Variability of morpho-anatomical features of needles of *Pinus nigra* (black pine) in the area of Jastrebac and Goč mountains in central Serbia

Kristina Živanović✉[1], **Dušan Jokanović**[2]

[1]University of Belgrade – Institute of Multidisciplinary Research, Belgrade, Serbia
[1]University of Belgrade - Faculty of Forestry, Belgrade, Serbia

✉ kristinazivanovic@imsi.bg.ac.rs

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Abstract

In this paper the variability of morphological (needle length and needle width) and anatomical (resin ducts width, epidermis thickness, hypodermis height, number of the hypodermis layers and needle thickness) properties of *Pinus nigra* J.F. Arnold (black pine) needles were studied at the intra- and inter-population levels. Two mountains in Serbia, Jastrebac and Goč, were selected as experimental plots. Three *Pinus nigra* trees were selected from both localities (6 in total). Obtained results showed that trees on Goč had bigger dimensions of the following elements: needle length, slightly wider needle width, epidermis thickness, hypodermis height, number of hypodermis layers and needle thickness. On the other hand, resin ducts were only slightly wider by the trees from Jastrebac. The results of the analysis of variance showed that variation between studied populations, as well as variation between trees within populations was statistically significant for all needle traits except resin ducts width and hypodermis height.

Keywords

Pinus nigra; Needles; Morphological traits; Anatomical traits; Variability

Contents

1 Introduction

Pinus nigra (black pine) is distributed along the Mediterranean basin (Sarić et al., 1992). Considering the environmental characteristics of the area, *P.nigra* has a very discontinuous range and is extremely variable in morphological, anatomical and physiological features, which means that this species has a broad ecological valence (Gajić et al. 1993, 1994; Liber et al. 2002). *P.nigra* is most abundant in the western part of Serbia (Tara, Povlen, Maljen, Zlatibor, Troglav, Čemerno, Crni Vrh near Priboj), in central Serbia (Goč, Stolovi, Kopaonik), and to the lesser extent in eastern Serbia (Zlotska gorge, Beljanica, Sukovska river gorge, Suva mountain and the surroundings of Bosilegrad), (Cvjetićanin and Perović 2010). It usually grows at an altitude of 400 to 1300 m above sea level, but some specimens can be found on Kopaonik at an altitude of 1650 m above sea level (Jovanović 1991; Jokanović 2021). *P.nigra* is a heliophilic and xerothermic type of wood. In Serbia it grows on basic geological substrates (limestone, sepentinite and dolomite), where it's pioneering role gets fully shown (Sarić et al. 1992; Cvjetićanin and Perović 2010).

P.nigra is of great importance both from ecological and economic point of view. Features that distinguish *P.nigra*: disjunctive (interrupted) distribution, high genetic variability, complex structure of populations, plasticity and the ability to survive in the most difficult habitats, as well as the ability to grow on sites with lower nutrients content, impose the need to provide a large amount of seeds and planting material of this species (Mataruga 2006). There are many papers that deal with complex taxonomy of *P.nigra* which may be considered based on geographic gradient (Bojović 1997), using of genetical markers (Fineschi 1984), content of terpens in needles (Bojović 1995) and all these references confirm the existence of a great species variability. There are also several studies related to intraspecies variability of *P.nigra* where different varieties were separated based on morphological features of the needles (Đorđević 1931; Schwarcz 1938; Delevoy 1949; Vidaković 1957). From an economic point of view, *P.nigra* is widely used as mining wood, carpentry wood, for railway sleepers, pillars, in the wood industry, for example in production of furniture, building materials and pulp, from stumps and scraps, tar and turpentine oil are obtained by distillation, tannin matter from bark, and essential oil from needles (Vilotić 2000).

