МАТИЦА СРПСКА
1826

МАТИЦА СРПСКА
ОДЕЉЕЊЕ ЗА ПРИРОДНЕ НАУКЕ
ЗБОРНИК
МАТИЦЕ СРПСКЕ ЗА ПРИРОДНЕ НАУКЕ

MATICA SRPSKA
DEPARTMENT OF NATURAL SCIENCES
MATICA SRPSKA J. NAT. SCI.

Покренут 1951 / First published in 1951.

Until volume 10, the journal was published under the title *Научни зборник Матице српске: Серија природних наука* (Scientific Proceedings of Matica Srpska: Natural Sciences Series) (1951–1955). Volume 11 was released under the title *Зборник Матице српске: Серија природних наука* (Matica Srpska Proceedings: Natural Sciences Series) (1956), volumes 12–65 under the title *Зборник за природне науке* (Proceedings for Natural Sciences) (1957–1983), and from volume 66 the journal was published under the title *Зборник Матице српске за природне науке* (Matica Srpska Proceedings for Natural Sciences) (1984–). From volume 84 (1993) the journal was published in English under the title *Matica Srpska Proceedings for Natural Sciences* (1993–2012), and since volume 125 under the title *Matica Srpska Journal for Natural Sciences* (2013–)

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Articales are available in full-text at the web site of Matica Srpska, Serbian Citation Index, CABI, EBSCO, Web of Science Master Journal List, AGRIS FAO (abstracts)

YU ISSN 0352-4906 UDK 5/6 (05)

MATICA SRPSKA JOURNAL FOR NATURAL SCIENCES

147
(2/2024)

NOVI SAD
2024

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UDC 633.81
UDC 582.929.4:581.4-022.53
https://doi.org/10.2298/ZMSPN2447007A
ORIGINAL ARTICLE
Received: 2023/11/14
Accepted: 2024/4/30

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CHARACTERIZATION OF PELTATE TRICHOMES OF COMMERCIALLY IMPORTANT Lamiaceae SPECIES GROWN IN SERBIA

ABSTRACT: Peltate trichomes have been the focus of botanical research for decades as the main secretory structures responsible for the production of essential oil and other specialized metabolites. For this study, samples of the plant material were obtained from the collection of the Institute for Medicinal Plant Research "Dr. Josif Pančić". This study was conducted to perform morphological characterization, determination of the diameter and analysis of the distribution of peltate trichomes on leaves and calyces of selected commercial Lamiaceae species. In addition, the density of peltate trichomes on the leaves was determined. The analyses were carried out using a stereomicroscope and a scanning electron microscope. The leaves of all plant species bear peltate trichomes on the adaxial and abaxial leaf sides, with the highest density observed in *Origanum heracleoticum* (24.3 and 17.4 per mm^2, respectively). Density was generally higher on the adaxial surface, with the exception of *Mentha* × *piperita* (higher density on the abaxial side) and *Satureja montana* (equal density on both leaf sides). The largest diameter of peltate trichomes was observed in leaves and calyces of *S. montana* (88.79 µm and 90.57 µm, respectively), while the smallest diameter was noticed in *Origanum vulgare* (about 65.00 µm). The plant species studied differ in appearance, diameter, distribution and density of the peltate trichomes. The results obtained indicate that micromorphological features could be valid parameters for the microscopic authentication of aromatic plants of which the representatives of the Lamiaceae are of particular commercial importance.

KEYWORDS: aromatic plants, Lamiaceae, leaves and calyces indumentum, peltate trichomes, scanning electron microscopy

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INTRODUCTION

The mint family (Lamiaceae) is the sixth-largest family of flowering plants and includes over 7,000 species (Drew and Sytsma, 2012). Due to the production of secondary metabolites, the Lamiaceae are considered one of the most economically important plant families. Certain taxonomic groups in the Lamiaceae family are rich in essential oils, which have a wide range of biological functions in plants and a high level of pharmacological activity (Dhifi et al., 2016).

The indumentum of Lamiaceae taxa, especially their leaves and floral parts, bear glandular trichomes that produce various useful secondary metabolites. Peltate trichomes, characterized by a short stalk and a broad secretory head consisting of 4–16 cells arranged in a single disc, are classified as long-term glandular hairs; the secreted substances were continuously accumulated as lipophilic droplets during the growth of the organ, and released after the cuticle ruptured (Werker, 1993). The density and diameter of peltate trichomes vary over a wide range depending on the species (Svidenko et al., 2018), plant origin and population (Fialova et al., 2015; Hanafy et al., 2019), and ecological or cultivation conditions (Kokkini et al., 1994; Bosabalidis and Kokkini, 1997; Shafiee-Hajiabad et al., 2014; Kosakowska et al., 2020; Stefan et al., 2021).

Table 1. Previous studies on the trichomes of selected Lamiaceae species

Species	Plant parts*	Glandular trichomes**	Place of collection	Reference
Hyssopus officinalis L.	L, S, Ca, Co	NG, C, P	Italy	Venditti et al. (2015)
(hyssop)	L, S, Ca, Co	NG, C, P	Ukraine	Kotyuk et al. (2016)
	L	P	Grown in Ukraine	Svidenko et al. (2018)
Lavandula angustifolia	L, S	NG, C, P (L)	Romania	Stefan et al. (2021)
Mill. (lavander)	L, Ca, Co	NG, C, P	Italy	Giuliani et al. (2023)
Mentha × *piperita* L.	L	P	Slovakia	Fialová et al. (2015)
(peppermint)	L	NG, C, P	Grown in Australia	Hanafy et al. (2019)
Ocimum basilicum L.	L	P	Grown in Ukraine	Svidenko et al. (2018)
(basil)	L	NG, C, P	Egypt	Azzazy (2019)
	L, S	NG, C, P	India	Sanoj and Deepa (2021)
Origanum heracleoticum	L, Ca	NG, C, P	Greece	Kokkini et al. (1994)
L. (Greak oregano)	L	P	Greece	Bosabalidis and Kokkini (1997)
Origanum vulgare L.	L, Ca	NG, C, P	Greece	Kokkini et al. (1994)
(common oregano)	L	NG, C, P	Grown in Germany	Shafiee-Hajiabad et al. (2014)
	L	NG, C, P	Grown in Germany	Bosabalidis and Kokkini (2014)
Rosmarinus officinalis L.	L	NG, C, P	Grown in Serbia	Marin et al. (2006)
(rosemary)	L	NG, C, P	Grown in Brasil	Boix et al. (2011)
Salvia officinalis L.	L, Ca	NG, C, P		Corsi and Bottega (1999)
(sage)	L	P	Grown in Ukraine	Svidenko et al. (2018)
Satureja montana L.	L, S, Ca	NG, C, P	Croatia	Marin et al. (2012)
(winter savory)	L	P	Grown in Ukraine	Svidenko et al. (2018)
Thymus vulgaris L.	L	P	Grown in Ukraine	Svidenko et al. (2018)
(thyme)	L	NG, C, P		Kosakowska et al. (2020)

*L – leaf, S – stem, Ca – calyx, Co – corolla **NG- non-glandular trichomes, C – capitate trichomes, P – peltate trichomes;

Data on micromorphology of many commercially used Lamiaceae species can be found in the literature (Table 1), but data on plants grown in Serbia are lacking. Since the aerial parts, which include leaves and inflorescences, are most commonly used as herbal remedies, the aim of this study was to morphologically characterize, determine the diameter and analyze the distribution of peltate trichomes on leaves and calyces of selected Lamiaceae species grown in Serbia. In addition, the density of peltate trichomes on the leaves was determined.

MATERIAL AND METHODS

Plant material

The plant material of ten commercial Lamiaceae species was obtained from the collection of the Institute for Medicinal Plant Research "Dr. Josif Pančić". The leaves and calyces were collected at the flowering stage, air-dried and carefully stored in paper bags until microscopic analysis.

Stereomicroscopic analysis

The dried leaves and calyces (N=10) of each species studied were separated and analyzed using a Nikon SMZ18 stereomicroscope (Tokyo, Japan). As for the peltate trichomes, photographs were taken of both the adaxial and abaxial sides of the leaf and the outer side of the calyx. The photos were evaluated with respect to the color and distribution of the trichomes. The diameter of the peltate trichomes and their density were determined using Digimizer 4.3.4 and presented as mean ± standard deviation.

Scanning electron microscopy (SEM) analysis

The adaxial and abaxial leaf side as well as the outer side of the calyx were coated with a thin gold layer (BALTEC SCD 005 Sputter Coater, 100 seconds, 30 mA) and then analyzed and photographed with JEOL JSM – 6390W.

RESULTS AND DISCUSSION

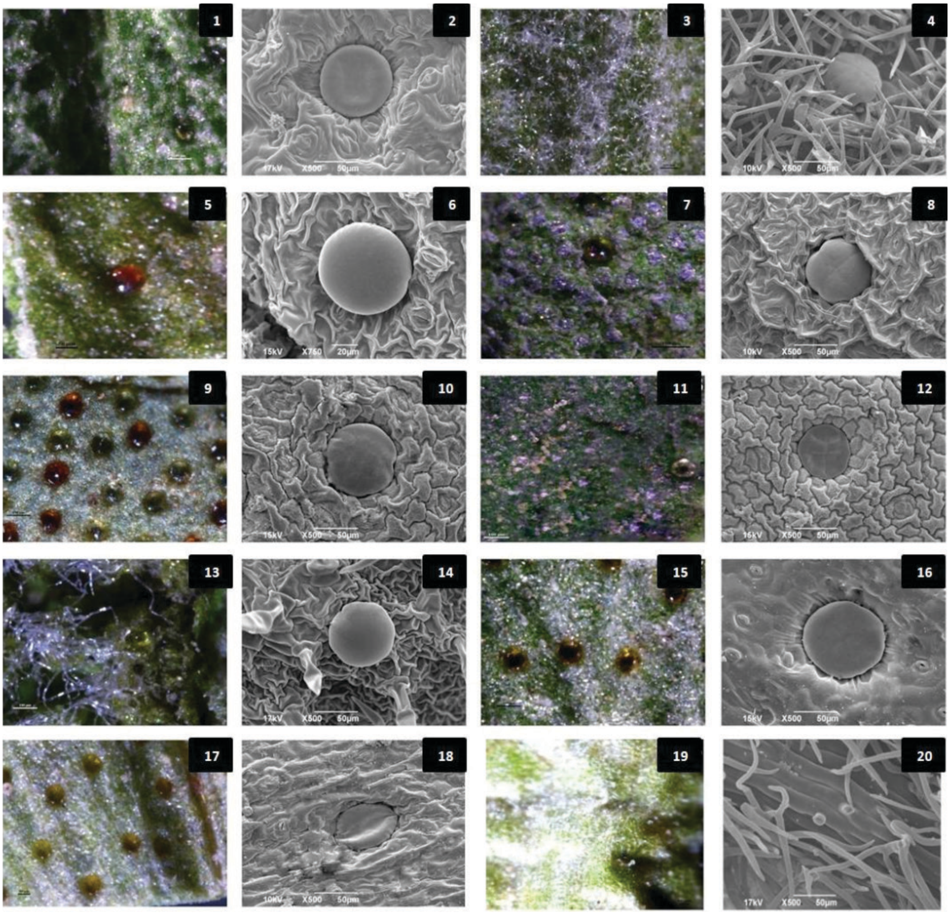
Glandular hairs, including those of peltate type, are distributed on the aerial vegetative and reproductive organs of Lamiaceae representatives (Werker, 1993). The results of the study confirmed that the plants grown in Serbia also had peltate trichomes on both the adaxial and abaxial sides of the leaves and on the outer side of the calyx of all the species studied (Table 2). In the current study, peltate trichomes were observed under the stereomicroscope as yellowish to brownish globular structures (Figures 5, 7, and other figures indicated by odd numbers), depending on the stage of trichome growth (Svidenko et al., 2018).

Table 2. Presence, diameter and density of peltate trichomes on the investigated species

Species	Leaves					Calyxes	
	Pr.		Diameter (µm)*	Density (per mm²)**		Pr.	Diameter (µm)*
	ad	ab		ad	ab		
Hyssopus officinalis	+	+	81.74±2.23	7.90±1.80	nd	+	78.00±4.63
Lavandula angustifolia	+	+	66.43±2.91	nd	nd	+	nd
Mentha × piperita	+	+	79.11±4.82	9.80±1.20	23.30±1.90	+	85.37±4.28
Ocimum basilicum	+	+	78.89±5.46	nd	nd	+	81.25±6.46
Origanum heracleoticum	+	+	82.34±3.34	24.30±1.30	17.4±1.70	+	88.84±4.75
Origanum vulgare	+	+	62.45±1.19	nd	nd	+	65.00±4.26
Rosmarinus officinalis	+	+	nd	nd	nd	+	73.68±1.37
Salvia officinalis	+	+	73.26±4.33	nd	nd	+	76.99±2.66
Satureja montana	+	+	88.79±2.80	11.30±1.80	11.80±2.20	+	90.47±3.36
Thymus vulgaris	+	+	66.89±2.04	16.00±3.70	6.50±1.90	+	66.83±4.21

*Obtained by measuring peltate trichomes on the adaxial leaf side and expressed as mean±standard deviation; **Obtained by counting peltate trichomes on abaxial leaf side of leaves; Pr. – presence; nd – The density and diameter of the peltate trichomes were not determined in the samples with the dense non-glandular trichomes in the indumentum.

The highest density of peltate trichomes was observed in the leaves of *O. heracleoticum* (Table 2; Figures 9–10 and 29–30). Our sample showed significantly higher density compared to *O. vulgare* subsp. *viridulum* (syn. *O. heracleoticum*) (1.5 and 1.2 per mm^2, respectively) (Bosabalidis and Kokkini, 1997) and *O. vulgare* subsp. *vulgare* (9.7 and 6.3 per mm^2, respectively) (Shafiee-Hajiabad et al., 2014). Density was generally higher on the adaxial surface, except for *M. × piperita* (peltate trichomes were denser on the abaxial than on the adaxial side) and *S. montana* (equal density on both leaf sides) (Table 2). Interestingly, the abaxial side of *M. × piperita* had a density of peltate trichomes as high as 23.30 per mm^2, which was almost more than twice the density of the adaxial leaf side. The results of previous studies (Fialová et al., 2015; Hanafy et al., 2019) also showed that the density on the abaxial than on the adaxial leaf surface of *M. × piperita* was significantly higher, ranging from about 8 to 16 peltate trichomes/mm^2. The adaxial side of *Th. vulgaris* leaves had more than twice the density of the abaxial (Table 2). Similar results were obtained for *Th. vulgaris* grown in Ukraine, with values of about 15 and 6 trichomes/mm^2, respectively (Kosakowska et al., 2020).

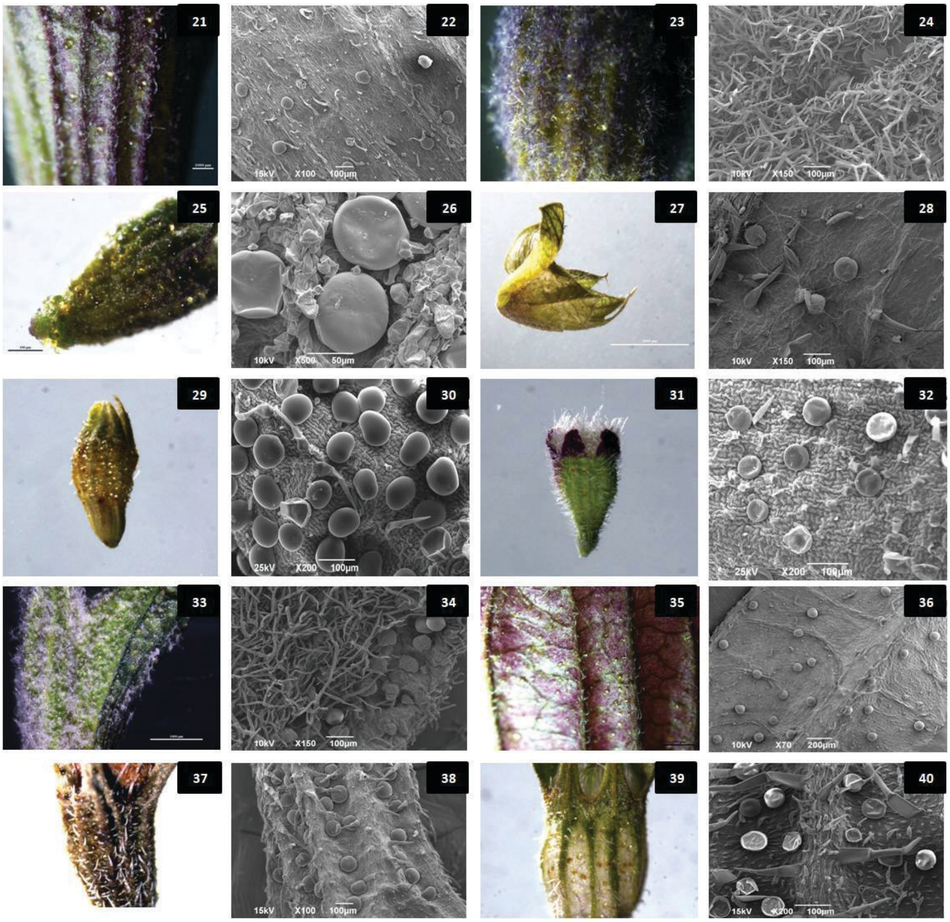


Figures 1–20. Peltate trichomes on the adaxial leaf side of investigated commercial Lamiaceae species[1]: 1–2. *H. officinalis* (135x); 3–4. *L. angustifolia* (100x); 5–6. *M.* x *piperita* (135x), 7–8. *O. basilicum* (135x); 9–10. *O. heracleoticum* (135x); 11–12. *O. vulgare* (135x), 13–14. *S. officinalis* (135x); 15–16. *S. montana* (135x); 17–18. *Th. vulgaris* (135x); 19–20. *R. officinalis* (80x).

The largest diameter of peltate trichomes was observed in leaves and calyces of *S. montana* (88.79 µm and 90.57 µm, respectively). Relatively large peltate trichomes (>80 µm diameter) were observed on the adaxial leaf side of *O. heracleoticum* and *H. officinalis*, and on the calyces of *O. heracleoticum*, *M.* × *piperita* and *O. basilicum*. The smallest diameter was found on leaves and calyces of *O. vulgare* (62.45 µm and 65.00 µm, respectively), followed by *Th. vulgaris* (about 67 µm for both plant parts) (Table 2). In general, the peltate

[1] Odd numbers are for the photographs obtained by stereomicroscope (magnification in brackets), and even numbers by SEM at magnification of 500x.

trichomes were slightly larger on the calyces than on the leaves of the corresponding species. As already indicated by Svidenko et al. (2018), the leaves of *Hyssopus angustifolius*, *H. officinalis* and *S. montana* bear the largest peltate trichomes among the Ukrainian Lamiaceae plants studied. Compared to our results, Kotyuk et al. (2016) found smaller and denser peltate trichomes (47.82 µm and 13.6 per mm^2, respectively) on the adaxial leaf surface of *H. officinalis* grown in Ukraine.



Figures 21–40. Peltate trichomes on the calyces of investigated commercial Lamiaceae species[2]: 21–22. *H. officinalis* (60x and 100x); 23–24. *L. angustifolia* (50x and 150x); 25–26. *M.* x *piperita* (50x and 500x); 27–28. *O. basilicum* (10x and 150x); 29–30. *O. heracleoticum* (30x and 200x); 31–32. *O. vulgare* (30x and 200x); 33–34. *R. officinalis* (30x and 150x); 35–36. *S. officinalis* (30x and 70x); 37–38. *S. montana* (50x and 100x); 39–40. *Th. vulgaris* (60x and 200x)

[2] Odd numbers are for the photographs obtained by stereomicroscope, and even numbers by SEM (magnification in brackets).

In contrast, Svidenko et al. (2018) observed peltate trichomes with a larger diameter on the *S. montana* leaves (as much as 105.12 μm). These authors also found that *Th. vulgaris* bear larger peltate trichomes compared to our sample. The diameter of peltate trichomes of *M.* × *piperita* determined in this study was in agreement with results reported for populations from Slovakia (Fialova et al., 2015) and the plants grown in Australia (Hanafy et al., 2019). The diameter of the subcuticular chamber of peltate trichomes observed on the adaxial leaf side of *O. vulgare* subsp. *viridulinum* had a lower value (70.27 μm) (Bosabalidis and Kokkini, 1997) than that determined for our sample. The size of the peltate trichomes on *O. vulgare* was in agreement with the value determined for the adaxial leaf side of *O. vulgare* subsp. *vulgare* grown in Germany (58.7 μm). The density and diameter of the peltate trichomes were not determined in the samples with the dense non-glandular trichomes in the indumentum, such as *R. officinalis* (Figures 19–20 abd 33–34) and *L. angustifolia* (Figures 3–4 and 23–24).

In most of the specimens examined, the peltate trichomes are observed in the secretory phase. The number of head cells varied from four cells in one cycle in *O. basilicum* (Figure 8) to 12 cells in *O. vulgare* (four in the inner and eight in the outer cycle) (Figure 12). The secretory head of various *Origanum* taxa consisted of four, occasionally two, central and eight to twelve peripheral cells (Shafiee-Hajiabad et al., 2014). Similar observations were made in the leaves of *S. officinalis* (Corsi and Bottega, 1999) and *S. montana* (Marin et al., 2012). The eight-celled secretory head was observed on the leaves of *Mentha* spp. (Fialova et al., 2015) and *R. officinalis* (Boix et al., 2011).

CONCLUSIONS

In this study, peltate trichomes in leaves and calyces indumentum of commercial Lamiaceae species grown in Serbia were examined for their density, diameter, distribution and morphology. The highest density of peltate trichomes was observed on the adaxial leaf side of *O. heracleoticum* and the largest diameter on leaves and calyces of *S. montana* (about 90 μm). The number of head cells varied between four and twelve, each arranged in one to two cycles. In addition, the results of this study suggest that differences in the size, density, appearance and distribution of peltate trichomes could be used for both macroscopic and microscopic authentication of aromatic Lamiaceae taxa.

ACKNOWLEDGEMENT

This work was supported by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grant No. 451-03-47/2023-01/200178 and 451-03-47/2023-01/200003). The authors would like to thank Prof. dr Ilinka Pećinar for professional and technical support.

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ОРИГИНАЛНИ ЧЛАНАК
Примљен: 14. 11. 2023.
Прихваћен: 30. 4. 2024.

