rotondi, 2003). In many species, when trichomes or wax layers reduce radiation absorbance, 2 or 3 layers of palisade parenchyma are present, presumably to provide better efficiency in photosynthetic light utilisation (Pritchard et al., 1998; Satil & Selvi, 2007). In almost every plant examined, stomata are sunken or well protected (Güvenç et al., 2011).

By DCA of anatomical properties of the leaves, it was established that epidermis characters are the major contributors to discrimination along the first 2 canonical axes. These are the thickness of the inner wall of front side epidermis and thickness of inner wall of back side epidermis. Front epidermis width and back side epidermis width contribute to discrimination, as well. In addition to epidermis characters, the wider conductive vessel diameter and back side cuticle thickness contribute to discrimination.

Analysis of the centroid position in the space of the first 2 canonical axes (Figure 3) shows that plants from 620 m (and those from 420 m, in part) are clearly differentiated along the first canonical axis. The samples from other altitudes are grouped. Only the sample from 1420 m is differentiated along

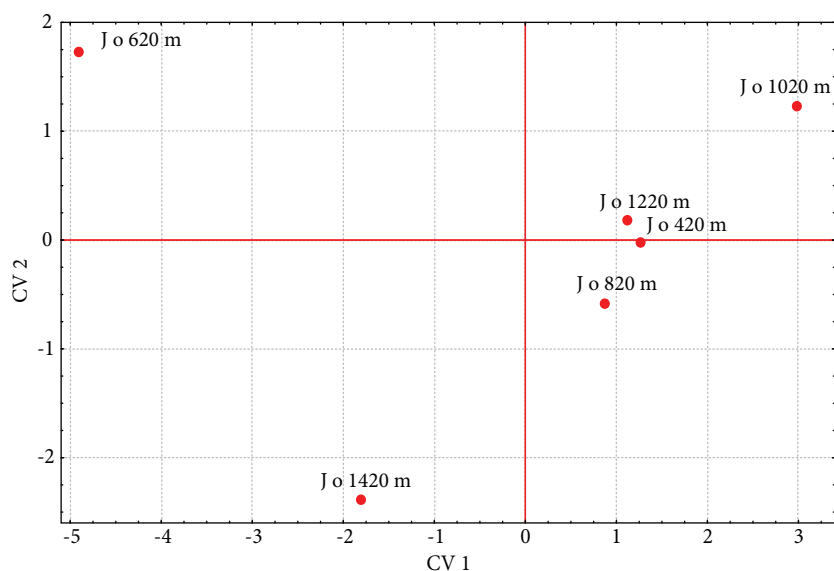


Figure 3. The position of centroids of analysed samples of red common juniper (*Juniperus oxycedrus*, J o) from different altitudes in the space of the first and second canonical axes.

the second axis. The results obtained in this way are in line with the results of the descriptive statistics, which also show that the sample from 620 m clearly differs from other samples of the genus.

The analysis of scores (Figure 4) shows clear differentiation in the sample from 620 m and partial

differentiation in a number of plants from 1020 and 1420 m. The other samples are grouped.

The results of cluster analysis also confirm that, on the basis of the obtained values of its anatomical characters, the sample from 620 m is differentiated from other samples. In Figure 5 it can also be observed

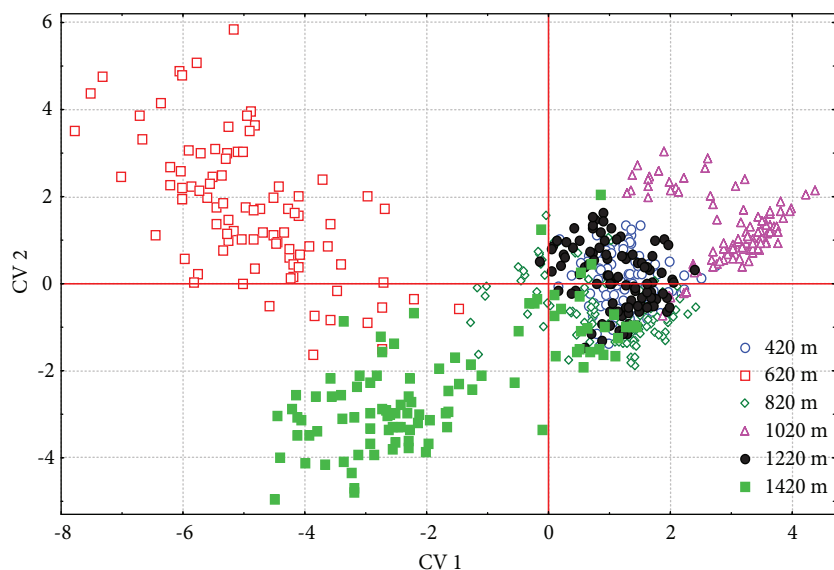


Figure 4. The position of scores of analysed samples of red common juniper (*Juniperus oxycedrus*) from different altitudes in the space of the first and second canonical axes.

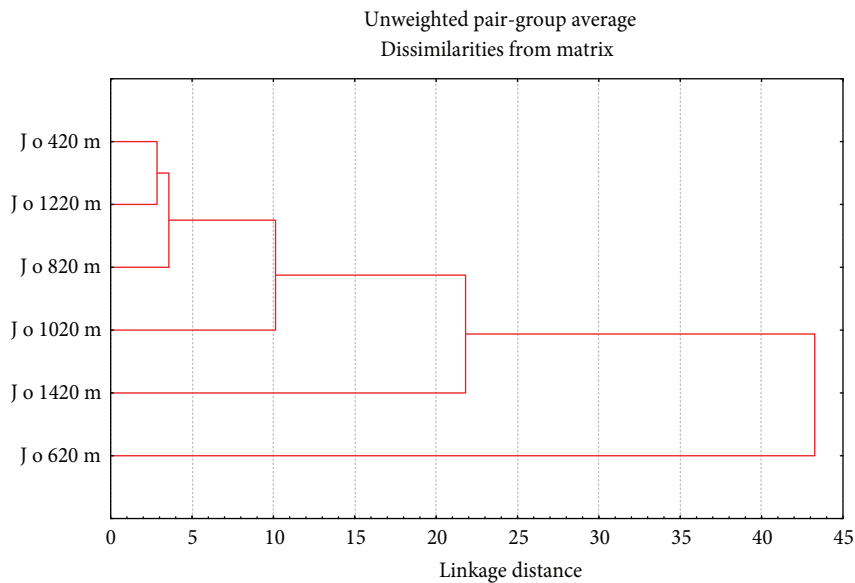


Figure 5. Cluster analysis of phenotype similarities of analysed common juniper (*Juniperus oxycedrus*, J o) samples from different altitudes.

that, phenetically, red common juniper from this altitude is clearly different from the red common junipers of other altitudes. Based on anatomical properties of the leaves, common junipers from 1420 and 1020 m are the most similar to it. It is interesting that plants from 420 m are located on the complete opposite end of the dendrogram, and they form a collective branch with red common juniper from 1220 m.

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