Many papers dealt with the analysis of the morphoanatomy of needles. Nikolić et al. (2019) investigated the morpho-anatomical structure of *P.heldreichii* (H.Christ.) needles from the Šar Mountain in Kosovo and Metohija and concluded that, compared to *P.heldreichii* needles from Dinaric Mountains; they have significantly shorter lengths and significantly narrowed resin ducts, while their needles are wider. *P.nigra* occupies a wide range of habitats (Lopez et al. 2008) and often grows in extreme conditions such as high temperatures, drought, and low soil nutrient levels. The ability of pine to develop in extreme forest conditions, such as fierce competition and excessive moisture, determines its potential for survival (Egorova and Kulagin 2007). Adaptation to different environmental conditions occurs through different modifications in morphological and anatomical structure, including needle proportions (Apple et al. 2002; Grill et al. 2004; Luomala et al. 2005). The aim of the research is to study the variability of morphological and anatomical features of *P.nigra* needles growing in different environmental conditions.

2 Material and methods

The research was conducted during growing season 2022. Two year old needles were collected from two natural *P.nigra* populations situated at mountain Goč (43° 32′ 11″ N; 20° 50′ 44″ E) and mountain Jastrebac (43° 23′ 31″ N; 21° 26′ 57″ E) (Figure 1). Field research included taking needles from 3 trees from each population. For sampling purpose, trees of approximate age and dimensions were selected. In order to get suitable sample, both the trees located at the edge of population and these grown under the dense canopy were not considered. It should be noted that needles were taken from the south side due to the large influx of direct sunlight and they were taken from the middle section of the crown. The collected material was then fixed. 50 needles were taken from each tree, 40 of them were used for morphological and the other 10 for anatomical analysis. The morphometric characteristics (total height, tree diameter at 1.3 m) as well as the geographical coordinates of all trees were also determined on the field.

Figure 1. Locations of analyzed populations of *P. nigra* (Source : Google Maps).

All the measured characteristics, apart from needle length, were investigated on mid-needle-cross-section (Figure 2A) since this is the region with the most resin ducts. Temporary anatomical preparations were made. As for measured elements, needle length was determined by digital nonius (Figure 2A), while all the other elements (Figure 2B and 2C) were measured with Boeco light microscope provided with appropriate software (Boeco Germany GmbH.). Before starting measurements, all prepared needle cross-sections were photographed by Scope Image camera (Boeco Germany GmbH.).

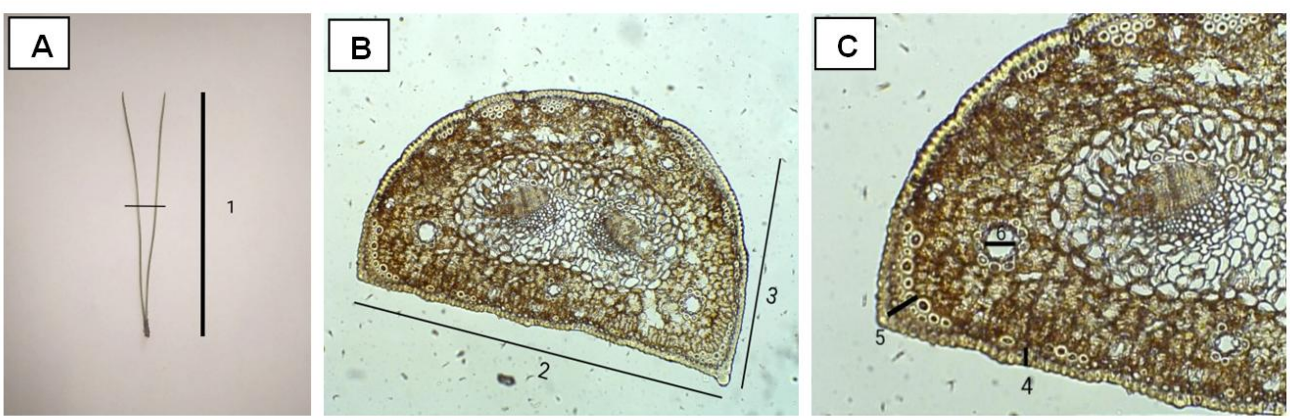

Figure 2. Cross-section of *P.nigra* needles (1) needle length; Numbers 2-6 denote the anatomical characteristics (2) needle width; (3) needle thickness; (4) epidermis thickness; (5) hypodermis thickness; (6) resin duct width (The author: Kristina Živanović).