КАРАКТЕРИЗАЦИЈА ПЕЛТАТНИХ ТРИХОМА КОМЕРЦИЈАЛНИХ ВРСТА УСНАТИЦА ГАЈЕНИХ У СРБИЈИ

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РЕЗИМЕ: Као најзначајније секреторне структуре одговорне за продукцију етарских уља и других специјализованих метаболита, пелтатне трихоме су деценијама у фокусу ботаничких истраживања. За потребе овог рада, узорци биљног материјала су добијени из колекције Института за проучавање лековитог биља „Др Јосиф Панчић”. Ово истраживање је спроведено у циљу морфолошке карактеризације, одређивања дијаметра и анализе дистрибуције пелтатних трихома на листовима и чашицама одабраних комерцијалних врста фамилије Lamiaceae. Поред тога, одређена је густина пелтатних трихома на листовима. Анализе су спроведене коришћењем бинокуларне лупе и скенирајућег електронског микроскопа. Листови свих испитиваних врста носе пелтатне трихоме на адаксијалној и абаксијалној страни, са највећом густином утврђеном код листова *Origanum heracleoticum* (24,3 односно 17,4 по mm^2). Густина пелтатних трихома је генерално била виша на адаксијалној страни, са изузетком *Mentha × piperita* (виша густина на абаксијалној страни) и *Satureja montana* (подједнака густина на обе стране листа). Највећи дијаметар пелтатних трихома уочен је на листовима и чашицама *S. montana* (88,79 µm, односно 90,57 µm), док су најмањег дијаматра биле код *Origanum vulgare* (око 65,00 µm). Густина и дијаметар пелтатних трихома није било могуће одредити код узорака са густим негландуларним трихомама у индументуму.

Проучаване врсте су се разликовале по изгледу, дијаметру, дистрибуцији и густини пелтатних трихома. Добијени резултати указују да би микроморфолошки карактери могли да буду валидни параметри за микроскопску аутентификацију ароматичних врста уснатица, од којих су неке од великог комерцијалног значаја.
КЉУЧНЕ РЕЧИ: ароматичне биљке, индументум листова и чашица, Lamiaceae, пелтатне трихоме, скенирајућа електронска микроскопија

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 147, 17—32, 2024

UDC 581.9(497.113 Titel)
https://doi.org/10.2298/ZMSPN2447017B
ORIGINAL ARTICLE
Received: 2023/11/16
Accepted: 2024/1/9

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NEW DATA FOR THE VASCULAR PLANTS IN THE SPECIAL NATURE RESERVE "TITELSKI BREG" (VOJVODINA, SERBIA)

ABSTRACT: In this paper we present results of the botanical research performed from October, 2022 to October, 2023 in SNR "Titelski breg". The Reserve has been legally protected from 2009 and ever since, active conservation measures, including mowing, grazing and removal of invasive plant species, have been implemented. Titelski breg is an isolated ellipsoid loess hill situated in the northern Serbia, between the Tisa River and several settlements. Flora and vegetation of the hill have been thoroughly studied in the past since 1896, and most of the data were published in the period 1980–1990. Our botanical research was conducted at 11 localities, which were previously prioritized, based on the number of published data on vasular plants and answers given by managers of the Reserve in the questionnaire. The collected plant samples were preserved, identified and stored in the herbarium of the University of Novi Sad (BUNS). After that, data on vascular plants were compared with a database compiled with available and relevant floristic and phytocoenological literature published between 1972 and 2022. As a result, there were 18 new vascular plant species, five new subspecies and one new variety. These results contribute to the general knowledge on their distribution and floristic diversity of Serbia, and are crucial for the evaluation of the protection and management in the Reserve.

KEYWORDS: invasive plants, loess, management, protected area, steppes

INTRODUCTION

Titelski breg is an isolated ellipsoid loess hill in the northern part of Serbia. From the eastern side it is bordered by the Tisa River, and on the southern, western and northern side it leans against the settlements Titel, Lok, Vilovo,

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Šajkaš and Mošorin. The plateau landform itself is 18 km long and 7.5 km wide, with minimum 72.5 m and maximum 130 m of altitude. The Titel plateau abruptly ends at the valley of the Tisa River, where it falls almost vertically toward the surrounding alluvial plain (Bjelajac et al., 2016). Particular geomorphology of these cliffs and gullies where natural vegetation, mainly open temperate grasslands, has been preserved to this day, along with the surrounding agricultural fields, represent a unique natural entity. Some authors claim that the Danube River separated Titelski breg from Srem loess plateau by cutting across its meander neck, while others consider that Titelski breg has always been isolated (Košćal et al., 2008). Even though there are still different theories about the origin of Titelski breg, it does not call the question on its specificity. Moreover, decades of research of the flora and vegetation, as well as geological and geomorphological studies of Titelski breg, have highlighted its uniqueness and exceptional value as a nature reserve. These investigations are vital and upon them this area was declared as the Special Nature Reserve (SNR) in Serbia in 2009.

SNR "Titelski breg" belongs to the first category of protected areas of exceptional national importance in Serbia (Anonymous, 2009, 2010a), while according to the IUCN categorization it is recognized as an area where protection is implemented through intervention measures. The Reserve covers an area of 496 ha, and its largest part is occupied by open grasslands such as steppes, meadows and pastures, followed by forests and roads, while the water channel network, reed beds and marshes are reduced to an almost negligible percent (Butorac et al., 2011).

With its grasslands, the SNR "Titelski breg" represents an important area for birds (BirdLife International, 2023), plants (Stevanović, 2005), and it is a part of the Ecological network of the Republic of Serbia (Anonymous, 2010b). Additionally, some of habitat types in the Reserve are evaluated as internationally important as stated in the EU Habitat Directive (2007), in particular Pannonian loess steppe grassland (code 6250). Furthermore, there are almost 30 plant species marked as important for biodiversity conservation due to their relic status (e.g. *Adonis vernalis*, *Crocus variegatus*, *Prunus tenella*, *Sternbergia colchiciflora*), although some of them, included in CITES convention (*Anacamptis coriophora*) or in Annex II of EU Habitat Directive (*Colchicum arenarium*) have not been observed in the Reserve for 50 or 100 years (Butorac et al., 2011; CITES Species, 2023; EU Habitat Directive, 2007). According to Jones et al. (2013), there are several reasons for long-term monitoring of biodiversity: 1) to improve understanding of complex interactions in natural systems, 2) to detect unexpected changes, 3) to raise awareness among the public and policy makers, 4) to audit management actions and 5) to inform management decisions. Moreover, Schalaepfer (2018) emphasized that biologists should include not only native species, but also non-native species in the monitoring plan. Overall, given the potential ecological and economic effects on biodiversity and ecosystem-services, a top priority of biodiversity research should be to collect, compile, and evaluate biodiversity data in space and time (Hines & Pereira, 2021). The best way to perform that in SNR "Titelski breg" is through thoroughly planned botanical research on previously defined localities.

Biogeographically, Titelski breg belongs to the Pontic-South-Siberian steppe region (Stevanović, 1992). Grasslands, such as steppes, pastures and meadows are under severe threats from ongoing degradation, undermining their capacity to support biodiversity, ecosystem service and human well-being. Yet, they are largely ignored in sustainable development agendas (Bardgett et al., 2021). Due to the negative effects of climate change, the spread of invasive plants and high nitrogen content in the substrate, these habitats are largely fragmented and altered, making less than 10% of the global area of terrestrial ecosystems (Henwood, 2010; Beck et al., 2015). Moreover, grasslands provide a wide range of ecosystem services, including biomass production, carbon storage and sequestration, water infiltration, purification and storage, erosion prevention and recreation, food for herbivores and other animals, as well as food and home for pollinators and birds (Török et al., 2020).

The flora and vegetation of the hill were thoroughly studied in the past, and botanical research can be divided into two periods. The first period covers the end of the 19th century and the beginning of the 20th century, while the second extends to the end of the 20th century, with the most active period from 1980 to 1990. In the former period, Prodán and Zorkóczy, stand out among the researchers, while in the latter Butorac, Igić, Stanojev and Stojanović must be highlighted. Owing to these systematic investigations (Zorkóczy, 1896; Prodán, 1915; Butorac and Igić, 1995; Butorac et al., 2008; Igić, 1988; Igić et al., 2019; Stanojev, 1981, 1983; Stanojev & Boža, 1984; Stanojev & Obradović, 1983, 1986; Stojanović, 1979, 1981) Titelski breg is considered a well-known floristic area in Serbia. However, there have been almost two decades since the last solid botanical investigation in the Reserve. Considering the time interval since the last floristic study, modern research approach was designed to assess the floristic richness, necessary for making a complete list of vascular plants of the Reserve, as well as to provide recommendations for sustainable conservation management planning in the future.

On the basis of these statements, the aims of this research were defined as follows: 1) to build a database with literature data on vascular flora published for the SNR "Titelski breg" in the period 1972–2022, 2) to design and conduct a questionnaire with the employees of the SNR "Titelski breg" about management measures that have been conducted in the past 20 years, 3) to define localities that are priorities for floristic research due to differences in management practice and existing floristic data, and 4) to perform botanical research and to evaluate floristic diversity on these localities.

MATERIALS AND METHODS

Design of the database and the questionnaire

Database of literature data was designed in Excel for Windows (Microsoft Office, 2007). It included floristic and phytocoenological data from the relevant references sources for the Titelski breg: Anačkov (2003, 2009), Butorac & Igić (1995), Butorac et al. (2008), Igić (1988), Igić et al. (2019), Josifović (1972–1976, 1977), Kostić (2005), Perić et al. (2018), Rat (2019), Sarić (1992), Sarić & Diklić (1986), Stevanović (2012, 2022), Stanojev (1981, 1983), Stanojev & Boža (1984), Stanojev & Obradović (1983, 1986) and Stojanović (1979, 1981). Although Titelski breg had already been botanically studied before 1972, this year was chosen as the "0-point" because it is the year when the first edition of the *Flora of the Republic of Serbia*, volume 3, was published. All the earlier botanical data on this area should have been summarized in this paper. The data extracted from the literature are distributed over 6 general variables (e.g. systematics, locality name and position, habitat, ecology, collection date and collector, and publication). After reviewing the literature, the data were critically analyzed and harmonized. Plant family affiliation is given according to the APG IV (2016), while the names of the vascular plant taxa were harmonized with the POWO (2023) and Euro+Med (2006+). Database of the literature data on the SNR "Titelski breg" includes 7.361 floristic records in total, for 672 recorded vascular plant taxa, which existed in the Reserve. Furthermore, almost 30% of data are imprecise, and are related to Titelski breg as a whole. After that, the questionnaire (Table 1) was made in order to get information about management measures that have been implemented in the Reserve for the past 20 years. Finally, the database and questionnaire were constructed with the purpose of designing botanical study on localities that can be labeled as "priority", as well as to compare new results with the previously published ones.

Table 1. The questionnaire about management measures that have been conducted in the Reserve for the past 20 years.

Measure	**Grazing**
Question 1	**Are there localities in the Reserve where grazing with sheep is conducted?**
Question 1.1	If YES – which are those localities?
Question 1.2	If YES – how large are those localities?
Question 1.3	If YES – how many sheep are there?
Question 1.4	If YES – in which period of the year grazing with sheep is conducted on those localities?
Question 2	**Are there localities in the Reserve where grazing with cows is conducted?**
Question 1.1	If YES – which are those localities?
Question 1.2	If YES – how large are those localities?
Question 1.3	If YES – how many cows are there?
Question 1.4	If YES – in which period of the year grazing with cows is conducted on those localities?
Measure	**Mowing**
Question 3	**Are there localities in the Reserve where mowing is conducted?**
Question 3.1	If YES – which are those localities?
Question 3.2	If YES – how many times per year the mowing is conducted on these localities?
Question 3.3	If YES – how large are those localities?

Definition of the localities as priorities for botanical research

During the prioritizing process concerning localities, there were three criteria to make decisions: 1) zone of protection (1^{st}, 2^{nd} and 3^{rd}), 2) number of previously published data on vascular plants, and 3) type (no measures, grazing by sheep, grazing by cows and mowing) and number (0, 1, 2 or 3) of management measures (Table 2). Different types of management measures were taken into account, because it has been proven that they have positive or negative effect on floristic diversity, as well as population size and abundance of grassland plants (e.g. Beck et al., 2015; Molina et al., 2020; Smith et al., 2018; Tälle et al., 2016; Zhang Y. et al., 2017; Zhang M. et al., 2023), and consequently affect other organisms. Additionally, the same long-term management measures may affect invasive plants (Porensky et al., 2020; Smart et al., 2017), which would be essential for future conservation steps in the Reserve. As for the number of previously published data on vascular plants, only those that can be certain were taken into consideration, i.e. when locality was named precisely, with or without coordinates. All data referred to larger area, cited with dash or hyphen (e.g. Lok–Vilovo, Vilovo–Titel, Šajkaš–Mošorin), could not be taken into account.

Upon given answers in questionnaire (Table 1) and floristic data on SNR "Titelski breg" in the database, 11 priority localities for botanical study were selected. There are three localities in the 1^{st} zone of protection, where management measures are forbidden: Demljankov gully, Dukatar and Fokini oraji and only one locality in 2^{nd} and 3^{rd} zone of protection where management measures are not implemented – Šajkaš. The rest of priority localities (7) are in the 2^{nd} and 3^{rd} zone of protection and these localities can be divided into three groups: 1) locality with only one management measure (grazing by sheep) – Mošorin, 2) five localities with two management measures (grazing by sheep and mowing) – Vilovo, 1^{st} gully from Topola to Vilovo, Grac, Kapetanov gully, Topola and 3) just one locality with three management measures (grazing by sheep and cows, and mowing) and small number of previous botanical data – pasture "Vodice" (Figure 1).

Table 2. Localities selected as priorities for botanical research in SNR "Titelski breg" during 2022–2023 with important characteristics

No	Locality abbreviation	Locality name	Zone of protection	Number of literature data	Management measures
1	FO	Fokini oraji	1st	39	n. a.
2	DEM	Demljankov gully		63	
3	DUK	Dukatar		56	
4	MOS	Mošorin	2nd and 3rd	827	grazing – sheep
5	SAJ	Šajkaš		1	no measures
6	VIL	Vilovo		232	grazing – sheep, mowing
7	KAP	Kapetanov gully		*	
8	GRA	Grac			
9	1_TOP-VIL	1st gully from Topola to Vilovo			
10	TOP	Topola		2	
11	VOD	Vodice		0	grazing – sheep and cows, mowing

Legend: n. a. – not applicable because the measures are forbidden in the 1st zone of protection; * it cannot be determined precisely

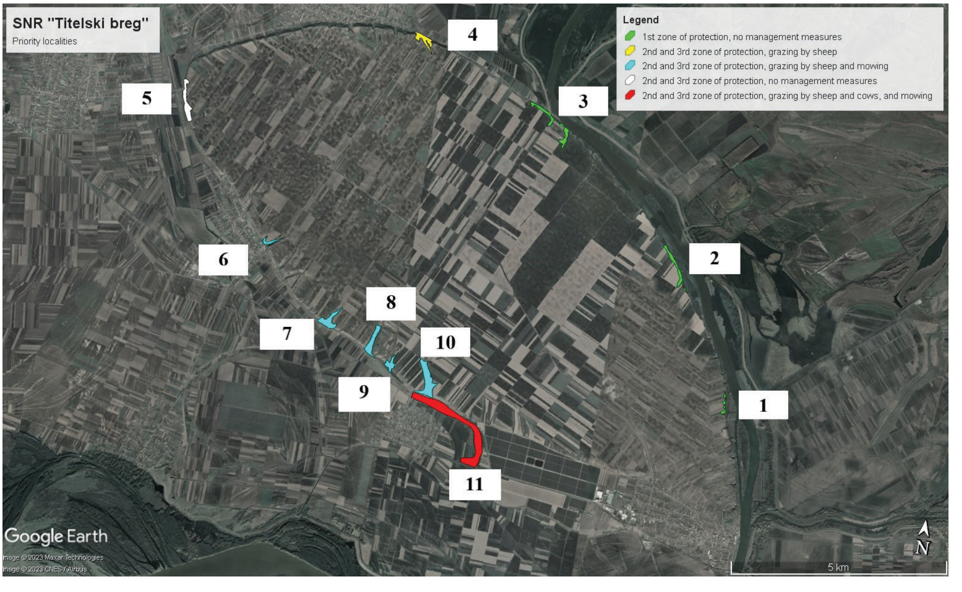

Figure 1. Study area with marked localities chosen as priorities based on defined criteria (Numbers of the localities follows legend given in the Table 2)

Botanical research

Botanical research included inventory on vascular plant taxa in 11 selected priority localities from October 2022 to October 2023. The research was performed in 25 m^2 sites, which were randomly chosen in each priority locality. Depending on the size of locality, number of sites varied from one to four. The vascular plant taxa were collected, herbarized by standard methods (Anačkov, 2021) and identified by relevant keys (Josifović, 1972–1976; Nikolić, 2020; Sarić, 1992; Stevanović, 2012, 2022; Tutin et al., 1964–1980) and iconographies (Jávorka and Csapody, 1975; Király et al., 2011). Plant specimens were deposited in Herbarium of the University of Novi Sad (BUNS). Plant family affiliation is given according to the APG IV (2016), while the names of the vascular plant taxa were harmonized with the POWO (2023) and Euro+Med (2006+).

In order to get a list of new taxa that have not been recorded in the SNR "Titelski breg", all vascular plant taxa from our research were compared with the database. Since the study was performed on the territory of the nature reserve, all alien plant taxa are considered invasive according to IAS Regulation (EU 1143-2014). List of invasive plant alien taxa was determined in comparison to Anačkov et al. (2013) and Lazarević et al. (2012). Names of collectors of plant material are presented with abbreviations: Bojana Bokić (BB), Kristina Đorđević (KĐ), Dragan Obradov (DO), Jovan Peškanov (JP) and Aleksa Vlku (AV).

RESULTS AND DISCUSSION

After comparing the data obtained during our botanical research at 11 priority localities, with the previously formed database, presence of 24 vascular plant taxa that were not previously recorded for the SNR "Titelski breg" was determined. Within this this number, 18 new species, 5 subspecies and 1 variety were found. Plant taxa names are listed alphabetically with name of plant family, locality, coordinates, collectors and dates (Table 3).

Table 3. New vascular plant taxa recorded in SNR "Titelski breg" during the botanical research in 2022 and 2023

No.	Taxon and family	Data on locality, collection date and legators
1	*Adonis flammea* Jacq. 1776 (Ranunculaceae)	Fokini oraji (N 45.23348, E 20.30181, leg. JP & DO, 11.05.2023.)
2	*Ailanthus altissima* (Mill.) Swingle 1916* (Simaroubaceae)	Vilovo (N 45.249265, E 20.162671, leg. JP, AV, KĐ & DO, 16.11.2022.)
3	*Althaea hirsuta* L. 1753 (Malvaceae)	Dukatar (N 45.28312, E 20.24219, leg. AV & DO, 05.06.2023.), Fokini oraji (N 45.23348, E20.30181, leg. JP & DO, 11.05.2023.; N 45.23348, E 20.30181, leg. AV & DO, 05.06.2023.)

4	*Arabidopsis thaliana* (L.) Heynh. 1842 (Brassicaceae)	Kapetanov gully (N 45.23557, E 20.18295, leg. AV & DO, 31.03. 2023), Demljankov (N 45.25389, E 20.28528, leg. AV & DO, 30.03.2023), Dukatar (N 45.28668, E 20.23276, leg. AV & DO, 30.03.2023), Mošorin (N 45.29586, E 20.19861, leg. AV & DO, 31.03.2023), Šajkaš (N 45.27992, E 20.12816, leg. AV & DO, 31.03.2023)
5	*Ballota nigra* L. 1753 subsp. *foetida* (Vis.) Hayek 1929 (Labiatae)	Topola (N 45.223229, E 20.214645, leg. JP, AV, KĐ & DO, 10.11.2022), Kapetanov gully (N 45.23741, E 20.185061, leg. JP, AV, KĐ & DO, 10.11.2022), Vilovo (N 45.249214, E 20.159358, leg. JP, AV, KĐ & DO, 16.11.2022), Dukatar (N 45.282786, E 20.236989, leg. JP, AV, KĐ & DO, 23.11.2022; N 45.28312, E 20.24219, leg. AV & DO, 3.10.2023), Mošorin (N 45.297324, E 20.194547, leg. JP, AV, KĐ & DO, 23.11.2022), Demljankov gully (N 45.25501, E 20.28314, leg. AV & DO, 05.06.2023; N 45.25501, E 20.28314, leg. JP & DO, 28.07.2023; N45.25501, E20.28314, leg. AV & DO, 03.10.2023)
6	*Carex distans* L. 1759 (Cyperaceae)	Vodice (N 45.21405, E 20.23321, leg. JP & AV, 28.03.2023; N 45.21405, E 20.23321, leg. KĐ & DO, 28.04.2023; N 45.21405, E 20.23321, leg. KĐ & DO, 02.06.2023; N 45.21309, E 20.23241, leg. JP & DO, 18.07.2023)
7	*Centaurea calcitrapa* L. 1753 subsp. *calcitrapa* (Compositae)	Vodice (N 45.22153, E 20.21897, leg. JP & DO, 18.07.2023)
8	*Cephalaria transsylvanica* (L.) Schrad. 1814 (Dipsacaceae)	Demljankov gully (N 45.25501, E 20.28314, leg. JP & DO, 28.07.2023)
9	*Cerastium brachypetalum* Pers. 1805 (Caryophyllaceae)	Demljankov gully (N 45.26057, E 20.27886, leg. JP & DO, 11.05.2023), Dukatar (N 45.28312, E 20.24219, leg. JP & DO, 11.05.2023), Fokini oraji (N 45.23348, E 20.30181, leg. JP & DO, 11.05.2023), Mošorin (N 45.2968, E 20.19442, leg. JP & DO, 11.05.2023), Grac (N 45.23145, E 20.19616, leg. KĐ & DO, 28.04.2023), Kapetanov gully (N45.23557, E20.18295, leg. KĐ & DO, 28.04.2023)
10	*Cerastium glomeratum* Thuill. 1799 (Caryophyllaceae)	Topola (N 45.22514, E 20.21581, leg. KĐ & DO, 28.04.2023), Šajkaš (N 45.27819, E 20.12843, leg. KĐ & DO, 28.04.2023), Vodice (N 45.22227, E 20.21647, leg. KĐ & DO, 28.04.2023)
11	*Cerastium pumilum* Curtis 1794 var. *glutinosum* (Fries) E. Rico 1987 (Caryophyllaceae)	1st gully from Topola to Vilovo (N 45.22804, E 20.20188, leg. KĐ & DO, 28.04.2023), Mošorin (N 45.2968, E 20.19442, leg. JP & DO, 11.05.2023), Šajkaš (N 45.27992, E 20.12816, leg. KĐ & DO, 28.04.2023), Topola (N 45.22589, E 20.21423, leg. KĐ & DO, 28.04.2023), Vodice (N 45.22153, E 20.21897, leg. KĐ & DO, 28.04.2023; N 45.21915, E 20.22819, leg. KĐ & DO, 28.04.2023; N 45.22227, E 20.21647, leg. KĐ & DO, 02.06. 2023)
12	*Chamaecytisus hirsutus* (L.) Link 1831 (Leguminosae)	Dukatar (N 45.287079, E 20.230881, leg. JP, AV, KĐ & DO, 23.11.2022)
13	*Cirsium vulgare* (Savi) Ten. 1835-1836 (Compositae)	Topola (N 45.22589, E 20.21423, leg. BB & DO, 29.09.2023), Vodice (N 45.2213, E 20.21809, leg. BB & DO, 29.09.2023)