Climate data is downloaded from the base CARPATCLIM Database European Commission – JRC, www.carpatclim-eu.org (Szalai et al. 2013). Average values displayed in the Table 1 are related to period 1961-2010. De Martonne Aridity Index was calculated according to the equation: $I_{dm} = P/(T+10)$, T-mean annual air temperature (ºC), P-annual precipitation sum (mm) (Baltas 2007).

The data was processed in a statistical program Statistica 13 (TIBCO Software Inc, 2017). Descriptive statistics elements such as: mean value ($\overline{x}$), standard deviation (SD) and coefficient of variation (CV [%]) were calculated for each parameter at both localities. Nested ANOVA was carried out to evaluate statistical significance of the following sources of variation: Locality (phenotypic variation caused by differences between localities) and Tree (Nested – within locality and represents variation between trees at one locality caused by differences in their genetical structure).

Table 1. Climate characteristics at study areas during 1961-2010: T- medium annual air temperature (ºC), T_{veg}- medium air temperature during growing season (April-September) (ºC), P-annual precipitation sum (mm), P_{veg}- precipitation sum during growing season (April-September) (mm), I_{dm}- De Martonne Aridity.

Locality	Elevation (m a.s.l.)	T	T_{veg}	P	P_{veg}	I_{dm}
Jastrebac	368	9.6	16.1	689	394	35.1
Goč	647	8.9	15.5	763	440	40.3

3 Results and discussion

Results of descriptive statistics showed greater values by trees on Goč when it comes to needle length, needle width, epidermis thickness, hypodermis height, number of the hypodermis layers and needle thickness (Table 2). On the other hand, needles on Jastrebac were characterized only by wider resin ducts. It should be noted that the width of the needles of the plants from Goč was slightly larger than the width of the plants from Jastrebac, only 0.26 mm. The resin ducts were slightly wider by trees on Jastrebac, and the difference was only 0.19 mm. Based on variation coefficient it can be deduced that following elements were more variable by trees on Goč: resic ducts width, epidermis thickness and needle thickness, while all the other elements are more variable on Jastrebac. If the degree of variability is observed at each locality, the most variable parameters on Goč are resin ducts width and epidermis thickness, unlike Jastrebac where hypodermis height and the number of hypodermis layers are the most changable.

The results of ANOVA indicated that variation between studied *P.nigra* populations, as well as variation between trees within populations were statistically significant for all examined needle traits, except resin ducts width ($F=1.18$; $p=0.282$) and hypodermis height ($F=1.32$; $p=0.255$) (Table 3).

By comparing the climatic characteristics between the two researched areas, a significant difference was established in elevation between two localities (Table 1). As for average air temperatures during a year and growing season (Table 1), they are a bit higher on Jastrebac, and it can be deduced that medium air temperatures during growing season are suitable for growth and development of *P.nigra* trees. If we analyze annual precipitation sum and precipitation sum during growing season, these values are a bit higher on Goč, as well as the value of De Martonne Aridity Index (Table 1).

Table 2. Descriptive variability indicators of investigated morphological and anatomical traits of *P.nigra* needles from Goč and Jastrebac: x̄ – mean value; SD – standard deviation; CV – coefficient of variation (%).