14	*Consolida regalis* Gray 1822 subsp. *paniculata* (Host) Soó 1922 (Ranunculaceae)	Grac (N 45.23145, E 20.19616, leg. JP & DO, 18.07.2023; N 45.23145, E 20.19616, leg. BB & DO, 29.09.2023), Demljankov gully (N 45.26057, E 20.27886, leg. AV & DO, 05.06.2023; N 45.26057, E 20.27886, leg. JP & DO, 28.07.2023), Dukatar (N 45.284629, E 20.237625, leg. JP, AV, KĐ & DO, 23.11.2022), Fokini oraji (N 45.23348, E 20.30181, leg. AV & DO, 05.06.2023; N 45.23348, E 20.30181, leg. JP & DO, 28.07.2023), Mošorin (N 45.295987, E 20.197513, leg. JP, AV, KĐ & DO, 23.11.2022; N 45.29674, E 20.19526, leg. AV & DO, 05.06.2023; N 45.29674, E 20.19526, leg. JP & DO, 28.07.2023), Šajkaš (N 45.27992, E 20.12816, leg. JP & DO, 28.07.2023), Topola (N 45.228618, E 20.214184, leg. JP, AV, KĐ & DO, 10.11.2022), Vilovo (N 45.250689, E 20.165175, leg. JP, AV, KĐ & DO, 16.11.2022)
15	*Crepis pulchra* L. 1753 (Compositae)	Fokini oraji (N 45.23177, E 20.30219, leg. AV & DO, 05.06.2023), Dukatar (N 45.28312, E 20.24219, leg. AV & DO, 05.06.2023)
16	*Festuca pratensis* Huds. 1762 (Gramineae)	Mošorin (N 45.29674, E 20.19526, leg. AV & DO, 05.06.2023), Vodice (N 45.21405, E 20.23321, leg. KĐ & DO, 02.06.2023)
17	*Galium spurium* L. 1753 (Rubiaceae)	Demljankov gully (N 45.25501, E 20.28314, leg. JP & DO, 11.05.2023; N 45.25501, E 20.28314, leg. AV & DO, 05.06.2023)
18	*Ipomoea purpurea* (L.) Roth 1787* (Convolvulaceae)	Vilovo (N 45.250597, E 20.164996, leg. JP, AV, KĐ & DO, 16.11.2023)
19	*Onopordum acanthium* L. 1753 (Compositae)	Demljankov gully (N 45.25501, E 20.28314, leg. JP & DO, 11.05.2023; N 45.25501, E 20.28314, leg. AV & DO, 05.06.2023; N 45.25501, E 20.28314, leg. JP & DO, 28.07.2023), Dukatar (N 45.28312, E 20.24219, leg. JP & DO, 11.05.2023; N 45.28312, E 20.24219, leg. AV & DO, 05.06.2023.; N 45.28312, E 20.24219, leg. JP & DO, 28.07.2023), 1st gully from Topola to Vilovo (N 45.22804, E 20.20188, leg. AV & DO, 05.06.2023; N 45.22804, E 20.20188, leg. JP & DO, 18.07.2023), Mošorin (N 45.29674, E 20.19526, leg. JP & DO, 11.05.2023; N 45.29674, E 20.19526, leg. AV & DO, 05.06.2023), Šajkaš (N 45.27992, E 20.12816, leg. KĐ & DO, 02.06.2023; N 45.27992, E 20.12816, leg. JP & DO, 28.07.2023), Topola (N 45.22589, E 20.21423, leg. KĐ & DO, 02.06.2023; N 45.22589, E 20.21423, leg. JP & DO, 18.07. 2023), Vodice (N 45.2213, E 20.21809, leg. JP & DO, 18.07.2023)
20	*Solanum nigrum* L. 1753 subsp. *schultesii* Wessely 1960* (Solanaceae)	Topola (N 45.228338, E 20.213723, leg. JP, AV, KĐ & DO, 10.11.2022), Kapetanov gully (N 45.237223, E 20.184823, leg. JP, AV, KĐ & DO, 10.11.2022), Vilovo (N 45.250818, E 20.165314, leg. JP, AV, KĐ & DO, 16.11.2022), Šajkaš (N 45.2789, E 20.127797, leg. JP, AV, KĐ & DO, 16.11.2022), Mošorin (N 45.296404, E 20.198629, leg. JP, AV, KĐ & DO, 23.11.2022), Dukatar (N 45.284503, E 20.238033, leg. JP, AV, KĐ & DO, 23.11.2022)
21	*Symphyotrichum lanceolatum* (Willd.) G.L. Nesom 1995* (Compositae)	Vilovo (N 45.249414, E 20.162991, leg. JP, AV, KĐ & DO, 16.11.2023), Kapetanov gully (N 45.237092, E 20.184707, leg. JP, AV, KĐ & DO, 10.11.2023)
22	*Trifolium fragiferum* L. 1753 subsp. *bonannii* (C. Presl) Sojak 1963 (Leguminosae)	Vodice (N 45.2213, E 20.21809, leg. JP & DO, 18.07.2023; N 45.21309, E 20.2324, leg. JP & DO, 18.07.2023)
23	*Veronica triloba* (Opiz) Opiz 1825 (Plantaginaceae)	Grac (N 45.23145, E 20.19616, leg. KĐ & DO, 28.04.2023)
24	*Vulpia myuros* (L.) C. C. Gmel. 1805 (Gramineae)	1st gully from Topola to Vilovo (N 45.22804, E 20.20188, leg. AV & DO, 05.06.2023)

Legend: *invasive alien plant

The majority of newly recorded plant taxa in SNR "Titelski breg" are autochtonous vascular plants (Figure 2) widely distributed in Serbia. On the other side, only for *Galium spurium* (Figure 2/j) and *Veronica triloba* (Figure 2/n) there is not much information on their distribution (Josifović, 1972–1976, 1977; Sarić, 1992; Sarić & Diklić, 1986; Stevanović, 2012). The explanation for the "absence" of certain taxa that could be regarded as common (e.g. *Arabidopsis*, *Cirsium*, *Cerastium*, *Onopordum*, *Symphyotrichum*, *Veronica*) is that the botanical investigations in the past were mostly not carried out during early spring and autumn.

Figure 2. Exsiccates of the selected vascular plant taxa recorded in the Reserve (**a** *Althaea hirsuta*; **b** *Ballota nigra* subsp. *foetida*; **c** *Cephalaria transsilvanica*; **d** *Cerastium brachypetalum*; **e** *Cerastium glomeratum*; **f** *Cerastium pumilum* var. *glutinosum*; **g** *Chamaecytisus hirsutus*; **h** *Consolida regalis* subsp. *paniculata*; **i** *Festuca pratensis*; **j** *Galium spurium*; **k** *Solanun nigrum* subsp. *schultesii*; **l** *Symphyotrichum lanceolatum*; **m** *Trifolium fragiferum* subsp. *bonani*; **n** *Veronica triloba*; **o** *Vulpia myuros*.

At 11 priority locations, four vascular plants were determined as invasive: *Ailanthus altissima* (Anonymous, 2014; Anačkov et al., 2013; Lazarević et al., 2012), *Ipomea purpurea* (Anačkov et al., 2013), *Solanum nigrum* subsp. *schultesii*

(Figure 2/k) (Anačkov et al., 2013) and *Symphyotrichum lanceolatum* (Figure 2/l) (Anačkov et al., 2013; Lazarević et al., 2012). The first one originates from East Asia, while the other three vascular plants were introduced from Americas (Josifović, 1973, 1974). Although *I. purpurea*, *S. nigrum* subsp. *schultesii* and *S. lanceolatum* are not included in the List of Invasive Alien Species of Union Concern (IAS Regulation 1143/2014), they could be considered as invasive plants due to their widespread distribution. Even though, *Centaurea calcitrapa* subsp. *calcitrapa* and *Consolida regalis* subsp. *paniculata* (Figure 2/h) were cited as neophytes of Pannonian part of Serbia (Anačkov et al., 2013), critical review of their natural range revealed that the area of the Pannonian Plain is also part of their natural range (POWO, 2023).

Generally, the largest number of vascular plant taxa during botanical research conducted at 11 priority localities in 2022 and 2023 was found at unmanaged sites in the 1st zone of protection (Dukatar and Demljankov), compared to managed sites in 2nd and 3rd zone of protection, with one major exeption – pasture "Vodice" (Figure 3). These results can be attributed to the fact that Dukatar and Demljankov are gullies with preserved natural vegetation, and more or less highly unapproachable, so less often botanicaly investigated in the past. On the other side, locality Vodice near Titelski breg was included within the borders of the Reserve as a buffer zone, in the first place with aim to conserve and protect the population of small rodent (ground squirrel), and more rarely visited by botanists, until now.

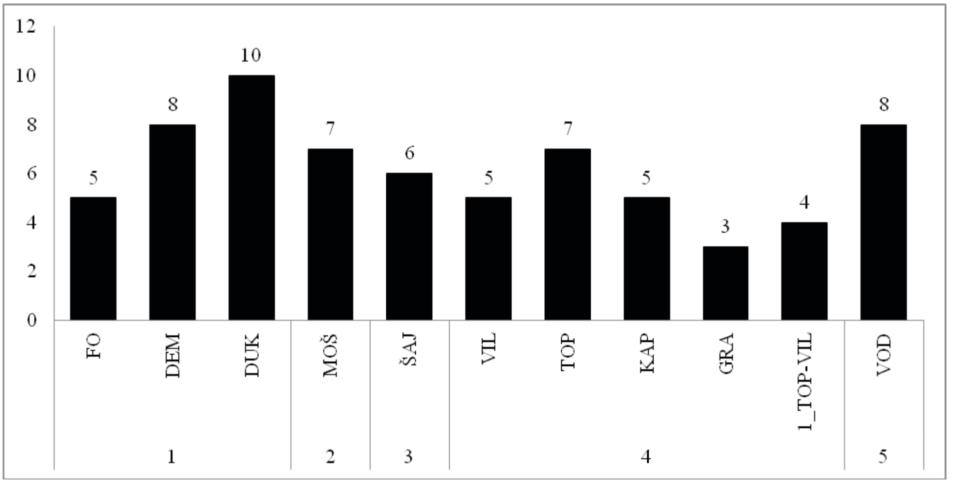

Figure 3. Number of recorded vascular plant taxa at 11 localities which differ according to the management measures (abbreviations of the localities follows Table 2; 1 – no measures; 2 – grazing by sheep; 3 – no measures; 4 – grazing by sheep and mowing; 5 – grazing by sheep and cows, and mowing).

CONCLUSION

During the recent botanical study performed in 2022–2023 at 11 localities, presence and distribution of 24 new vascular plant taxa in SNR "Titelski breg" is reported. These new records contribute to the knowledge of their general distribution, and are important for comprehensive publications that consider diversity of vascular flora of Serbia. Each data on vascular flora can be of great importance not only for making a complete list on vascular plants of the Reserve, but also for future managment steps in the Reserve, especially those regarding allochtonous plants. In other words, these data are crucial and must not be ignored in future processes of evaluation of protection of the Reserve. In the end, some areas such as Titelski breg, can be considered botanically well investigated, yet these kinds of results indicate need for constant field research, especially in the era of changes caused by direct and/or indirect human actions.

ACKNOWLEDGEMENT

Research was funded by Ministry of Science, Technological Development and Innovation of the Republic of Serbia (Grants no. 451-03-66/2024-03/200125 & 451-03-65/2024-03/200125) and SNR "Titelski breg". Authors are grateful to the colleague Dr Jernej N. Jogan (University of Ljubljana, Biotechnical Faculty, Department of Biology, Večna pot 111, 1000 Ljubljana, Slovenia) for determination, confirmation and/or revision of the taxa belonging to the family Gramineae.

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ОРИГИНАЛНИ ЧЛАНАК
Примљен: 16. 11. 2023.
Прихваћен: 9. 1. 2024.

НОВИ ПОДАЦИ О ТАКСОНИМА ВАСКУЛАРНЕ ФЛОРЕ НА ПОДРУЧЈУ СПЕЦИЈАЛНОГ РЕЗЕРВАТА ПРИРОДЕ „ТИТЕЛСКИ БРЕГ" (ВОЈВОДИНА, СРБИЈА)

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РЕЗИМЕ: У овом раду приказани су резултати ботаничког истраживања које је спроведено у периоду од октобра 2022. до октобра 2023. године на подручју Специјалног резервата природе „Тителски брег". Ово подручје је заштићено због његове јединствености и природних и културних вредности, а последњих неколико деценија заштита се спроводи активним мерама, укључујући кошење, испашу, уклањање инвазивних биљака и константну промоцију и едукацију заинтересоване јавности. Резерват је подручје важно за биљке и птице, део је еколошке мреже Републике Србије, а у оквиру њега се налазе и панонске степе на лесу, које су НАТУРА 2000 станишта. Флора и вегетација Тителског брега је у прошлости темељно и често била предмет истраживања, како страних, тако и домаћих ботаничара. Први подаци сежу из друге половине XIX века, при чему је највећи број података и публикација објављен током последњих двадесет година XX века, а прошло је више од две деценије од свеобухватног ботаничког истраживања. Због свега наведеног, у овом раду су обједињени ботанички подаци из свих релевантних и најзначајнијих публикација које се односе на Тителски брег у виду базе података. Потом је уз помоћ управљача утврђен интензитет и период спровођења мера управљања на локалитетима у резервату, јер је познато да оне значајно утичу на диверзитет и стање популација на отвореним травнатим стаништима. На основу спроведене анкете о примени мера и података у креираној бази, одабрано је 11 локалитета од значаја за ботаничко истраживање који се разликују према зони заштите, мерама које се спроводе и броју претходних података о забележеним биљкама. Након одабира, спроведено је ботаничко истраживање, а затим и поређење сакупљених података са претходно објављеним подацима који су синтетисани у виду базе података, како би се сачинила листа таксона који су нови за Резерват и како бисмо добили детаљне податке о њиховом распрострањењу. Укупно је забележено 18 врста, пет подврста и један варијетет који до сада нису познати за подручје резервата. Овакви резултати су веома значајни јер представљају доказ о постојању потребе за константним ботаничким истраживањима, посебно на подручјима која су у већој или мањој мери под антропогеним утицајем, како би у будућности евентуално утицали на дефинисање адекватних мера за одрживо управљање и очување природних вредности у Републици Србији. Такође, овакви подаци могу бити коришћени, како за свеобухватне студије флоре Србије, тако и за процес евалуације и управљања у СРП „Тителски брег".

КЉУЧНЕ РЕЧИ: заштићено подручје, инвазивне биљке, лес, степе, управљање

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 147, 33—45, 2024

UDC 551.583(497.11)
https://doi.org/10.2298/ZMSPN2447033V
ORIGINAL ARTICLE
Received: 2023/10/26
Accepted: 2023/12/12

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DYNAMICS OF CHANGE OF CLIMATE CONDITIONS AND EXTREME WEATHER EVENTS IN SERBIA

ABSTRACT: Analysis of climate change in Serbia was done using the data available from the Digital Climate Atlas of Serbia. It includes observed climate change and future climate change projections according to scenarios of greenhouse gases emissions, RCP4.5 and RCP8.5, until the end of 21st century, compared to the reference period 1961–1990. According to the main climate change indicator, change of average air temperature, climate change is accelerating. Results showed that climate conditions in the mid-century period are known with high reliability, while changes of climate conditions in the second half of the century depend on reductions of global net emissions of greenhouse gases, i.e. on the fulfillment of the Paris agreement. In this paper, the changes in climate hazards are presented. They are of highest significance for risk increase in health and safety and in agriculture. For the mid-century period, an expected increase of average air temperature for Serbia is about 3.1 °C. Expected frequency of extreme precipitation is at least once per year. Each year is most likely expected to be a year with drought. Average frequency of days per year, with temperatures above 35 °C, for the territory of Serbia, will be in the range of 9–13, compared to one day in the reference period. In the paper the summary of climate change characteristics in Serbia is also given, based on the presented results and results derived from the literature, categorized into groups of climate hazards, which cause: too warm conditions, too wet conditions, too dry conditions and storms.
KEYWORDS: climate change, climate hazards, adaptation, Serbia

INTRODUCTION

Climate change in Serbia, on national level, have been monitored over 10 years, through National Communications to UNFCCC, as well as through the research work (MOEP, 2010, 2017; Vuković et al., 2018; Đurđević et al., 2018; Vuković Vimić et al., 2022; Životić and Vuković Vimić, 2022; Tošić et al., 2023). Analyses of climate change are regularly updated according to the methodology of the latest published reports of the Intergovernmental Panel on Climate Change (IPCC Assessment Reports – ARs).

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According to the IPCC Sixth Assessment Report (IPCC, 2021) average global surface air temperature for the period 2011–2020, compared to the value in the preindustrial period, has increased by 1.1 °C. In the period 2011–2020 with respect to the period 1961–1990, average temperature for Serbia increased by 1.8 °C, while the average June–July–August (JJA) temperature increased by 2.4 °C (Životić and Vuković Vimić, 2022). Frequency of years with drought in Serbia has increased from one per decade during 1961–1990 to five per decade during 2011–2020 (Vuković Vimić et al., 2022). Agricultural producers identified drought, high temperatures and hail as most significant climate change risks.

Sectors with high priority for urgent planning and implementation of adaptation measures have been identified in the Nationally Determined Contributions of the Republic of Serbia (a document which was revised and adopted in 2022 by the government). According to this document, and according to the Law on Climate Change (adopted in 2021), the Republic of Serbia needed to develop and adopt a national plan for the implementation of adaptation. The Climate Change Adaptation Programme for the period 2023–2030 with the Action Plan for 2024–2026 was adopted in December 2023 (RS, 2023). Priority sectors for the implementation of adaptation to climate change are: agriculture, forestry, public health, infrastructure, biodiversity, as well as water management, which is considered as an integrated component in the risk assessment and planning of adaptation measures in other sectors. The program also considers the impact of climate change on the increasing risk of already existing problems with the degradation of natural resources (air, water and soil quality degradation).

In order to enable the integration of climate change adaptation to local level, and the application of climate change knowledge into different areas of science and fields of expertise, a web-portal has been created with available data for Serbia for both past and future climate analysis – Digital Climate Atlas of Serbia (atlas-klime.eko.gov.rs). It enables easy access to data and various products (indices) adjusted for different users (basic to advanced). Here, the analysis of selected parameters is presented, done by using data from the Digital Climate Atlas. Results show the main characteristics of climate change over the territory of Serbia, including the assessments of climate hazards on the national level. Further, a summary of climate change in Serbia has been presented, which is compiled from various literature sources.

METHODOLOGY

Analysis of the climate change in Serbia has been done using the observed daily data form the EOBS database, which provides interpolated daily temperature and precipitation at a resolution of about 10 km, and the data from an ensemble of 8 climate models form the EURO-CORDEX database. All data are also included in the web-portal Digital Climate Atlas of Serbia (atlas-klime.eko.gov.rs), where details of the sources of data can be found. Climate change analysis was done for the near past climate period 2011–2020 and for the decade 2011–2020 (because of the increasing rate of changes), and for the future climate periods: near future 2021–2040, mid-century 2041–2060, and end of century 2081–2100. Observed and future changes of selected parameters are represented with respect to the values obtained for the reference climate period 1961–1990. Future climate change is considered according to the two scenarios of greenhouse gasses emissions RCP4.5 and RCP8.5 (IPCC, 2021).

There are many climate indices which show different aspects of climate change and changes in climate hazards in Serbia. In order to systematize the representation of the results, four groups of climate hazards have been made, according to the conditions they contribute to. It is important to understand that some changes of climate conditions can contribute to different groups of climate hazards (for example, increased climate variability can contribute to more humid and to more dry conditions). Table 1 presents groups of climate hazards and climatic impact-drivers identified to contribute to these four groups. Group "excess of heat" shows the changes which cause, under climate change, too warm conditions in average for some period or during an extreme event, compared to the reference period. Group "excess of precipitation" shows the changes over shorter or longer period which cause problems with too much water/moisture and includes changes in intensive precipitation and accumulated precipitation over some period. Group "too dry" shows the changes which indicate the deficit of precipitation in some period, which causes problems because of lack of water/moisture. Group "storms" includes information about severe and short-in-duration weather events which cause hazards related to wind gusts and intensive precipitation including hail. In the Table 1 there are marked parameters which are analyzed in this study, while for other information they are derived from the literature. Analysis and representation of data by group of hazards follow the methodology of the IPCC AR6 (IPCC, 2021; IPCC, 2022).

Table 1. Groups of climate hazards and climatic impact-drivers which contribute to each group.

Group of climate hazards	Climatic impact-drivers
Too warm (excess of heat)	• Increased climate variability • Increase of average temperatures* • Increase in frequency/intensity of heat waves, increase in frequency of extreme (high) temperatures*
Too wet (excess of water/moisture)	• Increased climate variability • Change in annual distribution of precipitation • Change in distribution of precipitation by intensity, Increase in extreme precipitation*
Too dry (deficit of water/moisture)	• Increased climate variability • Change in annual distribution of precipitation • Droughts* • Level of aridity/dryness of climate
Storms	• Change in distribution of precipitation by intensity

* Climatic impact-drivers which are analyzed on the national level in this study. For the frequency of the extreme high temperatures, analysis is done using the information on the number of days with temperatures above 35 °C. For the analysis of changes in extreme precipitation, data on days with precipitation above 30 mm have been used. For drought indicator is used SPEI6a (Standardized Precipitation Evapotranspiration Index, for 6 months, ending with August).

RESULTS

Figure 1 shows anomalies of the observed surface air temperature, averaged for Serbia, compared to the 1961–1990, and the ranges of their values for the future periods compared to the same reference period, derived according to the scenarios RCP4.5 (red lines) and RCP8.5 (purple lines). For the future periods, values from the results of the ensemble of models are given: median value (bold lines), lower (25th percentile) and upper (75th percentile) thresholds of the most probable range (thinner lines). Most probable expected outcomes for the mid-century period are marked as green circles. These values are considered as most probable, if observed trends of changes are considered. In the assessment of the average temperature, they correspond to the 75th percentile of the climate models ensemble. In the period 2001–2020 the average temperature was 1.4 °C higher than the average for 1961–1990, while in second decade of this period 2011–2020 increase was 1.8 °C. Most probable increase in the future periods compared to the same reference period are: 2.1 °C (1.9–2.1) for the period of near future 2021–2030, 3.1 °C (2.5–3.1) for the mid-century period 2041–2060 and in the range 3.2–5.8 °C (most probable according to RCP4.5 and RCP8.5) in the period end of the century 2081–2100. Significant difference between outcomes according to the different scenarios is in the second half of the 21st century, when global reductions of net greenhouse gasses emissions are expected to have larger effects.

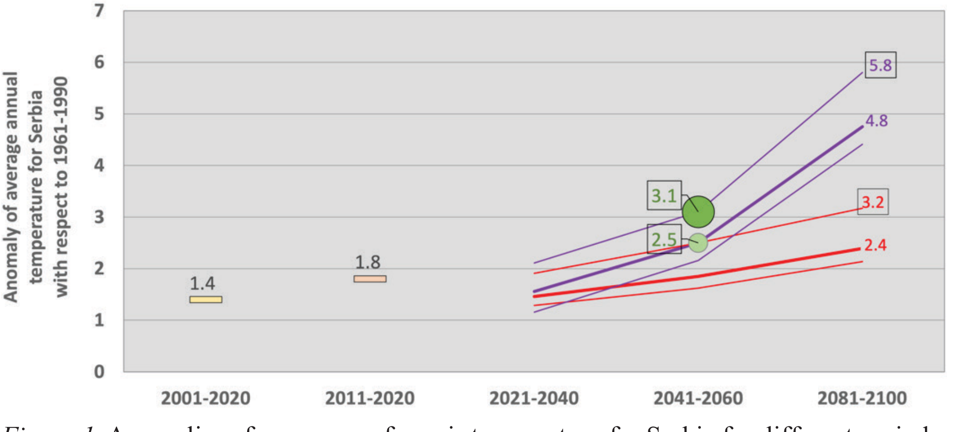


Figure 1. Anomalies of average surface air temperature for Serbia for different periods, compared to the reference period 1961–1990. For the future climate periods, values are presented according to the RCP4.5 (in red) and RCP8.5 (in purple), with bold line for models ensemble median values, and with thinner lines values for 25th and 75th percentiles. With green circles are highlighted most probable values assessed for the mid-century period.

Significant change of average annual accumulated precipitation is not expected, but there is a change in the precipitation distribution by intensity, with an increasing frequency of extreme precipitation (here defined as days with precipitation over 30 mm). Figure 2 shows observed and future values in the average number of days per year with extreme precipitation, for the territory of Serbia, for the observed (past) periods and future periods according to the different scenarios. These events were rare during the reference period. Their frequency in 1961–1990 was 0.4 (the number of appearances, on average, was less than every second year). In the period of near past their frequency increased (+0.4 for 2001–2020 and +0.5 for 2011–2020) and reached the values of 0.9 in the decade 2011–2020. For the future, all models according to both emission scenarios show a trend of increase for this index, but mainly underestimate the intensity of change, meaning that observed changes exceed the values projected for the future according to the median of the models ensemble. In the mid-century period, most probably, the average frequency of days with extreme precipitation is expected to be 1.4 (if observed trends are considered), meaning that this climate hazard can be expected to happen every year, at least once and possible more than once per year.

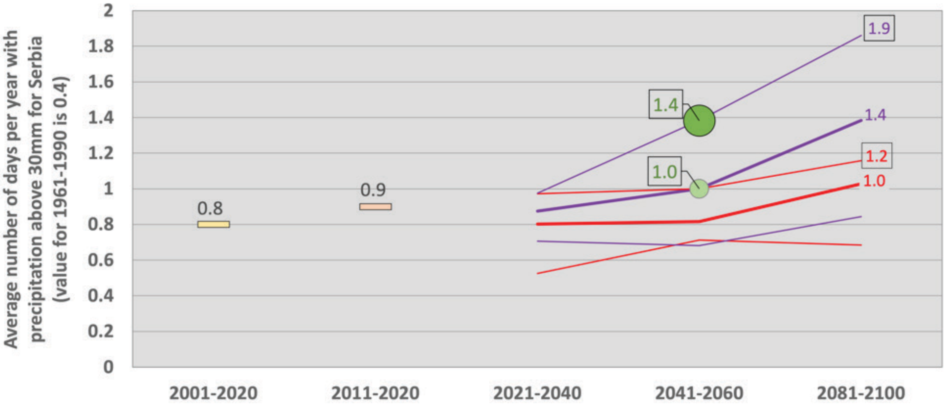
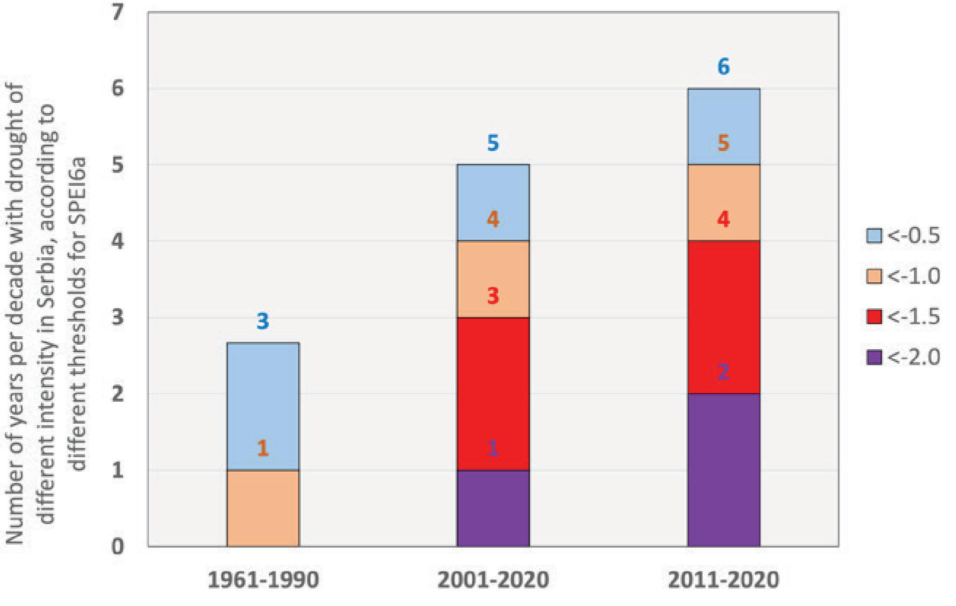


Figure 2. Frequency of days with extreme precipitation, averaged for Serbia, for different periods with respect to the reference period 1961–1990. For the future climate periods, values are presented according to the RCP4.5 (in red) and RCP8.5 (in purple), with bold line for the median values of the models ensemble, and with thinner lines for the values of the 25th and 75th percentiles. Most probable values assessed for the mid-century period are highlighted with green circles.

Frequency and intensity of droughts in Serbia are increasing. SPEI6a has been selected as an indicator for drought in this study because its values correspond well with dry conditions which caused damages and losses in Serbia (Đurđević, 2020). Using the average SPEI6a value for the territory of Serbia, the following categories are introduced: a value below -0.5 means that the "conditions are drier" (to which level depends on following categories), a value bellow -1 means that "year was with drought" in Serbia, a value bellow -1.5

means that “year was with severe drought”, and a value below -2.0 means that “year was with extreme drought”. Frequency of years per decade for different periods in the past, defined according to the adopted categorization of SPEI6a values, are given in Figure 3 – left. In the period 1961–1990, a “year with drought” was happening once per decade, while more extreme drought conditions (SPEI6a values bellow -1.5) were not recognized in average for Serbia or were very rare events. In the period 2001–2020, the frequency of “years with drought” per decade was four, out of which three years per decade were with severe drought, and one of them had extreme drought. In the second decade of this period (2011–2020), the frequency is larger, meaning that five years were with drought, four years were with more intensive drought conditions and two of them were with extreme drought. Those years were 2012 and 2017, when the largest losses were recorded in agriculture (for these two years about 3.5 billion US dollars; Vuković Vimić et al., 2022). Observed and future frequency of years with drought per decade are presented in Figure 3 – right. Future increase in the frequency of “years with drought” in Serbia is highly probable because all models, according to both scenarios, show an increasing trend. It is expected to reach maximum in the mid-century climate, meaning that drought can be expected each year. By the end of the century, a high risk of drought will remain according to both scenarios.



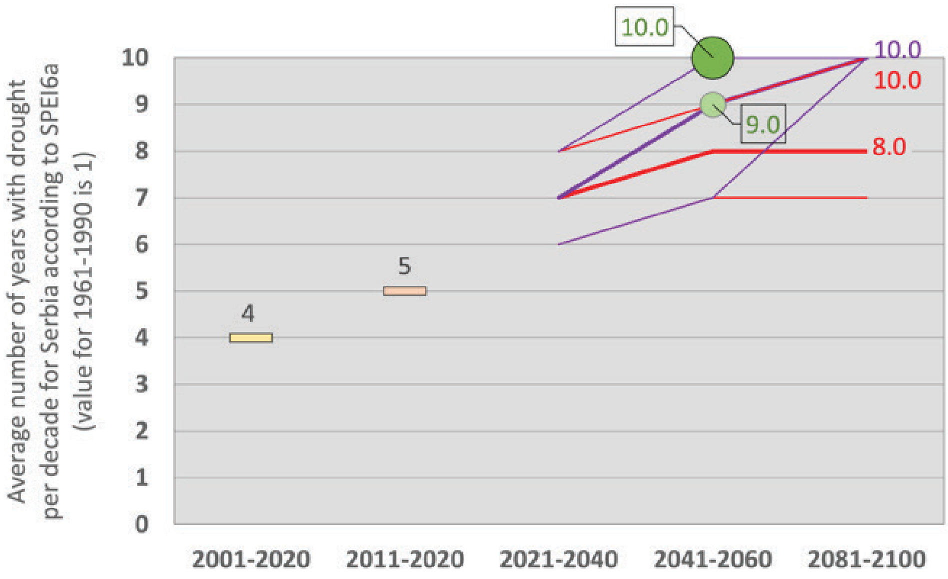
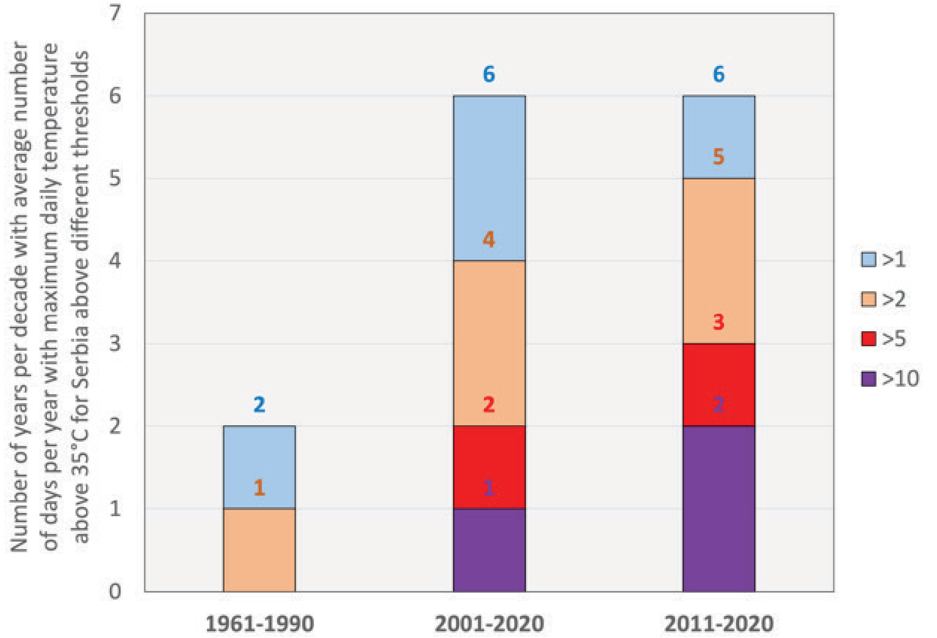


Figure 3. Frequency of years per decade with values of average SPEI6a for Serbia bellow different thresholds, for different past periods (panel left), and the frequency of years with drought (SPEI6a < -1) in the period of near past and in the future climate periods according to the different greenhouse gasses emission scenarios (panel right). For the future climate periods, values are presented according to the RCP4.5 (in red) and RCP8.5 (in purple), with bold line for the median values of the models ensemble, and with thinner lines for the values of the 25th and 75th percentiles. Most probable values assessed for the mid-century period are highlighted with green circles.

Average number of days with maximum daily temperatures above 35 °C per year, averaged for Serbia, was chosen as an indicator for the analysis of change of the high temperature conditions. Average value for Serbia was calculated as an average of the frequency derived for all points in Serbia, including areas at lower altitudes, as well as mountain areas. According to the values of this indicator we define: values over 1 – there is a risk of extremely high temperatures, over 2 – high risk, over 5 – very high risk, over 10 – extremely high risk. Frequency of years per decade defined according to the given criteria are presented in Figure 4 – left. In the period 1961–1990 there was, in average, one "year with risk" of high temperatures per decade, but years with very high/ extreme levels of risks did not exist (or were very rare, with an average frequency equal or close to 0). In the period 2001–2020, in average, four years per decade had "high risk", out of which two had "very high" and one of them had "extremely high risk". In the second decade of this period (2011–2020), five years were with "high risk", out of which three were with "very high risk" (two of them had "extremely high risk"). Years with extremely high risks in the period 2001–2020 were 2007, 2012 and 2017. In these three years record



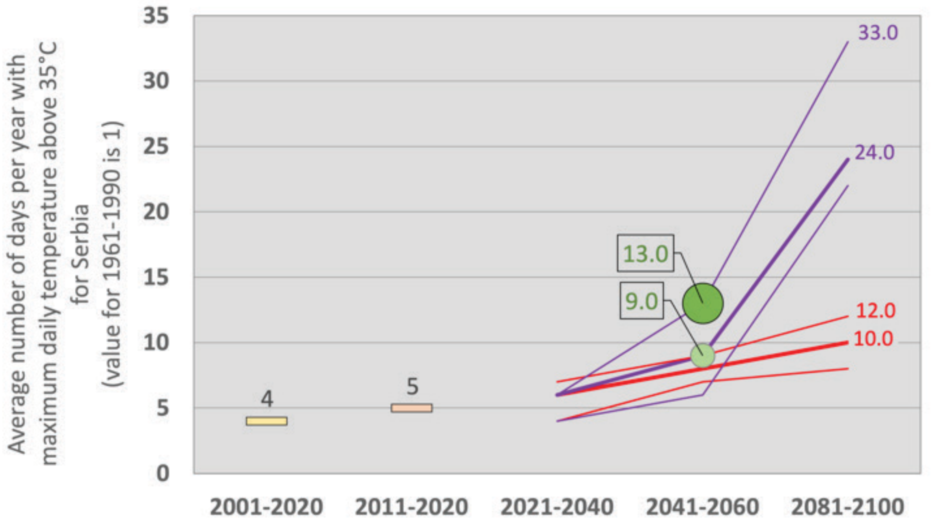


Figure 4. Frequency of years per decade with values of average number of days with temperatures above 35 °C for Serbia above different thresholds, for different periods in the past (left panel) and average number of days per year with maximum daily temperature above 35 °C for Serbia in the period of near past and for the future periods according to different emission scenarios (right panel). For the future climate periods, values are presented according to the RCP4.5 (in red) and RCP8.5 (in purple), with bold line for the median values of the models ensemble, and with thinner lines for the values of the 25th and 75th percentiles. Most probable values assessed for the mid-century period are highlighted with green circles.

high temperatures were measured at meteorological stations in Serbia (RHMSS annual reports available at https://www.hidmet.gov.rs/ciril/meteorologija/klimatologija_produkti.php, in Serbian). In Figure 4 (right panel) there are presented values of the average number of days per year with maximum daily temperature over 35 °C, for Serbia, for the near past and the future climate periods, according to different emission scenarios. As mentioned before, for the reference period 1961–1990 the value of this level of risk was one. For the period 2001–2020 it was four and for 2011–2020 it was five. In the future, further increase of this indicator is certain because all models according to both scenarios show an increasing trend. In the mid-century period, 2041–2060, expected values are in the range 9–13 (more probably 13), while at the end of the century according to RCP8.5 is expected to be over 30. This means that frequency of years with very high and extremely high risks will most probably reach maximum values in the mid-century period (they can be expected each year).

DISCUSSION

Presented results additionally confirm increasing risks of the climate hazards caused by climate change, which are also recognized in the previous studies. Results are given for the national level, but it is important to understand that the assessments of risks of climate hazards, in order to develop and implement effective adaptation measures, should be done at the level of implementation (local level). Short summary of collected information on climate change in Serbia, derived from presented results and comprehensive analysis done within the National Communications, studies and scientific research (Đurđević et al., 2018; Rädler et al., 2019; Vuković Vimić et al., 2022; Životić and Vuković Vimić, 2022; Vujadinović Mandić et al., 2022), classified into the groups of climate hazards (as in Table 1) is presented in Table 2. Changes are presented with respect to the values in the reference period 1961–1990. Review is given here only for the mid-century period (2041–2060) because for this period outcomes are known with high reliability, and afterwards climate change will stabilize in case of RCP4.5 or will further increase according to RCP8.5, so the ranges of possible outcomes are wide.

Climatic impact-drivers, which are presented in this study, as other derived from different literature in Table 2, also show the favourable conditions for increasing risks of other hazards, such as land degradation, floods, landslides, fires, etc. (with more details given in MOEP, 2023). In planning adaptation measures, it is important to consider the nexus of climate-water-land, meaning that impacts on water resources and land degradation should be considered when finding the solution for mitigation of negative impacts.

Table 2. Short summary on information on climate change in Serbia distributed in the main climate hazards groups

Group of climate hazards	2001–2020 (2011–2020)	2041–2060
Too warm (excess of heat)	Average temp. increase +1.4 °C (+1.8 °C). Highest increase in temperature in JJA. High risk of high temperatures in four years per decade (in five years). Extremely high risk of high temperatures in one year per decade (in two years).	Average temperature increase: +3.1 °C Highest increase during JJA. High risk of high temperatures expected each year.
Too wet (excess of water/moisture)	Shift of annual maximum of precipitation toward earlier period in the year. Larger amount of precipitation falls in form of intensive precipitation. Average number of days with extreme precipitation per year increased by +0.4 (+0.5).	Events with high precipitation continue to increase. Increase of average number of extreme precipitations per year +1. Level of risk of extreme precipitation increases, and larger area of Serbia is under risk of these events.
Too dry (deficit of water/moisture)	Drier and warmer JJA. Increased frequency of years with drought per decade: +3 (+4). Increased intensity of droughts. Increasing level of aridity of climate conditions, mainly because of the increase of temperature.	Further drying and heating of JJA. Years with drought in Serbia can be expected each year. In average for Serbia, climate is dry sub-humid.
Storms	Increase of events with very high and extreme precipitation – shows the increase of events with wind gusts and hail. Increase of average size of hail and of the area affected is expected.	

CONCLUSION

Climate change is identified through the values of slowly evolving changes, like changes in average values of temperatures and accumulated precipitation, and through changes in the intensity and frequency of extreme events, both contributing to the increasing risks of climate hazards. This means that general characteristics of climate conditions are changing, which implies average temperatures increase and change in annual distribution of precipitation, so that general climate conditions shift toward the characteristics of a subtropical climate, with longer and drier summers and annual precipitation maximum shifted toward colder part of the year. Because of the large increase of temperature expected in the future, significant change in the level of aridity will happen. This could lead to persistent problems with water deficit and land degradation caused by climate change could become permanent, if not managed properly. Addressing the increasing frequency and intensity of climate hazards (heat waves, and especially of increasing risks of high summer temperatures, extreme precipitation and storms, droughts, and consequently floods, fires, etc.) is the priority for mitigation of impacts in planning of adaptation measures, in order to reduce damages and losses and to enable the implementation of sustainable process of adaptation in the future changing climate.

Analysis presented in this paper, as well as in other literature which deals with assessments of climate change and its impacts in Serbia on the national level, including the Programme for Adaptation to Climate Change of the Republic of Serbia, shows that the increasing risks by the mid-century are certain for high temperatures, drought, extreme precipitation and storms (with wind gusts and hail). These increasing hazards have already been identified to have impacts on agricultural producers. Level of risk for different climate hazards varies depending on the location (characteristics of the terrain, population density, economic activities, etc.). Therefore, it is strongly recommended to conduct climate change risk analyses on the local level, with the engagement of local self-governments and other interested parties (producers, contractors from different sectors, the general public, etc.).

ACKNOWLEDGEMENTS

This research had technical support realized under the contract on realization and financing of science and research in 2023 between the Ministry of Science, Technological Development and Innovations of the Republic of Serbia and the Faculty of Agriculture, University of Belgrade (contract no. 451-03-47/2023-01/200116). This research was also the part of the project Advancing medium and long-term adaptation planning in the Republic of Serbia, (Ministry of Agriculture, Forestry and Water Economy and UNDP) under which was developed Programме for adaptation to climate change of Republic of Serbia for the period 2023–2030.

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ОРИГИНАЛНИ ЧЛАНАК
Примљен: 26. 10. 2023.
Прихваћен: 12. 12. 2023.

ДИНАМИКА ПРОМЕНЕ КЛИМАТСКИХ УСЛОВА И ЕКСТРЕМНИХ ВРЕМЕНСКИХ ПОЈАВА У СРБИЈИ

Ана ВУКОВИЋ ВИМИЋ, Мирјам ВУЈАДИНОВИЋ МАНДИЋ

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РЕЗИМЕ: Анализа климатских промена у Србији урађена је коришћењем података доступних из Дигиталног атласа климе Србије. Укључује уочене климатске промене и будуће пројекције климатских промена према сценаријима емисије гасова стаклене баште, РЦП4.5 и РЦП8.5, до краја 21. века, у поређењу са референтним периодом 1961–1990. Према главном индикатору климатских промена, а то је промена просечне температуре ваздуха, климатске промене се убрзавају. Резултати су показали да су климатски услови средином века познати са великом поузданошћу, док промене климатских услова у другој половини века зависе од смањења глобалне нето емисије гасова стаклене баште, односно од испуњења Париског споразума. У овом раду су приказане промене климатских хазарда које су од највећег значаја за повећање ризика по здравље и безбедност, као и у пољопривреди. За период средине века, очекивано повећање просечне температуре ваздуха за Србију је око 3,1 ºC. Очекивана учесталост екстремних падавина је најмање једном годишње. Највероватније се очекује да ће свака година бити година са сушом. Просечна учесталост дана у години, са температуром изнад 35 °C, за територију Србије биће у распону од 9–13, у односу на један дан у референтном периоду. У раду је дат и сажетак карактеристика климатских промена у Србији, на основу приказаних резултата и резултата изведених из литературе, категорисаних у групе климатских хазарда, које изазивају: превише топли услови, превише влажни услови, сувише суви услови и олује.

КЉУЧНЕ РЕЧИ: климатске промене, климатски хазарди, адаптација, Србија

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 147, 47—57, 2024

UDC 631.51:582.635.8
https://doi.org/10.2298/ZMSPN2447047S
ORIGINAL ARTICLE
Received: 2024/2/26
Accepted: 2024/4/29

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BIOSTIMULATORY ACTIVITY OF ROOT-ASSOCIATED BACILLUS ISOLATES FROM NETTLE (*Urtica dioica* L.)