Characteristic		Goč	Jastrebac
Needle length (cm)	x̄	12.20	8.84
	SD	1.11	1.21
	CV	9.09	13.74
Needle width (mm)	x̄	1.39	1.13
	SD	0.14	0.12
	CV	10.34	11.02
Resin ducts width (µm)	x̄	4.91	5.10
	SD	0.89	0.84
	CV	18.15	16.49
Epidermis thickness (µm)	x̄	4.88	3.30
	SD	2.19	0.48
	CV	44.94	14.56
Hypodermis height (µm)	x̄	7.69	7.28
	SD	1.03	1.76
	CV	13.44	24.22
Hypodermis layers number	x̄	3.20	2.80
	SD	0.55	0.76
	CV	17.21	27.18
Needle thickness (µm)	x̄	101.12	84.62
	SD	18.15	6.72
	CV	17.95	7.76

Table 3. Results of ANOVA test for investigated morphological and anatomical traits of *P.nigra* needles.

Source of variation	F test	Needle length	Needle width	Resin canals width	Epidermis thickness	Hypodermis layers number	Hypodermis height	Needle thickness
Location	F	632.30	283.80	1.18	88.28	8.20	1.32	20.14
	p (≤)	0.001	0.001	0.282	0.001	0.005	0.255	0.001
Tree	F	16.81	18.54	9.65	72.69	8.37	3.18	3.83
	p (≤)	0.001	0.001	0.001	0.001	0.001	0.020	0.008

Nikolić et al. (2016) investigated the morpho-anatomical properties of *P.heldreichii* needles in natural populations in two locations in the territory of Serbia and Montenegro each and confirmed that in terms of needle anatomy, *P.heldreichii* differs from other pines from the subgenus *Pinus*, section *Pinus* and subsection *Silvestres*. On the other hand, in the analysis of the needle cross-section, Nikolić et al. *(2016)* noticed that *P. heldreichii* is the most similar to black pine, especially to two spatially close subspecies – *P. nigra subsp. dalmatica* (Vis.) Franco and *P. nigra subsp. pallasiana* (D. Don) Holmboe.

Our results are in agreement with a number of studies showing that abiotic factors, such as climate conditions, affect not only morphological and anatomical characteristics of *P.nigra* needles (Schoettle and Rochelle 2000; Jankowski et al. 2017), but also geographical distribution of the species (Niinemets et al. 2001; Urbaniak et al. 2003; Pensa et al. 2004). Similarly, studying morpho-anatomical structure of *P.heldreichii* needles in two natural populations situated in Serbia and North Macedonia, Nikolić et al. (2019) reported that population from Serbia was characterized by significantly shorter, but wider and with narrower resin ducts. Moreover, Popović et al. (2022) reported significant differences of needle length, needle width and needle thickness in 16 *Abies alba* Mill. natural populations across the region of Balkan, as well as that local climatic conditions significantly influenced studied traits. This coincides with the results obtained in this paper that the geographical area and the existing habitat conditions, together with the genetic specifications of the species, largely determine the quantitative characteristics of needles.

4 Conclusions

In the paper morphological (needle length and needle width) and anatomical (needle thickness, resin ducts width, epidermis thickness, hypodermis height and number of the hypodermis layers) traits of *P.nigra* needles from Goč and Jastrebac mountains were studied. The ANOVA analysis showed significant differences at intra- and inter-population levels for majority of studied traits, indicating that site conditions together with genetic structure of the species affect quantitative needle characteristics. Descriptive statistics displayed that *P.nigra* on Jastrebac have only slightly wider resin ducts, while all other measured characteristics (needle length, slightly larger needle width, epidermis thickness, hypodermis height, number of hypodermis layers and needle thickness) were greater by trees on Goč Mountain, which could be the result of more suitable climatic conditions for the growth of this species. The taxonomy of *P.nigra* is very complicated, as there are numerous subspecies, varieties, and ecotypes, and this problem is closely related to the variability of the morpho-anatomical structure of the needles. In the future a wide range of *P.nigra* populations from different habitats should be included in order to obtain more purposeful results on the morpho-anatomical structure of their needles.

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