ABSTRACT: The objective of this research was to isolate bacteria belonging to the genus *Bacillus* from the nettle rhizosphere, conduct biochemical and plant-growth-promoting (PGP) characterization of isolates, and examine their biostimulatory effects on the seeds of medicinal plants. Microorganisms were isolated using the agar plate method for bacterial isolation. Biochemical characterization involved evaluating the isolates' ability to produce enzymes such as lipase, amylase, pectinase, protease, cellulose, urease and gelatinase. Plant-growth-promoting (PGP) characterization included evaluation of indole-3-acetic acid (IAA), siderophore and hydrogen cyanide (HCN) production, as well as the ability to mineralize organic phosphorus compounds and solubilize phosphate. The impact of isolates on seed germination and plant growth was studied using summer savory (*Satureja hortensis* L.) and parsley (*Petroselinum crispum* L.) seeds. The experiment was conducted under laboratory conditions. The number of germinated seeds, the shoot and root length of seedlings, vigor index and root and shoot length of the plant were measured. According to the morphological description of the colony and cells, five *Bacillus* bacteria (denoted as Bac4, Bac5, Bac6, Bac7, Bac8) were selected for further examination. All isolates showed good PGP potential. The isolates that stand out are isolates Bac5, Bac7 and Bac8. The best effect on the seed germination was exerted by B5 and Bac8 isolates. Isolates Bac7 and Bac8 had the greatest stimulatory effect on seedling and plant growth. Having great PGP traits, these isolates may represent a biological alternative for the application of harmful pesticides and chemical fertilizers in the production of summer savory and parsley.

KEYWORDS: inoculation, medicinal plants, microorganisms, plant-growth-promoting rhizobacteria

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INTRODUCTION

The rhizosphere serves as a dynamic habitat for a diverse range of microorganisms. A large number of these microorganisms play a crucial role in supporting plants growth by suppressing pathogenic invasions and facilitating nutrient uptake from the soil (Leach et al., 2017). The diversity and composition of the rhizosphere bacterial community are influenced by both plant species and soil properties (Vorholt et al., 2017). Although different plant species or genotypes tend to assemble relatively distinct rhizobacterial communities (Matthews et al., 2019), these communities can be largely similar even in different environments across geographical regions.

A specialized group of bacteria residing in the plant rhizosphere is called plant growth promoting rhizobacteria (PGPR). These are bacteria that predominantly exist in the rhizosphere, rhizoplane or between cells of the root cortex (Bhattacharya and Jha 2012). They include bacteria from genera like *Agrobacterium*, *Azotobacter*, *Bacillus*, *Caulobacter*, *Chromobacterium*, *Pseudomonas*, *Rhizobium*, *Streptomyces*, etc. (Gray and Smith 2005). A significant number of these bacteria enhance the growth of plants through various mechanisms, including nitrogen fixation, phosphate solubilization, production of siderophores, phytohormones, enzymes, etc. (Zhang et al., 2013; Whang et al., 2014).

The study of rhizosphere bacteria from important medicinal plants is crucial (Bafana and Lohiya, 2013). Medicinal plants host a unique microbiome due to their distinct and structurally divergent bioactive secondary metabolites, likely responsible for the high specificity of associated microorganisms (Rios and Recio, 2005). The diversity and richness of rhizosphere microorganisms are closely related to medicinal plants, impacting their growth, development, yield, quality and resilience (Peng et al., 2020).

Nettle (*Urtica dioica* L.) is a perennial herbaceous plant with spiny leaves, belonging to the nettle family Urticaceae. Utilized as a wild vegetable for centuries, it is renowned for its medicinal properties, antiviral activity, antioxidant effects, and pharmacological properties (Bhusal et al., 2022). Despite the significant importance and potential of nettle, our findings suggest that the rhizosphere microbial community of nettle has been insufficiently researched to date.

Among medicinal plants, summer savory (*Satureja hortensis* L.) and parsley (*Petroselinum crispum* L.) hold significant importance. Natural products derived from summer savory, such as extracts and essential oils, are rich in polyphenols and flavonoids, which contribute to their wide array of beneficial properties including antioxidant, antimicrobial, antiparasitic, pesticidal, anti-inflammatory, analgesic, hepatoprotective and anticancer effects, among others (Fierascu et al., 2018). Parsley is notable for its pharmacological uses, which encompass hepatoprotective, nephroprotective, antiurolithiatic, neuroprotective, cardioprotective and antineoplastic effects as evidenced by animal and cell-based studies (Bahramsoltani et al., 2024).

Therefore, the objective of this research was to isolate bacteria belonging to genus *Bacillus* from the nettle rhizosphere, conduct biochemical and PGP characterization of isolates and examine their biostimulatory effects on seed germination and initial growth of summer savory (*Satureja hortensis* L.) and parsley (*Petroselinum crispum* L.).

MATERIAL AND METHODS

Microorganisms were isolated from the rhizosphere of nettle (*Urtica dioica* L.) using the agar plate method for bacterial isolation. For this purpose Nutrient agar (HIMEDIA) was used.

Biochemical and PGP characterization

Enzyme production (lipase, amylase, pectinase, protease, cellulose, urease and gelatinase) was assessed following established protocols outlined in Benson (2002). For this purpose, isolates were cultivated on the appropriate medium. After 48 hours of inoculation at 28 ºC, changes appeared in the medium around the colonies of the isolates, such as the presence of transparent or cloudy zones and changes in the color or consistency of the medium, indicating the isolate's capacity to produce specific enzymes.

The characterization of PGP traits included the determination of indol-3-acetic acid (IAA), siderophores and hydrogen cyanide (HCN) production, as detailed by Slimani et al. (2023). The ability to mineralize phosphorus organic compounds was evaluated using Menkina medium (Liu et al., 2011), while inorganic phosphate solubilization capacity was examined through Pikovskaya's agar plates (Wahyudi et al., 2011).

The effect of isolates inoculation on seed germination

The impact of isolates application on seed germination and plant growth was studied using summer savory and parsley seeds. The experiment was conducted under laboratory conditions. Fifty seeds were submerged for 20 minutes in the bacterial inocula (medium Nutrient broth HIMEDIA), titer 10^9 CFU/ml, and then placed on filter paper and put in a thermostat at 22 ºC for germination. The seeds in the control were not treated with bacterial inocula. The effects of selected isolates on seed germination and initial growth were measured after seven days, while on the plant growth after 14 and 21 days.

Statistical analysis

Statistical analysis was performed using Statistics 13.3 software (TIBCO Software Inc.) and the significance of differences between applied treatments was determined using Fisher's LSD test.

RESULTS AND DISCUSSION

From the rhizospheric soil of nettle, twelve bacterial isolates belonging to *Bacillus* genus were isolated. According to the morphological description of the colony and cells, five *Bacillus* bacteria (denoted as Bac4, Bac5, Bac6, Bac7, Bac8) were selected for further examination.

The results of the examination of the isolates ability to produce lipase, amylase, pectinase, protease, cellulase, urease, and gelatinase are presented in Table 1.

Table 1. Enzyme production of *Bacillus* isolates from nettle

Isolates	Lypase[1]	Amylase	Pectinase	Protease	Cellulase	Urease	Gelatinase
Bac4	+	-	-	-	+	+	+
Bac5	+	+	-	-	+	+	+
Bac6	+	-	+	-	+	+	+
Bac7	+	+	+	-	+	+	+
Bac8	+	+	+	-	+	+	+

[1] (+) positive reaction / produces; (-) negative reaction / does not produce

It was found that all isolates had the ability to produce lipase, cellulase, urease, and gelatinase. The production of amylase was detected in isolates Bac5, Bac7 and Bac8. Proteolityc enzyme was not detected in any of the isolates, while pectinolytic enzyme was identified in isolates Bac6, Bac7 and Bac8.

Microorganisms that produce hydrolityc enzymes have a significant role in the formation and maintenance of soil fertility. These enzymes hydrolyze complex compounds such as polysaccharides, proteins and urea into simpler forms, positively influencing soil fertility (Reddy et al., 2022). Besides improving soil fertility, hydrolytic enzymes can also induce the death or at least paralysis of the pathogenic microorganisms, particularly fungi (Panicker and Sayyed, 2022). Thus, microorganisms capable of producing hydrolytic enzymes may have a crucial role in suppressing various plant-pathogenic fungi and bacteria (Tasnim et al., 2020). In this research, isolates Bac7 and Bac8 produced the highest number of tested enzymes, suggesting that these isolates could be further investigated as potential bioagents for promoting plant growth and controlling phytopathogens.

Isolates were observed to possess multiple plant growth promoting traits such as production of IAA, siderophores, HCN and mineralization and solubilization of phosphates (Table 2).

Table 2. Plant growth promoting characteristics of the isolates

Isolates	IAA[1]	Siderophores	HCN	Mineralization of phosphorus	Solubilize phosphates
Bac4	+	-	+	+	+
Bac5	+	+	+	-	+
Bac6	+	-	+	+	+
Bac7	+	+	+	+	-
Bac8	+	-	+	-	+

[1] (+) positive reaction / produces/ performs decomposition; (-) negative reaction / does not produce/ does not performs decomposition

In these studies, the ability to produce IAA and HCN was determined in all isolates. One of the most important PGP properties is the ability to produce IAA, a hormone from the auxin group. This hormone produced by microorganisms in plants can increase the number of root hairs and the size and number of adventitious roots in plants (Ribeiro and Cardoso, 2012). It can also control various physiological processes, including cell elongation, tissue differentiation, and response to light, gravity and stressful environmental conditions (Gupta et al., 2015). It has been found that isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Antonius et al., 2016). According to the research by Datta et al. (2011), the production of HCN by microorganisms has a favorable impact on plants. HCN is volatile product that exhibit antifungal action by playing essential role in the biological control of plant pathogens or acting as inducer of plant resistance (Agustiyani et al., 2022).

The isolates Bac 5 and Bac7 were found to have the ability to produce siderophores. Iron is an essential element for the growth, metabolism and survival of most cell types on Earth. Although it is the fourth most abundant element in the soil, it rarely occurs in a free form. There is sufficient evidence regarding iron uptake by plants through microorganism-produced siderophores, which convert the insoluble form of iron into a soluble form (Prasad, 2022). In addition to aiding plant iron supply, siderophores produced by microorganisms also play a significant role in the biocontrol of numerous plant diseases caused by phytopathogens, especially phytopathogenic fungi (Prasad et al., 2019).

Isolates Bac4 and Bac6 are the only isolates that demonstrated the ability to mineralize and solubilize phosphates. Other isolates possessed at least one of these abilities. Phosphorus (P) is an essential nutrient for plant development. Microorganisms play a crucial role in enhancing the accessibility of inorganic phosphorus by producing protons, organic acids and ligands. These components are commonly found among rhizosphere microorganisms specializing in phosphorus solubilization (Hinsinger et al., 2011). Additionally, they facilitate the mobilization of phytate (organic P), likely through the production of phytase (Jorquera et al., 2008).

The data in Table 3 revealed the potential of the isolates to increase the degree of seed germination, root length, shoot stem and vigor index (VI) of summer savory and parsley seeds after seven days.

Table 3. The effect of isolates on seed germination (No, %), root and shoot length (mm), and VI (%) after 7 days

Isolates	Summer savory		Parsley		Summer savory		Parsley		Summer savory	Parsley
	seed germination				root and shoot length				VI	
	No.[1]	%	No.	%	root	shoot	root	shoot		
Bac4	13.3^{a}	66.5	15.6^{cb}	78.0	7.7^{e}	18.8^{d}	9.5^{d}	24.1^{d}	1762	2620
Bac5	14.7^{a}	73.5	14.3^{cd}	71.5	8.5^{b}	19.5^{d}	9.1^{d}	16.2^{b}	2058	1809
Bac6	10.3^{b}	51.5	14.3^{cd}	71.5	6.1^{c}	14.6^{a}	14.7^{a}	26.2^{a}	1066	2924
Bac7	11.3^{b}	56.5	13.0^{d}	65.0	9.1^{b}	25.1^{c}	12.1^{b}	23.4^{ed}	1932	2307
Bac8	10.0^{b}	50.0	17.3^{b}	86.5	11.2^{a}	30.0^{c}	9.5^{d}	22.1^{e}	2060	2733
Control	9.7^{b}	48.5	4.0^{a}	20.0	3.8^{d}	7.5^{b}	4.6^{c}	11.0^{c}	548	312

[1] Number of germinated seeds
Values in the same row followed by different letters indicate significant differences (p < 0.05) between the means

Seven days after the inoculation of summer savory and parsley seeds, there was an increase in seed germination in all variants for both plants. The highest germination percentage for summer savory seeds (73.5%) was observed in the variant where isolate Bac5 was applied, while for parsley seeds, this effect was achieved with the application of isolate Bac8 (86.5%).

For summer savory, all applied isolates led to a statistically significant increase in root and shoot length, with the best effect achieved through the application of isolate Bac8 (Figure 1).

Figure 1. The Influence of Isolates Bac8 and Bac7 on the initial seedling growth in summer savory

In parsley, seven days after inoculation, a statistically significant increase in root and shoot length was observed in all variants, and the best effect was achieved with the application of Bac6 (Figure 2).

All isolates contributed to increased seedling vigour. The highest response for vigour index was observed with isolates Bac5 and Bac8 for summer savory and Bac6 and Bac8 for parsley.

The effect of isolates on root and shoot length of summer savory and parsley after 14 and 21 days are presented in Table 4.

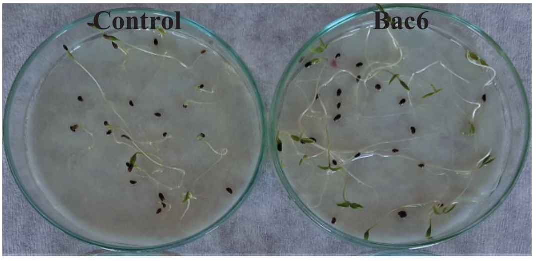

Figure 2. The influence of the Bac6 isolate on the Initial Seedling Growth in parsley

Table 4. The effect of isolates on root and stem length (mm).

Isolates	Summer savory		Parsley		Summer savory		Parsley	
	14 days				21 days			
	root	shoot	root	shoot	Root	shoot	root	shoot
Bac4	8.2^{e}	26.5^{c}	13.2^{d}	28.2^{a}	15.5^{c}	45.1^{d}	17.1^{f}	41.2^{e}
Bac5	9.1^{b}	19.7^{e}	18.7^{e}	17.4^{b}	9.5^{b}	44.3^{d}	30.2^{b}	21.3^{c}
Bac6	7.5^{e}	23.4^{d}	19.5^{e}	35.1^{c}	9.2^{b}	42.2^{b}	23.7^{d}	38.8 ^{b}
Bac7	10.6^{b}	34.6^{b}	28.2^{a}	35.8^{c}	16.0^{c}	44.3^{d}	35.5^{a}	42.1^{e}
Bac8	15.3^{a}	38.2^{a}	25.5^{b}	34.5^{c}	16.4^{c}	55.2^{a}	26.6^{c}	48.2^{a}
Control	4.2^{d}	14.4^{f}	16.0^{c}	17.1^{b}	5.0^{a}	16.1^{c}	21.1^{e}	19.0^{d}

Values in the same row followed by different letters indicate significant differences ($p < 0.05$) between the means

After 14 days from inoculation, all applied isolates showed a statistically significant increase in both root and shoot lengths for summer savory. Notably, Bac8 exhibited the most significant impact on root length, while Bac7 and Bac8 had the highest impact on shoot length. For parsley, a significant increase in root length was observed in all variants, except when Bac4 was applied, resulting in a statistically significant decrease. The optimal effect on parsley root length was achieved with Bac7. Parsley shoot length significantly increased in all variants, except in the case of Bac5 isolate, where the increase was not statistically significant.

After 21 days, for summer savory, all applied treatments led to a statistically significant increase in root and shoot length. The best effect on root and shoot length was observed with isolate Bac8. Parsley root length significantly increased in all variants after 21 days, except when isolate Bac4 was applied. The best effect was achieved with the application of Bac7, where a 168% increase in root length was observed. The shoot length of parsley was statistically significantly increased in all variants. The best effect was observed with the application of isolate Bac8.

The obtained results in this study align with the conclusions of other researchers, indicating that the effects of PGP bacteria depend on the nature of the isolate, its population, inoculum concentration and plant-bacteria interactions

(Dobbelaere et al., 2002; Sahin et al., 2004). Furthermore, the results of this research are consistent with those of Cappellari et al. (2013) and Çakmakçı (2016), who observed a positive effect, namely an increase in the biomass of shoots and roots of various medicinal and aromatic plants inoculated with PGP bacteria. The results of present study suggest that isolates labeled as Bac5, Bac7, and Bac8 could serve as bioagents in the production of summer savory and parsley.

CONCLUSION

In this study, every *Bacillus* isolate derived from the nettle rhizosphere exhibited significant potential as a plant growth promoter. The isolates that stand out are isolates Bac5, Bac7 and Bac8. The isolate Bac5 had the most significant impact on summer savory seed germination, while the application of the isolate Bac8 led to the highest increase in both root and shoot length of summer savory during all three measurements. Concerning the improvement of parsley seed germination rate, the application of the isolate Bac8 had the most significant effect. On average, for enhancing root and shoot length of parsley across all three measurements, the Bac7 and Bac8 isolates showed the greatest influence. Having great PGP traits, these isolates may represent precious biological alternative for harmful pesticides and chemical fertilizers application in the production of summer savory and parsley.

ACKNOWLEDGEMENT

This work was supported by Ministry of Education, Science and Technological Development of the Republic of Serbia (451-03-65/2024-03/200117).

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ОРИГИНАЛНИ ЧЛАНАК
Примљен: 26. 2. 2024.
Прихваћен: 29. 4. 2024.

БИОСТИМУЛАТОРНА АКТИВНОСТ ИЗОЛАТА БАЦИЛУСА ИЗ РИЗОСФЕРЕ КОПРИВЕ (*Urtica dioica* L.)

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РЕЗИМЕ: Циљ овог истраживања био је изоловати микроорганизме из ризосфере коприве, спровести биохемијску и ПГП карактеризацију изолата и испитати њихово биостимулативно дејство на семе лековитог биља. Микроорганизми су изоловани методом агар плоче за изолацију бактерија. Биохемијска карактеризација је укључивала процену способности изолата да продукују ензиме као што су липаза, амилаза, пектиназа, протеаза, целулоза, уреаза и желатиназа. ПГП карактеризација је укључивала испитивање способности изолата да продукују индол-3-сирћетну киселину (ИАА), сидерофоре и цијановодоник, као и способност минерализације органских фосфорних једињења и солубилизације фосфата. Утицај изолата на клијавост семена и раст биљака испитиван је коришћењем семена чубрице (*Satureja hortensis* L.) и першуна (*Petroselinum crispum* L.). Експеримент је спроведен у лабораторијским условима. Одређиван је број проклијалог семена, дужина изданака и коренка, вигор индекс, као и дужина корена и стабла биљака. Према морфолошком опису колоније и ћелија, за даље испитивање је одабрано пет *Bacillus* бактерија (означених као Bac4, Bac5, Bac6, Bac7, Bac8). Сви изолати су показали добар ПГП потенцијал. Изолати који се издвајају су изолати Bac5, Bac7 и Bac8. Најбољи утицај на клијавост семена имали су изолати Bac5 и Bac8. Највећи стимулативни ефекат на раст расада имали су изолати Bac7 и Bac8. Са одличним својствима ПГП-а, ови изолати могу представљати биолошку алтернативу за примену штетних пестицида и хемијских ђубрива у производњи чубрице и першуна.

КЉУЧНЕ РЕЧИ: микроорганизми, инокулација, лековите биљке, ризобактерије промотори-биљног-раста

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 147, 59—65, 2024

UDC 551.583:634.8
https://doi.org/10.2298/ZMSPN2447059K
ORIGINAL ARTICLE
Received: 2023/10/4
Accepted: 2024/3/20

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IMPORTANCE OF NEW GRAPEVINE CULTIVARS IN MITIGATION OF NEGATIVE EFFECTS OF CLIMATE CHANGE

ABSTRACT: The aim of this work was to investigate the differences in yield and grape quality among three grapevine cultivars Dionis, Morava and Panonia during the period 2015–2022. The results suggest that season highly affects grape quality of all tested cultivars. Among the all tested cultivars, Morava had the lowest variation in bunch weight, while the lowest variation in acidity was demonstrated by Panonia. On average, these cultivars had satisfying yield (0,91–1,41 kg/m^2) and even the production was performed according to ecological principles, without the use of pesticides. Colored cultivar Dionis and its parent Panonia, showed high sugar content in the grape must. Panonia and Morava had significantly higher acidity of the must (8.4 and 7.8 g l^{-1}), compared to Dionis (6.30 g l^{-1}).
KEYWORDS: climate conditions, phenology, grape quality, new cultivars, yield

INTRODUCTION

For successful grapevine production, it is highly important to grow a proper cultivar in a given wine region. However, climate change makes it difficult to select suitable cultivars for growing in a given wine region (Bai et al., 2022). Climate conditions highly affect the incidence of fungal diseases and insects which is dependent on the amount and frequency of rainfall during the season. Cultivars such as Pinot blanc and Chardonnay are rather sensitive to pathogens and therefore new cultivars should be cultivated.

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The main goal of grapevine breeding programs in different countries around the world is creation of varieties that are resistant to fungal diseases while yielding adequate grape quality. In the vineyards of the resistant grapevine varieties, only few spraying treatments are required to prevent diseases. Therefore, these varieties minimize the long-term risk of environmental pollution and human health hazards. The first resistant varieties obtained through traditional breeding carried a high percentage of non-*Vitis vinifera* varieties in their genetic background and were considered as interspecies hybrids. Multiple backcrossing with *Vitis vinifera* varieties allowed subsequent development of resistant varieties that retained more than 85% of the *Vitis vinifera* genes. Three resistant white varieties released in Serbia (Panonia and Morava) and one colored skin variety (Dionis) have high percentage of *Vitis vinifera* genes and should therefore not be considered as hybrids. Panonia yields full-bodied wines similar to Riesling while the wine of Dionis is similar to the wine of Cabernet franc. At present, the grape quality of these varieties is at the level of *Vitis vinifera*. Unfortunately, these wines are still not fully embraced by consumers, suggesting that further effort is required in both production and marketing.

The aim of this study was to examine the grape quality of three grapevine cultivars Dionis, Panonia and Morava during seven consecutive years (2015–2022) in ecological production.

MATERIAL AND METHODS

The experiment was conducted at the Experimental field for viticulture, University of Novi Sad, Faculty of Agriculture, situated in Sremski Karlovci (45º10' N; 20º10' E) during seven years (2015–2022). All vines were pruned to Karlovac training system (modified Guyot, one arched cane of 12 buds and one spur of 2 buds), with an average of 14 buds per vine. Rows had an E–W orientation. The vines were trained ecologically i.e. without use of pesticides and mineral fertilizers. Grass mixture was established as cover crop in between the rows. Every second row was ploughed. Weeds in between the vines were controlled mechanically. Fifteen vines were included in the treatment of each cultivar, with three replicates per treatment.

Phenological observations

Phenological observations were performed at three specific moments, i.e. the most important grapevine phenological stages: (BBCH scale – stage 07) beginning of bud-burst, (BBCH scale – stage 60) beginning of flowering and (BBCH scale – stage 80) beginning of veraison – when cultivars develop their characteristic berry color (Coombe, 1995).

Yield and grape quality

Yield was determined at harvest by weighing all harvested bunches of each replicate. Average bunch weight was obtained by dividing the weight of all bunches by the number of bunches per replicate. After crashing, the sugar content in the grape must was measured with Oechsle hydrometer. Titratable acidity was measured by titration of grape juice sample (10 ml) by adding 0,1 M NaOH, drop by drop, in the presence of bromothymol blue as an indicator.

RESULTS AND DISCUSSION

In Serbia, there have been extreme weather conditions in recent years affecting grape production: the year of 2014 was very rainy which reduced yield and quality of some *Vitis vinifera* L. cultivars (Ivanišević et al. 2020). In 2017, sunburn damages on the berries were observed in the field due to hot weather conditions (Kuljančić and Božović, 2018). Extreme climate events in second decade of the XXI century and the negative effects of climate change on grapevine cultivars were also observed in Germany (Topfer and Trapp, 2022). In most of the examined seasons (5/7), June (2016, 2017, 2019, 2021 and 2022), July (2015, 2017, 2020, 2021 and 2022) and August (2015, 2017, 2018, 2019 and 2022) were hotter compared to the average temperatures for these months during the period 2000–2022 (Figure 1/1a).

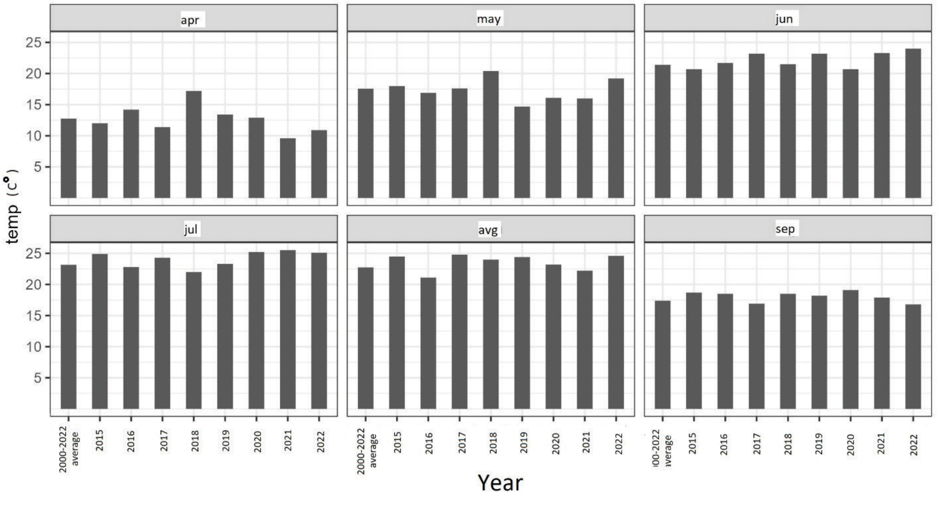

Figure 1. Average monthly temperatures during 2015–2022

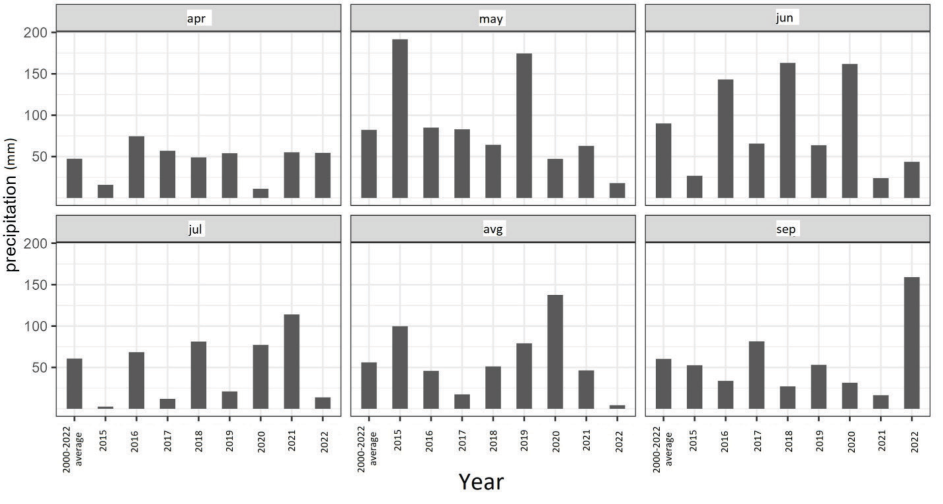

Figure 1a. Average monthly sum of precipitation during 2015–2022

Phenology was highly affected by the weather conditions. On average, bud-break was observed at the beginning of April (Table 1). The first occurrence of bud-break in Sremski Karlovci was observed in 2017 in the vineyard of Dionis (27th March), while in 2021, bud-break occurred very late in the vineyard of Morava (18th May). The highest shift in phenology was observed in 2018, when flowering occurred 20 days earlier compared to average 2015–2022. An earlier occurrence of flowering in 2018 was a consequence of hot temperatures during April and May (Figure 1). The beginning of veraison i.e. the beginning of ripening was recorded at the end of July for all cultivars with high variations from year to year.

Table 1. Phenology of examined cultivars during 2015–2022.

Cultivar	Year	Beginning of bud-break (Day of year)	Beginning of flowering (Day of year)	Beginning of veraison (Day of year)
Dionis	2015	105	144	201
	2016	91	147	203
	2017	86	146	204
	2018	92	128	191
	2019	92	150	212
	2020	94	143	216
	2021	107	160	215
	2022	88	151	218
	Average 2015–2022	94 (04.04)	146 (26.05)	207 (26.07)

Morava	2015	106	145	207
	2016	93	147	208
	2017	88	146	206
	2018	93	128	195
	2019	93	155	216
	2020	98	148	220
	2021	108	160	227
	2022	92	153	217
	Average 2015–2022	96 (06.04)	147 (27.05)	212 (31.07)
Panonia	2015	105	145	199
	2016	92	147	199
	2017	88	145	197
	2018	93	127	190
	2019	92	154	207
	2020	98	143	206
	2021	107	160	209
	2022	91	154	213
	Average 2015–2022	96 (06.04)	147 (27.05)	202 (21.07)

On average, these cultivars had satisfied yield, despite the fact that the production was performed according to ecological principles, without the use of pesticides. Panonia and Morava had higher yield compared to Dionis (Table 2). Standard deviation values calculated for yield of these fungus tolerant cultivars are similar to the values calculated for several *Vitis vinifera* L. cultivars (Cindrić et al., 1992; Cindrić et al., 2000).

All cultivars had the lowest bunch weight in 2020 (Table 2). Panonia and Morava had significantly higher bunch weight compared to Dionis.

Sugar content in the must of Dionis and Panonia exceeded 20% in all seasons (Table 2). In 2015, sugar content in the must of Morava was below 20%. The highest variation in the sugar content in the must showed Dionis, while the lowest was observed in Panonia.

Table 2. Yield and grape quality of examined cultivars during 2015–2022

Cultivar	Year	Harvest date	Yield (kg m^{-2})	Bunch weight (g)	Total soluble solids (%)	Titratable acidity (g l^{-1})
Dionis	2015	01.10.	1.01	160.0	23.3	4.7
	2016	10.10	1.02	160.8	21.7	4.7
	2017	01.10	1.24	183.4	24.7	6.9
	2018	11.09.	1.06	186.8	22.3	6.8
	2019	25.09.	0.60	125.0	24.1	6.2
	2020	01.10.	0.53	87.4	26.8	5.3
	2021	05.10.	0.55	142.9	27.9	8.3
	2022	15.09.	1.32	152.3	24.1	7.5

Morava	2015	20.09.	1.49	267.0	18.9	8.1
	2016	25.09.	2.57	240.2	20.5	9.7
	2017	25.09.	1.05	210.3	22.7	4.6
	2018	06.09.	0.90	209.7	20.4	7.0
	2019	20.09.	1.31	180.4	21.2	7.4
	2020	30.09.	1.47	165.8	21.5	7.7
	2021	05.10.	1.05	150.0	22,3	9.5
	2022	15.09.	1.11	200.3	20.9	9.1
Panonia	2015	01.09.	0.97	240.3	24.0	8.4
	2016	07.09.	1.54	156.7	24.9	9.4
	2017	23.08.	1.54	186.7	24.1	7.8
	2018	20.08.	1.05	241.7	25.6	6.9
	2019	28.08.	1.14	176.1	22.4	8.0
	2020	31.08.	1.21	141.9	23.2	8.8
	2021	06.09.	1.82	182.2	24.9	9.1
	2022	12.08.	1.31	164.1	26.3	9.0
Dionis	2015–2022	27.09.	0.91	149.6	24.4	6.3
Morava	2015–2022	22.09.	1.41	209.0	21.1	7.8
Panonia	2015–2022	28.08.	1.31	186.2	24.4	8.4

Panonia and Morava had significantly higher acidity of the must, compared to Dionis (Table 1). The lowest variation in acidity showed Panonia (below 1.0) (Table 3). Cindrić et al. (1992) observed that grapevine cultivar Grašac during the period 1968–1980, had standard deviation for titratable acidity only 0.7. The same author (Cindrić et al., 1992) observed that Cabernet franc, the parent of Dionis, also showed high variation in acidity (SD=1.3).

Table 3. Standard deviation of examined cultivars during 2015–2022 (average)

Cultivar	Yield (kg m^{-2})	Bunch weight (g)	Total soluble solids (%)	Titratable acidity (g L^{-1})
Dionis	0.32	32.3	2.11	1.32
Morava	0.51	32.1	1.29	1.57
Panonia	0.29	36.7	1.27	0.83

CONCLUSIONS

New cultivars released in Sremski Karlovci, Dionis, Morava and Panonia, grown without the use of pesticides, showed low variation in yield despite variable climate conditions. In all seasons, Dionis and Panonia accumulated more than 20% of sugars in grapes at harvest. These cultivars are suitable for growing in such a modified and unpredicted climate conditions in Serbia, particularly in Fruška Gora wine region.

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ОРИГИНАЛНИ ЧЛАНАК
Примљен: 4. 10. 2023.
Прихваћен: 20. 3. 2024.

ЗНАЧАЈ НОВИХ СОРТИ ВИНОВЕ ЛОЗЕ У УБЛАЖАВАЊУ НЕГАТИВНИХ ЕФЕКАТА КЛИМАТСКИХ ПРОМЕНА

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РЕЗИМЕ: Циљ овог рада је да се испита разлика у приносу и у квалитету грожђа сорти Дионис, Морава и Панониа, током периода од 2015. до 2022. године. Резултати су показали да је година испитивања значајно утицала на квалитет грожђа код свих испитиваних сорти. Поређењем испитиваних сорти, Панониа се издвојила као сорта која је имала најмање варијације у маси грозда, док је најмања варијација у садржају киселина у шири такође забележена код сорте Панониа. У просеку, све испитиване сорте имале су задовољавајући принос (0,91–1,41 kg/m^2) иако је производња заснована на еколошким принципима, без примене пестицида. Црна винска сорта Дионис и њен родитељ Панониа, имали су висок садржај шећера у шири. Сорте Панониа и Морава имале су значајно већи садржај киселина у шири (8,4 и 7,8 $g\ L^{-1}$) у поређењу са сортом Дионис (6,30 $g\ L^{-1}$).

КЉУЧНЕ РЕЧИ: фенологија, климатски услови, квалитет грожђа, принос, нове сорте

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 147, 67—79, 2024

UDC 551.583:634.8(497.11)
https://doi.org/10.2298/ZMSPN2447067R
ORIGINAL ARTICLE
Received: 2023/11/1
Accepted: 2024/1/30

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PRIORITY MEASURES OF ADAPTATION TO CLIMATE CHANGE IN THE VITICULTURE SECTOR IN SERBIA

ABSTRACT: Viticulture production, like the entire agriculture, is subject to climate changes related to a significant increase in air temperature. High air temperatures during the ripening period negatively affect the yield and quality of grapes. Apart from the increase in air temperature, grape production is also affected by rainfall and the frequency of extreme events. Climate projections for the future predict a further increase in air temperature and a change in the most important viticultural indices. The analysis shows that there has been a change in the climate category according to WI and HI values, and in some areas also CI. The Drought Index (DI) does not show significant changes in the first two periods (2021–2040; 2041–2060), in contrast to the increased frequency of droughts and the extension of the duration of the dry period during the year. Since the most significant risks in viticulture come from high temperatures during the grape ripening period and from hail and stormy weather during the growing season, appropriate adaptation measures are needed.

KEYWORDS: adaptation, exposure, climate change, viticultural indices, vulnerability, risk

INTRODUCTION

Gases with the greenhouse effect as a consequence of the irrational use of natural resources and the linear economy (take – make – use – throw away) cause climate changes that manifest themselves to a significant extent in Serbia, causing great damage in all spheres of the economy with a particularly negative effect in the sector of agriculture. The European Environment Agency (EEA), announced that agriculture directly contributes to around 10% of greenhouse gas emissions (GHG) in the EU (EEA, 2022). However, when all emissions related to food production are taken into account, it is estimated that this pro-

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duction is responsible for around 21–37% of global emissions (IPCC, 2019). Viticulture production, like the whole agriculture, is vulnerable to climate change due to a significant increase in air temperature, which amounted to 1.2 °C in Serbia for the period 1996–2015 compared to the period 1961–1980 when the largest increase in summer maximum daily temperature was 2.2 ºC (Vuković Vimić et al., 2018, 2022). As already confirmed in large number of works as well as in various projects, high air temperatures during the ripening period adversely affect the yield and quality of grapes (Ranković-Vasić et al., 2014; Ranković-Vasić et al., 2022a; NAP, 2022; IAPS, 2022; FAO, 2023). All phenological stages of grapevine development are subject to the effects of changes in air temperature. Flowering, the ripening and harvesting of grapes were significantly shifted, while a smaller change was observed in bud burst development (Ruml et al., 2016; Vujadinović Mandić et al., 2020). In addition to the increase in air temperatures, the precipitation and frequency of extreme events also affected agricultural production and food security over the last few decades (Vujadinović Mandić, 2022). Climate projections for the future predict a further rise in air temperature both globally and in Serbia. In the second half of the 21st century, in the period 2081–2100, compared to 1961–1980, the average increase in air temperature in Serbia will most likely be over 2.5 ºC according to the RCP4.5 scenario (lower risk assessment scenario) and over 5 ºC according to RCP8.5 (higher scenario) (Vuković Vimić et al., 2018). The probability of number of days with a temperature higher than 35 ºC will increase significantly in the future (Ranković-Vasić et al., 2022b). As the negative impacts of climate change are already expected to intensify in the future, especially in the viticulture sector, adaptation measures should be mandatory, not an alternative.

MATERIALS AND METHODS

For the analysis of climate changes in Serbia, there were used observed data from the EOBS database of daily temperature and precipitation at a resolution of about 10 km and the results of an ensemble of 8 regional climate models selected from the EURO-CORDEX project database (https://www.euro-cordex.net/), included in the Digital Climate Atlas of Serbia (atlas-klime.eko.gov.rs). In this work, the RCP 8.5 scenario was chosen (Relative Concentration Pathway) from the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (IPCC, 2014), for the analysis of climate change in the future. In accordance, the three twenty-year future periods, 2021–2040, 2041–2060 and 2081–2100, were selected. Chosen bioclimate viticultural indices for analysis are: Mean temperature during the vegetation period (Tweg – Average mean daily temperature for the standard vegetation period from 1 April to 31 October), Winkler Index (WIN, Winkler et al., 1974), Huglin Index (HI, Huglin, 1978), Cool Nigth Index (CI, Tonietto and Carbonneau, 2004), Drought Index (DI, Riou et al., 1994), High summer temperatures (N35 – Number of days with a maximum daily temperature higher than 35 °C). Exposure, vulnerability and risks from high temperatures and hail are analyzed in this paper. Exposure assessment means estimating the frequency, i.e. how often the defined potential hazards occur related to climate change and extreme weather events in the past and future. The scale of probability and frequency of occurrence of hazards is: Probability of event: Small – Frequency of occurrence: once in 20 years; Probability of event: Moderate – Frequency of occurrence: once in 10 years; Probability of event: High – Frequency of occurrence: once in 4 years; Probability of event: Very high – Frequency of occurrence: once in 2 years. Vulnerability assessment involves assessing the severity of the socio-economic consequences that defined hazards may have according to the scale: Vulnerability: Small – Degree of consequence: with little or no negative consequences; Vulnerability: Moderate – Degree of consequence: with negative consequences that can lead to smaller economic losses; Vulnerability: Great – Degree of consequence: with negative consequences that may lead to significant economic losses and/or may affect certain social categories. The risk quantification is performed based on assessments of exposure levels and vulnerabilities. Low risk are those events that occur with low or moderate probability and cause small or moderate consequences. Moderate risk are those events that occur with low or moderate probability and cause large or critical consequences, as well as those that occur with high or very high frequency, and cause small or moderate consequences. High risk are those events that occur with a high or very high probability and cause great or critical consequences.

RESULTS AND DISCUSSION

Climatic factors exert their influence on the grapevine throughout the annual cycle, mostly during the vegetation period. Based on the analysis of projected values of temperatures for the vegetation period (*Tveg*) it can be concluded that in the last 40 years of this century (periods 2041–2060 and 2080–2100) there will be significant changes in these temperatures in relation to the end of the 20th century. Changes in the mean temperature of the growing season have been projected, which will be higher, for the 75th percentile in the entire wine-growing region of Vojvodina and certain parts of the wine-growing region of Tri Morave, as well as the Niš and Nišava regions (Figure 1). Knowledge of phenological changes during the vegetation of the grapevine, on one hand, and climatic factors on the other, are important factors in the selection of cultivars in the wine-growing region. Meteorological conditions have a greater influence on the phenological dynamics than the genetic characteristics of cultivars (Ruml et al., 2013). According to Ruml et al. (2016), the most significant change is the period of earlier blooming and ripening, precisely because of the increase in air temperatures. Climate exerts the strongest effect on grape berry composition during ripening period (Jones and Davis, 2000; Zoecklein and Gump, 2022). The analysis shown in the Figure 1 indicates that there has been a change in the climate category according to WI and HI values and in some areas also CI (Vujadinović Mandić et al., 2022). It is predicted that in the next twenty years the Winkler Index will move to the WI III zone (1668–1944 °C) for most wine-growing regions of Vojvodina and zone IV (1945–2222 °C) for Belgrade and parts of Banat. In the second period (2041–2060) entire Vojvodina, except for small parts of the Subotica region and parts around Fruška Gora, will be WIN IV (1945–2222 °C), and in the last projected period of the future (2081–2100) the region of Vojvodina, eastern and southern parts of Serbia, the wine-growing region Tri Morave, Cer-Valjevo, Belgrade wine-growing region, the far east and south of the contry will be in the highest WI V zone with over 2222 °C. The Huglin Index (HI) also predicts a move towards a warmer climate that almost all wine-growing regions will have. Thus, in the 2081–2100 period, all regions of Vojvodina, except Subotica, will be in the HI+3 zone (> 2700 °C). Moreover, Cer-Valjevo and the wine-growing region Tri Morave will be in this highest class, as well as parts of Niš, Nišava, the wine-growing region of Negotinska Krajina, etc. The mean minimum temperature in September (CI) in the end of the 20^{th} century belongs to the category of very cold nights (CI<12 °C) in the entire territory of Serbia, except for separate parts of Belgrade, Banat and the far east where CI is 12–14 °C (cold nights). In the near and distant future, significant changes will occur in this index as well, so that in the 2081–2100 period in entire Vojvodina, parts of Tri Morave, Knjaževac, Niš, Leskovac, Toplica and Vranje wine-growing regions nights will be moderate (CI 14–18 °C, and in the same period (75^{th} percentile), the region of Belgrade would have warm nights (CI>18 °C). The Dryness Index (DI) does not show significant changes in the first two periods (2021–2040; 2041–2060), in contrast to the increased frequency of droughts and the prolongation of the duration of the drier period during the year.

The three viticultural climates, in the Geoviticulture MCC system (Tonietto and Carbonneau, 2004), are present in Serbia in the past: HI–1 DI–1 CI+2 temperate, sub-humid with very cool nights, regions: Subotica-Horgoš, Srem and Nišava-Južna Morava; HI–1 DI–2 CI+2 temperate, humid with very cool nights, regions: Banat, Šumadija-Velika Morava and Zapadna Morava; HI+1 DI–1 CI+2 temperate warm, sub-humid with very cool nights, Timok region (Ruml et al., 2012). Significant changes are predicted for the end of the 21^{th} century (2081–2100). As already confirmed in various papers, high air temperatures during the ripening period adversely affect the yield and quality of grapes (Conde et al., 2007; Ranković-Vasić et al., 2014; Meggio, 2022; Ranković-Vasić et al., 2022). Climate change is a major challenge in wine production. Temperatures are increasing worldwide, and most regions are exposed to water deficits more frequently. Higher temperatures trigger advanced phenology. This shifts the ripening phase to warmer periods in the summer, which will affect grape composition, in particular with respect to aroma compounds (Van Leeuwen and Darriet, 2016; Gutiérrez-Gamboa and Moreno-Simunovic, 2018; Modina et al., 2023).

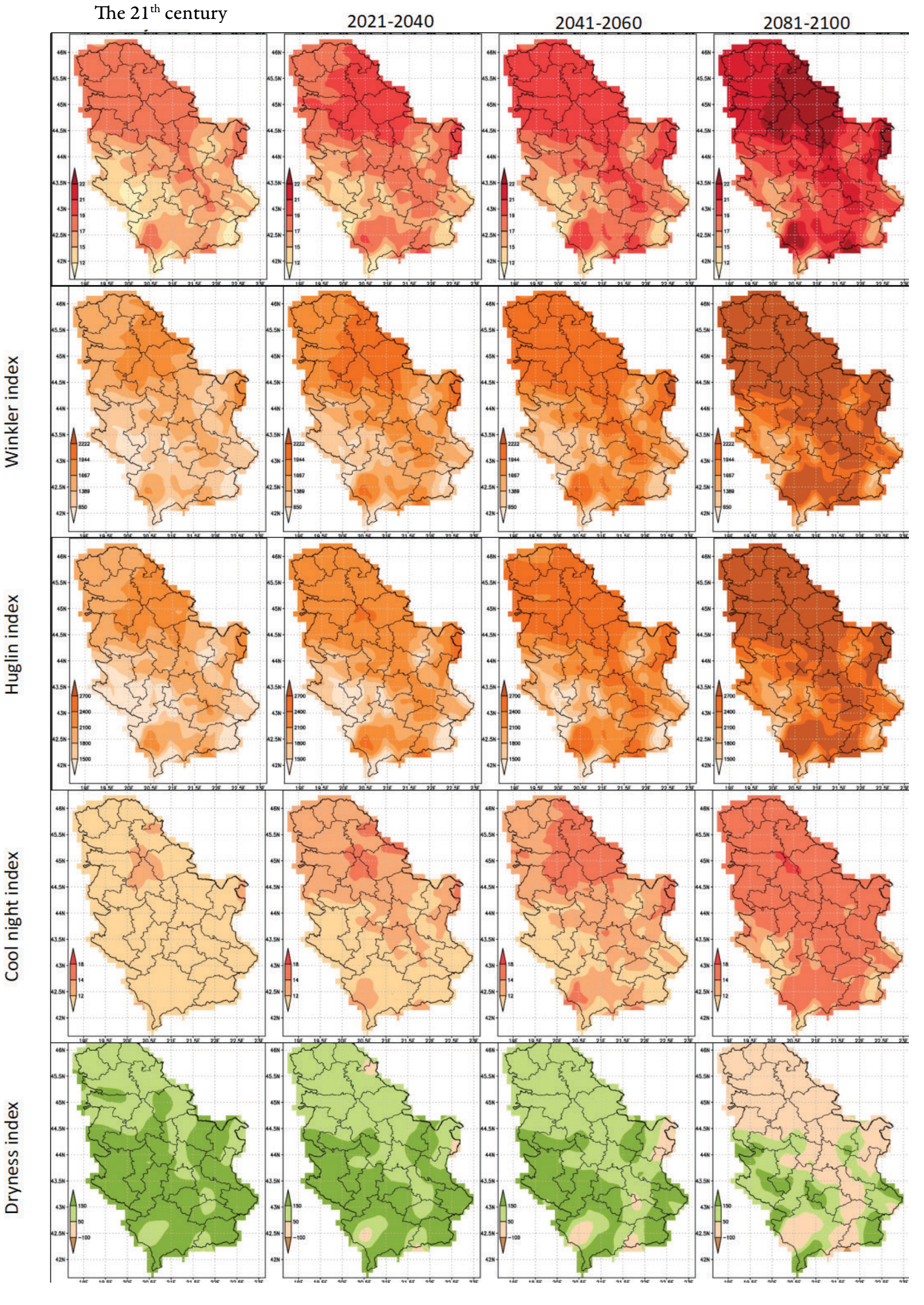

Figure 1. Values of viticultural indices (Tveg – mean vegetation temperature, WI – Winkler Index, HI – Huglin Index, CI – Cool Night Index, DI – Dryness Index) for the period of the end of the 20th century and future climatic periods (2021–2040; 2041–2060; 2081–2100)

Table 1. Exposure and vulnerability of high temperatures in grapevines (Tx > 35 °C)

Administrative district	Exposure			Vulnerability
	Present 2000–2019	Near future 2021–2040	Mid-century 2041–2060	
North Banat	Moderate	Large	Large	Moderate
Central Banat	Moderate	Large	Large	Moderate
South Banat	Moderate	Large	Very large	Large
North Bačka	Moderate	Large	Large	Moderate
West Bačka	Moderate	Large	Large	Moderate
South Bačka	Small	Large	Large	Moderate
Srem	Small	Large	Large	Moderate
City of Belgrade	Moderate	Large	Very large	Large
Danube River Basin	Moderate	Very large	Very large	Large
Braničevo	Moderate	Large	Very high	Large
Bor	Moderate	Large	Large	Moderate
Zaječar	Moderate	Very high	Very high	Moderate
Nišava	Moderate	Very high	Very high	Large
Pirot	Small	Large	Large	Moderate
Toplica	Moderate	Very high	Very high	Large
Jablanica	Small	Very high	Very high	Large
Pčinja	Small	Large	Large	Moderate
Šumadija	Small	Large	Large	Moderate
Pomoravlje	Moderate	Very high	Very large	Large
Raška	Small	Small	Small	Small
Rasina	Small	Large	High	Moderate
Mačva	Small	Large	High	Moderate
Kolubara	Small	Large	High	Moderate
Moravica	Small	Moderate	Moderate	Small
Zlatibor	Small	Small	Small	Small
National level	Small, Moderate	Large	Large	Moderate

The planning and implementation of adaptation measures in a particular wine-growing region or vineyard depends on the local impacts of climate change and risk levels of a certain abiotic factor. Table 1 shows the exposure and vulnerability of high temperatures (Tx > 35 °C), which can significantly reduce the accumulation of anthocyanins in the skin of the berry. This risk is of particular importance for red wine cultivars, the quality of grapes and the quality of the wine produced. The tendency of increasing temperatures during the vegetation period in the grape ripening period is projected for the regions of central, southern and eastern Serbia where the risk is assessed as very high (Figure 2). Temperatures over 30 °C and 35 °C are especially risky. The selection of the position, use of shade nets, monitoring chemical parameters in the bunch during ripening, shifting the harvest date are just some of the adaptation measures that must be observed during intensive viticulture production, in order to obtain quality grapes and wine. Hailstorms are typically localized events, and very little is known about their effect on crops (Petoumenou et al., 2019). Hail is extremely dangerous in viticulture, because for various reasons using anti-hail nets in the vineyards in Serbia is not a part of practice. Damage from hail on the grapevine in one production year can be extremely large and cause a decrease in the yield and quality of grapes, sometimes the loss of the entire yield (Baniță et al., 2020). Hail have a destructive effect on vineyards, depending on the intensity, size and timing of the fall, and influence next year's grape production (Teodor, 2018). In addition to the use of anti-hail rockets in breaking hailstorm clouds, on which currently fighting against hail relies, which sometimes does not give satisfactory results, investments in the installation of anti-hail nets, as well as appropriate conditions when insuring vineyards, must be of state interest. The average frequency of wind gusts stronger than 25 m/s, which can cause damage to the grapevine, in most of the territory of Serbia during the reference period was from 0.8 to 1.2 days. For the end of the 21st century (2071–2100), an increase in the frequency of hail is predicted in Serbia, from 40 to 80% in Vojvodina and from 20 to 40% in the rest of Serbia for hail larger than 2 cm in diameter (Table 2). This fact is confirmed by a study designed to assess changes in the appearance and size of hail at the European level (Radler et al., 2019). High risks from hail with a tendency to increase are present in 5 administrative districts (Jablanica, Pčinja, Raška, Pomoravlje and Moravica) (Figure 3). Protecting the vineyards with the anti-hail net is an ideal solution for table grape growers who want to obtain a high quality grape production. The use of different anti-hail net may be an alternative of protection against hail and other storms, with no important negative effects on the development and quality of grapes (Forte et al., 2022).

Table 2. Hail larger than 2 cm in diameter and natural disasters in vineyard. Exposure assessment and vulnerability by regions

Administrative district	Exposure		Vulnerability
	Present 1971–2000*	The end of the century 2071–2100**	
North Banat	Small	Large	Moderate
Central Banat	Small	Large	Moderate
South Banat	Moderate	Very large	Large
North Bačka	Small	Large	Moderate
West Bačka	Small	Large	Moderate
South Bačka	Moderate	Large	Moderate
Srem	Moderate	Large	Moderate
City of Belgrade	Moderate	Large	Large
Danube River Basin	Moderate	Large	Large
Braničevo	Moderate	Large	Large
Bor	Moderate	Large	Moderate
Zaječar	Moderate	Large	Moderate

Nišava	Moderate	Large	Large
Pirot	Moderate	Large	Moderate
Toplica	Moderate	Large	Moderate
Jablanica	Moderate	Very large	Large
Pčinja	Large	Very large	Large
Šumadija	Moderate	Large	Moderate
Pomoravlje	Moderate	Very large	Large
Raška	Large	Very large	Large
Rasina	Moderate	Large	Moderate
Mačva	Moderate	Large	Moderate
Kolubara	Large	Very large	Moderate
Moravica	Large	Very large	Large
Zlatibor	Large	Very large	Large
National level	Moderate	Large	Moderate

* reference period; ** RCP8.5 scenario (Radler et al., 2019)

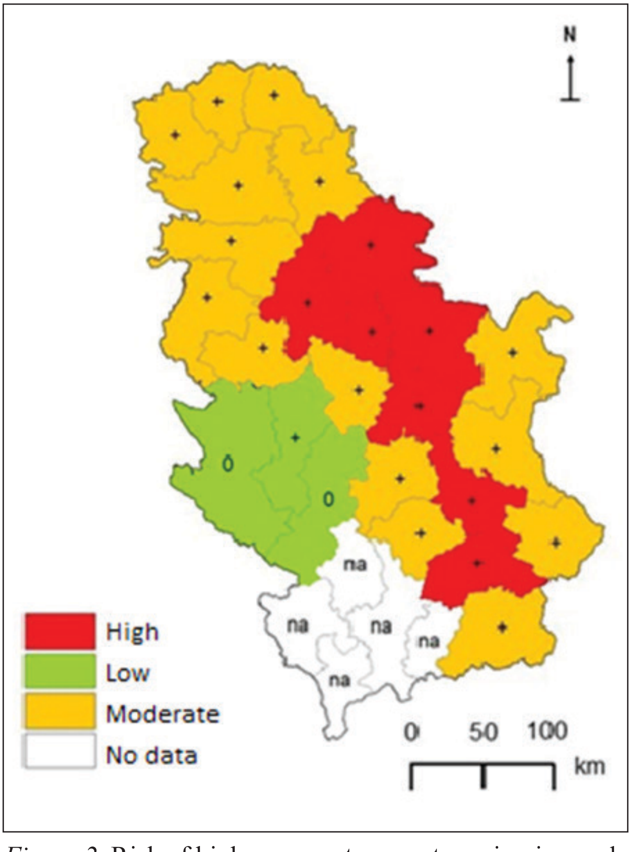

Figure 2. Risk of high summer temperatures in vineyards in different administrative regions in Serbia

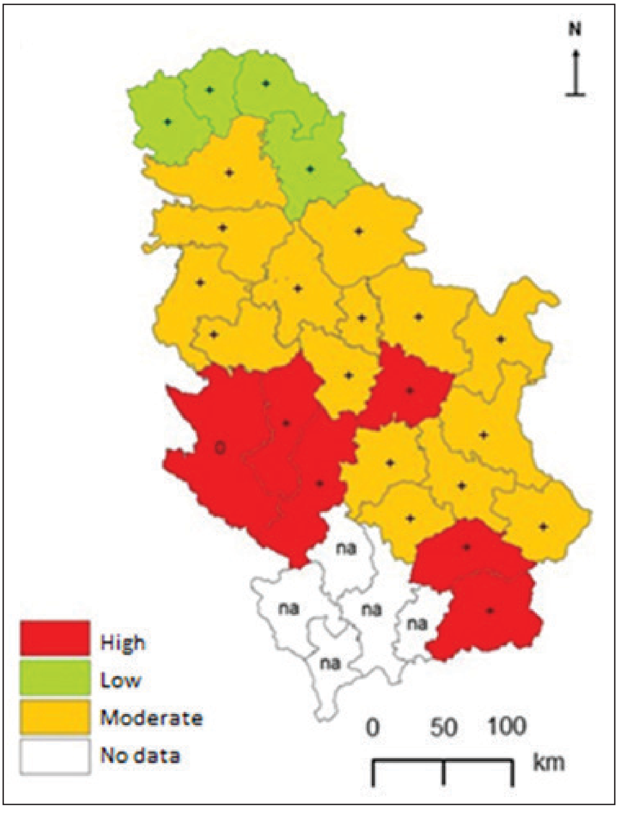

Figure 3. Risk of hail in vineyards in different administrative regions in Serbia

CONCLUSION

Changes in the climatic categories for growing vines show a shift in the optimal conditions for growing grapes, as well as the production of high-quality wine. In the case of production optimization, viticulture can benefit from climate change in the coming decades. The increase in the prevalence and frequency of the risk of hail indicates a high risk of this phenomenon for viticultural production. To use the potential for cultivation of grapevine, it is necessary to regularly renew the viticulture zoning of Serbia, including assessments of potential risks from climate change, with recommendations for the selection of varieties, locations, methods of cultivation and various agrotechnical measures that would ensure the quality of the yield. In areas with a high risk, it is necessary to protect the plantings from hail, high temperatures and other unfavourable factors. Measures for adapting viticulture to climate change at the global level are: moving vineyards to higher altitudes, natural shading of vineyards (cultivation on north-facing sides, setting up nets), adequate protection against diseases and pests due to more frequent adverse weather conditions, adequate defense against extreme events (droughts, floods) and the appropriate selection of varieties and rootstocks. Appropriate agro and ampelotechnical measures such as tillage and pruning can also be applied in vineyards with the aim of mitigating negative extreme events in the appropriate phenophases of grapevine development and concept of organic viticulture.

ACKNOWLEDGEMENT

This research was funded by the Project: "Advancing medium and long-term adaptation planning in the Republic of Serbia – NAP", funded by Green Climate Fund (GCF) and implemented by UNDP, in partnership with the Ministry of Agriculture, Forestry and Water Management Republic of Serbia and research within the Agreement on the realization and financing of scientific research work in 2023 between the Ministry of Science, Technological Development and Innovation of the Republic of Serbia and the Faculty of Agriculture of the University of Belgrade, agreements. /2023-01/200116.

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ОРИГИНАЛНИ ЧЛАНАК
Примљен: 1. 11. 2023.
Прихваћен: 30. 1. 2024.

ПРИОРИТЕТНЕ МЕРЕ АДАПТАЦИЈЕ НА КЛИМАТСКЕ ПРОМЕНЕ У СЕКТОРУ ВИНОГРАДАРСТВА У СРБИЈИ

Зорица РАНКОВИЋ ВАСИЋ, Ана ВУКОВИЋ ВИМИЋ,
Мирјам ВУЈАДИНОВИЋ МАНДИЋ

Универзитет у Београду, Пољопривредни факултет,
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РЕЗИМЕ: Виноградарска производња, као и цела пољопривреда, рањива је на климатске промене, које између осталог условљавају и значајно повећање температуре ваздуха. Високе температуре ваздуха у периоду зрења негативно утичу на принос и квалитет грожђа. Осим повећања температуре ваздуха, на производњу грожђа утичу и падавине и учесталост екстремних догађаја. Климатске пројекције за будућност предвиђају даље повећање температуре ваздуха и промену најважнијих виноградарских индекса. Анализа показује да је дошло до промене категорије климе према вредностима WI и HI, а у неким областима и CI. Индекс суше (DI) не показује значајније промене у прва два периода (2021–2040; 2041–2060), за разлику од повећане учесталости суша и продужења трајања сушног периода током године. Како су најзначајнији ризици у виноградарству од високих температура у периоду сазревања грожђа као и од града и олујног времена током вегетације, потребне су одговарајуће мере адаптације.

КЉУЧНЕ РЕЧИ: адаптација, изложеност, климатске промене, виноградарски индекси, рањивост, ризик

Зборник Матице српске за природне науке / Matica Srpska J. Nat. Sci. № 147, 81—93, 2024

UDC 634.8+663.2(497.11)
https://doi.org/10.2298/ZMSPN2447081U
ORIGINAL ARTICLE
Received: 2023/10/31
Accepted: 2023/11/6

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CHARACTERISTICS OF GRAPE MUST OF PERSPECTIVE GENOTYPES IN AUTOCHTHONOUS GRAPEVINE VARIETY *PROKUPAC*

ABSTRACT: The autochthonous, i.e. local, red wine variety *Prokupac* is one of the most important varieties in Serbia. In the past, this variety was the leading one in the largest number of wine-growing areas of our country. The *Prokupac* variety is currently the most represented in the Tri Morave wine-growing region (Central Serbia), while the largest number of vineyards with this variety are located in the Župa wine-growing subregion. Due to its importance as an old and traditional variety, certain researches of this variety have been initiated in recent years in wine-growing areas where *Prokupac* is traditionally grown. Certain scientific activities were initiated to isolate different genotypes with improved production and use value, all with the aim of clonal selection of this variety. In this paper, some characteristics and quality parameters of the more promising *Prokupac* genotypes that were isolated in the Župa wine-growing subregion in the Aleksandrovac municipality are presented. More extensive research was carried out on grapes from the harvest of 2019, 2020 and 2021 with 16 selected genotypes, namely eight genotypes from the Latkovac locality and eight genotypes from the Drenča locality. In addition to laboratory analyses of sugar, acid and pH content in the grape must, the theoretical alcohol content was also calculated. Furthermore, spectrophotometric methods were used to determine colour intensity A-420, colour intensity A-520, colour intensity A-620 and total colour intensity, as well as colour tonality. The analyses showed that certain main quality parameters from the research are within the value limits as in earlier research, but that the acid content is generally more optimal for red wine varieties compared to the research of that parameter carried out in the earlier period in other wine-growing areas (Fruška Gora and Čegar wine-growing subregions).

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Moreover, in our research, the sugar content of the grape is higher than the established values of that parameter in the grape must from the Čegar wine-growing subregion.
KEYWORDS: grape must, *Prokupac*, Župa wine-growing subregion

INTRODUCTION

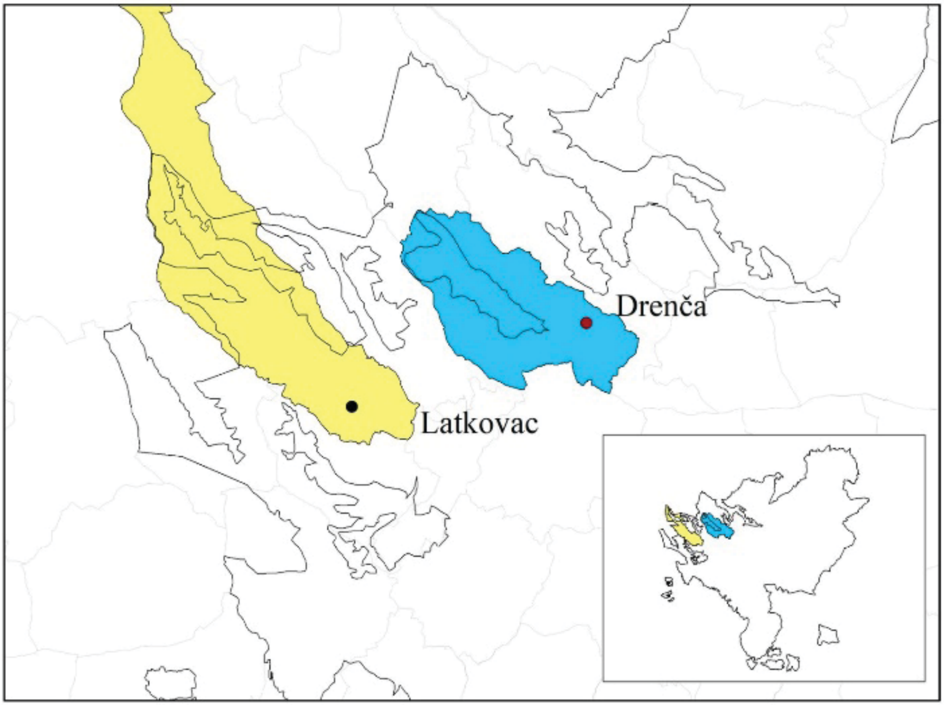
The *Prokupac* grapevine variety is a Serbian autochthonous variety (Zirojević, 1974; Radojević et al., 2013; Marković and Atanacković, 2013; Petrović et al., 2019) that, according to most authors, originates from the vicinity of Prokuplje, a historic vineyard centre in Serbia (Zirojević, 1974; Radojević et al., 2013). This red wine variety belongs to the *Convarietas pontica* group, *subconvarietas balcanica*, and has numerous synonyms such as *Rskavac*, *Kameničarka*, *Niševka*, *Crnka*, *Prokupk*a, and others (Zirojević, 1974; Cindrić et al., 2000; Jakšić et al., 2019). In terms of total vineyard area, *Prokupac* is the leading local and regional grapevine variety in Serbia compared to 31 other old local grapevine varieties. Additionally, when considering the distribution of vineyard areas for this variety across wine-growing regions, the Tri Morave region (Tomić et al., 2019) stands out with the largest areas of this local variety, with the Župa wine-growing subregion being particularly notable (Jakšić, 2019). The precondition for valorizing wine from local grapevine varieties and expanding vineyards with such varieties is the identification of valuable genotypes and research aimed at clonal selection of the best and most promising genotypes of local grapevine varieties. However, one of the challenges facing contemporary scientific practice is the insufficient representation of vineyards with local varieties. Over the years, vineyards with such varieties in Serbia have been largely cleared, and their share in the total vineyard area (excluding the widespread local variety Grašac) is just over 15%, with a share in the total number of vineyards being slightly less than 34% (Jakšić et al., 2022a). For this reason, the preservation and development of local varieties, such as the *Prokupac* variety, pose a special challenge to today's domestic science. One of the first steps in this process is the selection of promising genotypes in old vineyards that are the subject of research for clonal selection. Such a scientific-professional process has been initiated by the Centre for Viticulture and Oenology in Niš for the *Prokupac* variety and other local grapevine varieties in the Župa wine-growing subregion and other wine-growing areas in Serbia. Given that the analysis of current and future climate change has shown that climate change will have a positive impact on the *Prokupac* variety (Jakšić et al., 2022b; Jaksic et al., 2022c), as a variety with a later grape ripening time, special attention is paid to the selection of promising genotypes of this variety.

The aim of this paper is to present the results of laboratory analyses of the quality parameters of the grape must in selected individual promising genotypes of the *Prokupac* variety from the Župa wine-growing subregion.

MATERIALS AND METHODS

Data on Vineyards with Selected Genotypes

The production vine plantations where the selection of prospective genotypes of the *Prokupac* grapevine variety was carried out are located within the Tri Morave wine-growing region, Župa wine-growing subregion. The vineyards are situated in two localities, within two cadastral municipalities, namely Latkovac and Drenča (Map 1).



Map 1. Localities of vineyards where *Prokupac* genotypes were selected

The vineyard in Latkovac (Lat/Lon: 43º 27' 57.8496" N, 21° 01' 00.8199" E) was planted in 1972, situated at an altitude of 543 metres, with a slope of 17.2º and a southern exposure. The rootstock used is *Rupestris* du Lot, and the vineyards follow the traditional (Župski) training system. Promising genotypes from this locality are marked with numbers from 1 to 8.

The vineyard in Drenča consists of two parts, Lukarevina (Lat/Lon: 43° 28' 22.5215" N, 21° 02' 31.9569" E) and Gubovac (Lat/Lon: 43° 28' 36.5154" N, 21° 03' 26.5526" E). The genotypes marked from 9 to 13 are from the part of the vineyard planted in 1920, while those marked from 13 to 16 are from the section planted in 1900. This vineyard is located at an altitude of 403 metres, with a slope of 16.7º and a southern exposure. Similar to the Latkovac vineyard, it also uses *Rupestris* du Lot rootstock and follows traditional (Župski) vine training system.

The methodology for preparing grape must samples

Grapes from the selected 16 genotypes, with eight genotypes from each of the two localities, were harvested in the years 2019, 2020, and 2021. Before harvesting, the analysis determined that the grapes were 100% healthy. After harvesting the entire grape crop from the vines of the selected genotypes, they were transported and temporarily stored under cold conditions. Subsequently, the grapes were crushed, ensuring that the grape must extraction was moderate. Laboratory analysis of the grape must quality parameters was conducted at the Center for Viticulture and Oenology in Niš.

The laboratory analyses

Determination of sugar content in grape must be carried out using a digital refractometer following the method specified by the International Organization of Vine and Wine (OIV): Method OIV-MA-AS2-02 (OIV, 2016).

Determination of total acidity in grape must was performed through titration using a titrator in accordance with the OIV method: Method OIV-MA-AS313-01 (OIV, 2016).

Determination of pH of grape must was carried out potentiometrically following the OIV method: Method OIV-MA-AS313-15 (OIV, 2016).

The theoretical alcohol content in grape must was determined computationally by multiplying the sugar content in percentage by the factor 0.59 (fermentation of 1 gram of sugar yields approximately 0.59 ml of alcohol) (Jakšić, 2023).

The colour intensity and colour intensity analyzed using spectrophotometric methods following the OIV method: Method OIV-MA-AS2-07B (OIV, 2016).

RESULTS AND DISCUSSION

Grape Must Quality Parameter Analysis

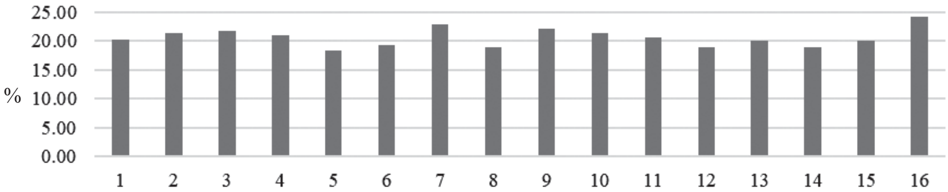
All the data for the selected and analyzed quality parameters of grape must from prospective genotypes of the *Prokupac* variety for the period 2019–2021 are presented in Appendix 1, Table 1. Laboratory analyses have shown that there are certain differences in parameter values among prospective genotypes.

Table 1. The main quality parameters of the grape musts of selected perpective genotypes of the Prokupac variety (2019–2021)

Genotypes	Vineyard localities	Sugar content (%)	Total acids (g/l)	pH	Theoretical alcohol content (%Vol.)
1	Latkovac	20.29	6.05	3.36	11.96
2	Latkovac	21.33	5.89	3.56	12.59
3	Latkovac	21.72	5.84	3.54	12.81
4	Latkovac	21.00	5.62	3.51	12.38
5	Latkovac	18.39	9.10	3.37	10.85
6	Latkovac	19.23	7.35	3.32	11.33
7	Latkovac	22.83	6.27	3.62	13.47
8	Latkovac	19.00	7.37	3.41	11.20
Average	Latkovac	20.47	6.69	3.46	12.07
9	Drenča	22.18	6.12	3.71	13.08
10	Drenča	21.30	4.95	3.82	12.56
11	Drenča	20.67	5.78	3.71	12.18
12	Drenča	18.98	5.66	3.66	11.19
13	Drenča	20.16	7.26	3.46	11.88
14	Drenča	18.93	5.81	3.49	11.17
15	Drenča	20.08	5.60	3.65	11.83
16	Drenča	24.20	4.82	3.68	14.25
Average	Drenča	20.81	5.75	3.65	12.27
Total Average	Latkovac and Drenča	20.64	6.22	3.55	12.17

Sugar Content

The average sugar content (%) in the grape must of the investigated prospective genotypes of the *Prokupac* variety under the conditions of the Župa wine-growing subregion at the Latkovac locality was determined to be 20.47%, while at the Drenča locality, it was 20.81%. Laboratory analysis revealed that among the eight genotypes at each of the two localities, the highest average sugar content at the Latkovac locality was found in genotype 7 (22.83%), and at the Drenča locality, it was in genotype 16 (24.20%) (Graph 1).



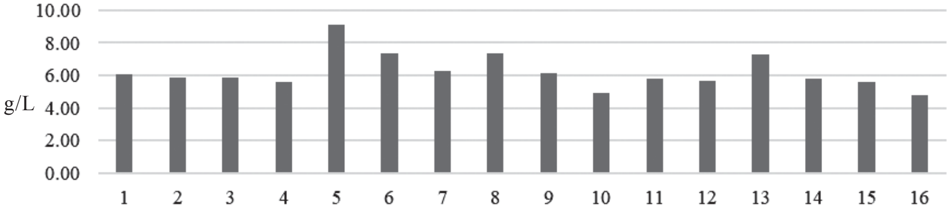
Graph 1. Average sugar content (%) in grape must of *Prokupac* genotypes (1–16) (2019–2021)

Comparing the average sugar content in the grape must from prospective genotypes at both localities, it can be concluded that this quality parameter of the grape must is slightly better in the genotypes from the Drenča locality. This is somewhat understandable, given that the vineyards at that locality are older and located at a slightly lower altitude.

The results of sugar content obtained in this research are similar to previous studies, falling within the approximate range of results from earlier investigations of this grape must parameter. Specifically, the similarity of these analyses aligns with the average sugar content in grape masts in the Čegar wine-growing subregion (Niš wine-growing region) during the period 1958–1972 (21.06%) (Zirojević, 1974) and the Fruška Gora wine-growing subregion (Srem wine-growing region) during the period 1981–1998 (20.10%) (Cindrić et al., 2000). However, the sugar content in the grape must in our research is higher compared to more recent studies conducted in the Čegar wine-growing subregion during the period 2009–2010 (18.95%) (Radojević et al., 2013).

Total acidy content

Regarding the average total acidity content (g/L) in the grape must is always expressed in grams of tartaric acid per liter, it amounted to 6.69 g/L for the investigated prospective *Prokupac* genotypes at the Latkovac site, and 5.79 g/L for the genotypes at the Drenča site. The highest average total acidity content was observed in the grape must of genotype 5 at the Latkovac locality (9.10 g/L) and genotype 13 at the Drenča locality (7.26 g/L) (Graph 2).



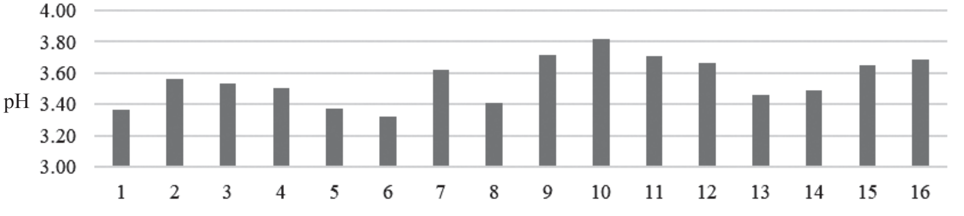
Graph 2. Average amount of total acids in grape must genotypes (1–16) of the *Prokupac* variety (g/L) (2019–2021)

The results of the research on the average acidity content (g/L) of the grape must indicate that this parameter is higher in the grape must from the prospective genotypes of the *Prokupac* variety located in the Latkovac locality.

The results of the total acidity content (g/L) of the grape must obtained in this research are similar to previous studies on this parameter, specifically in line with earlier research conducted on grape must from the Čegar wine-growing subregion during the period 1958–1972 (6.97 g/L) (Zirojević, 1974) and in the period 2009–2010 (6.84 g/L) (Radojević et al., 2013). However, the average acidity content obtained in our research is much lower compared to the high acidity content in the grape must of the *Prokupac* variety determined for the period 1981–1998 in the conditions of the Fruška Gora wine-growing subregion (9.10 g/L) (Cindrić et al., 2000).

pH of grape must

The average pH of the grape must in the investigated prospective genotypes of the *Prokupac* variety under the conditions of the Župa wine-growing subregion in the Latkovac locality was 3.46, while in the Drenča locality, it was 3.65. Laboratory analysis revealed that among the eight genotypes at each locality, the grape must from genotype 6 in the Latkovac locality had the lowest average pH (3.32). In the Drenča locality, the grape must from genotype 13 had the lowest pH (3.46) (Graph 3).



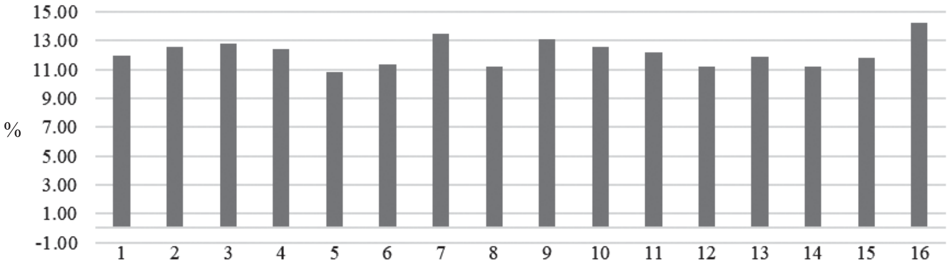
Graph 3. Average pH of grape must genotypes (1–16) of *Prokupac* variety (2019–2021)

The results of the pH of grape must indicate a slightly better pH value in the grape must from the genotypes located in the Latkovac locality compared to the genotypes from the Drenča locality.

The theoretical alcohol content

The average value of the calculated theoretical alcohol content (%Vol.) for future wines in the investigated prospective genotypes of the *Prokupac* variety in the Latkovac locality was 12.07%Vol., while for the genotypes in the Drenča locality, it was 12.27%Vol.

Laboratory analysis revealed that among the eight genotypes at each locality, genotype 7 from the Latkovac locality had the highest average value of theoretical alcohol content (13.47%Vol.), and from the Drenča locality, genotype 16 had the highest (14.25%Vol.) (Graph 4).



Graph 4. The average theoretical alcohol content in grape must genotypes (1–16) of *Prokupac* variety (%Vol.) (2019–2021)

As with the average sugar content in the grape must, the average theoretical alcohol content in future wines is slightly higher for the genotypes from the Drenča locality compared to the genotypes from the Latkovac locality.

Colour intensity and colour tonality of grape must

All results of the grape must colour intensity analysis (including color intensity – A_{420}, color intensity – A_{520}, and color intensity – A_{620}) and the colour tonality of the grape must from the investigated prospective genotypes of the *Prokupac* variety for the period 2019–2021 are presented in Appendix 1, Table 2.

Table 2. Color intensity and color tonality in grape musts of perspective genotypes of the *Prokupac* variety (2019–2021)

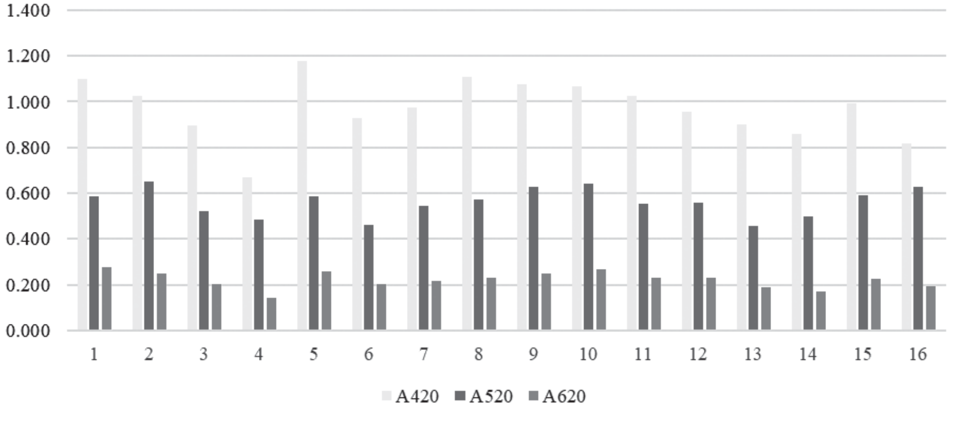
Genotypes	Vineyard localities	Color intensity – A_{420}	Color intensity – A_{520}	Color intensity – A_{620}	Color intensity	Color tonality
1	Latkovac	1.099	0.587	0.275	1.961	1.872
2	Latkovac	1.026	0.652	0.246	1.925	1.573
3	Latkovac	0.894	0.521	0.201	1.615	1.716
4	Latkovac	0.671	0.485	0.142	1.298	1.383
5	Latkovac	1.176	0.586	0.259	2.021	2.007
6	Latkovac	0.929	0.459	0.203	1.591	2.024
7	Latkovac	0.975	0.544	0.215	1.733	1.793
8	Latkovac	1.110	0.573	0.231	1.914	1.938
Average	Latkovac	0.985	0.551	0.221	1.757	1.788
9	Drenča	1.076	0.628	0.250	1.954	1.712
10	Drenča	1.067	0.640	0.267	1.974	1.667
11	Drenča	1.024	0.554	0.231	1.809	1.848
12	Drenča	0.958	0.559	0.228	1.745	1.714

13	Drenča	0.900	0.457	0.188	1.544	1.970
14	Drenča	0.857	0.500	0.169	1.526	1.714
15	Drenča	0.993	0.590	0.223	1.807	1.683
16	Drenča	0.817	0.629	0.194	1.639	1.300
Average	Drenča	0.961	0.570	0.219	1.750	1.688
Total Average	Latkovac and Drenča	0.973	0.560	0.220	1.753	1.738

Colour intensity analysis of grape must

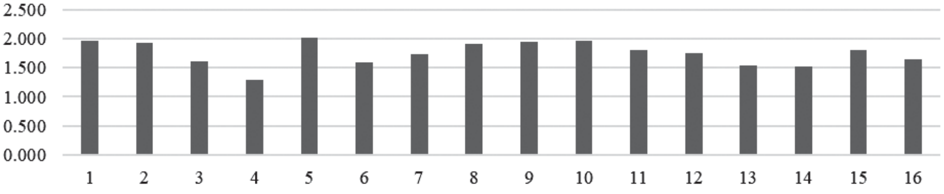
From 2019 to 2021, the average value of colour intensity – A_{420} in the grape must of the investigated prospective genotypes of the *Prokupac* variety in the Latkovac locality was 0.985, while in the Drenča locality, it was slightly lower at 0.961. The average value of colour intensity – A_{520} in the grape must from the genotypes in the Latkovac locality was 0.551, and in the Drenča locality, it was slightly higher at 0.570. The average value of colour intensity – A_{620} in the grape must from the genotypes in the Latkovac locality was 0.221, while in the Drenča locality, it was slightly lower at 0.219.

Analysis results of the grape must from individual prospective genotypes showed that the highest average value of colour intensity – A_{420} within the Latkovac locality was found in the grape must from the prospective genotype 5 (1.176), while in the Drenča locality, it was genotype 9 (1.076). The highest average value of colour intensity – A_{520} within the Latkovac locality was determined in the grape must from the prospective genotype 2 (0.652), and in the Drenča locality, it was genotype 10 (0.640). The highest average value of colour intensity – A_{620} within the Latkovac locality was found in the grape must from the prospective genotype 1 (0.275), while in the Drenča locality, it was genotype 10 (0.267) (Graph 5).



Graph 5. Average colour intensity of grape must genotypes (1–16) of *Prokupac* variety (2019–2021)

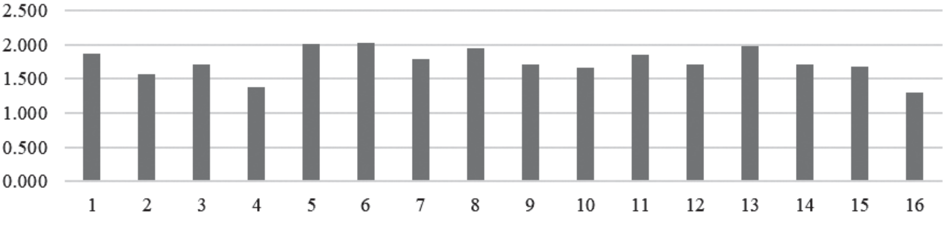
Analyzing the total colour intensity of the grape must from prospective genotypes of the *Prokupac* variety, it can be concluded that the highest average value of total colour intensity was observed in the grape must from genotype 5 from the Latkovac locality (2.021) and genotype 10 from the Drenča locality (1.974) (Graph 6).



Graph 6. The average value of the total colour intensity of grape must genotypes (1–16) of *Prokupac* variety (2019–2021)

The average value of the total colour intensity of the grape must from the investigated prospective genotypes of the *Prokupac* variety for the period 2019–2021 in the Latkovac locality was 1.757. This average value was slightly lower in the grape must from the genotypes in the Drenča locality, specifically 1.750.

Results of the analysis of the colour tonality of the grape must showed that genotype 6 had the highest average value of colour tonality (2.024) within the Latkovac locality. In the Drenča locality, the grape must from genotype 13 exhibited the highest colour tonality value (1.970) (Graph 7).



Graph 7. The average value of the color tonality of grape must of selected genotypes (1–16) of the *Prokupac* variety (2019–2021)

CONCLUSION

Based on the analyses of various quality parameters of grape must from 16 selected prospective genotypes of the autochthonus Prokupac variety for the period 2019–2021 from two localities (Latkovac and Drenča, with 8 genotypes each), the following conclusions can be drawn regarding the overall quality of the Prokupac grapevine variety in the Župa wine-growing subregion.

The average sugar content (%) in grape must of the investigated prospective *Prokupac* genotypes ranged from 18.39% to 24.20%, with genotype 16 from the Drenča locality showing the highest average sugar content. The average total acidity (g/L) in the grape must ranged from 4.82 g/L to 9.10 g/L, with genotype 5 from the Latkovac locality having the highest value. The average pH of grape must were in a range from 3.32 to 3.82. The pH values suggest slightly better acidity levels in the grape must of genotypes from the Latkovac locality compared to those from the Drenča locality. The calculated theoretical alcohol content (%Vol.) of future wines from the investigated *Prokupac* genotypes ranged from 10.85%Vol. to 14.25%Vol., and similar to the average sugar content, the theoretical alcohol content in future wines was slightly higher for genotypes from the Drenča locality. The average colour intensity – A_{420} in the grape must of prospective *Prokupac* genotypes was 0.973, with genotype 5 from the Latkovac locality showing the highest value. The average colour intensity – A_{520} in the grape must was 0.560, with genotype 2 from the Latkovac locality exhibiting the highest value. The average colour intensity – A_{620} in the grape must was 0.220, with genotype 1 from the Latkovac locality having the highest value. Analyzing the total colour intensity of the grape must, it can be concluded that the average value was 1.753, with genotype 5 from the Latkovac locality showing the highest intensity.

Regarding the average colour tonality, the results indicated that this parameter was higher for genotypes from the Latkovac locality compared to those from the Drenča locality. Genotype 6 from the Latkovac locality had the highest average colour tonality.

In summary, the prospective genotypes from both locations exhibited main quality parameters of grape must that fall within general limits based on available data from previous research in the Čegar and Fruška Gora wine-growing subregions. However, certain main parameters have more favourable values, and above all the acid content in the grape must, which is within optimal limits for red wine varieties compared to earlier research conducted in the Fruška Gora wine-growing subregion, when the acid content was somewhat less favourable. In addition to this broader quality parameter, the sugar content was higher in this research compared to the analyzed values of that parameter determined in the conditions of the Čegar wine-growing subregion.

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ОРИГИНАЛНИ ЧЛАНАК
Примљен: 31. 10. 2023.
Прихваћен: 6. 11. 2023.

КАРАКТЕРИСТИКЕ ШИРЕ ПЕРСПЕКТИВНИХ ГЕНОТИПОВА АУТОХТОНЕ СОРТЕ ВИНОВЕ ЛОЗЕ *ПРОКУПАЦ*

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РЕЗИМЕ: Аутохтона, односно локална црна винска сорта винове лозе *прокупац* једна је од најважнијих сорти Србије. Некада је та сорта била водећа сорта у највећем броју виноградарских подручја наше земље. Сорта *прокупац* је тренутно најзаступљенија у виноградарском рејону Три Мораве (Централна Србија), док се највећи број винограда са овом сортом налази у Жупском виногорју. Због значаја који има као веома стара и традиционална сорта, последњих година су покренута извесна истраживања ове сорте у виноградарским подручјима у којима се *прокупац* традиционално гаји. Одређене научне активности су покренуте на издвајању различитих генотипова са побољшаном производном и употребном вредношћу, а све у циљу клонске селекције ове сорте. У овом раду представљене су неке карактеристике, односно параметри квалитета шире перспективних генотипова сорте *прокупац* који су издвојени у Жупском виногорју у општини Александровац. Испитивање шире извршено је од грожђа из бербе 2019, 2020. и 2021. године са 16 издвојених генотипова, и то 8 генотипова са локалитета Латковац и 8 генотипова са локалитета Дренча. Осим лабораторијских анализа садржаја шећера, киселина и pH, извршен је и обрачун теоретског садржаја алкохола. Такође, спектрофотометријским методама је одређен интензитет боје А – 420, интензитет боје А – 520, интезитет боје А – 620 и укупни интезитет боје шире, као и тоналитет боје шире. Анализе су показале да су одређени главни параметри квалитета шире из наших истраживања у границама вредности као и у ранијим истраживањима, али да је садржај киселина у шири оптималнији за црне винске сорте у односу на истраживања тог параметра вршена у ранијем периоду у другим виноградарским подручјима (Фрушкогорско и Чегарско виногорје). Такође, садржај шећера у шири је већи у нашим истраживањима у односу на утврђене вредности тог параметра шире од грожђа из Чегарског виногорја.

КЉУЧНЕ РЕЧИ: *прокупац*, Жупско виногорје, шира

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Matica Srpska Journal for Natural Sciences
Published twice a year
Editorial and publishing office:
1 Matica Srpska Street, 21000 Novi Sad, Serbia
Phone: +381 21/6615798

E-mail: ljdrazic@maticasrpska.org.rs
zmspn@maticasrpska.org.rs
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The editors of the *Matica Srpska Journal for Natural Sciences*
completed the selection for Issue 147 (2/2024) on may 15, 2024

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Language editor: Ljiljana Tubić
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Technical design: Vukica Tucakov

Published in july 2024
Computer set: Vladimir Vatić, GRAFIT, Petrovaradin
Printed by: SAJNOS, Novi Sad

Публиковање овог Зборника помогло је Министарство науке,
технолошког развоја и иновација Републике Србије

Publication of this volume was supported by the Ministry of Science,
Technological Development and Inovation of the Republic of Serbia

CIP – Каталогизација у публикацији
Библиотека Матице српске, Нови Сад

5/6(082)

ЗБОРНИК Матице српске за природне науке = Matica Srpska Journal for Natural Sciences / главни и одговорни уредник Ивана Максимовић. – 1984, св. 66– . – Нови Сад : Матица српска, Одељење за природне науке, 1984– . – 24 cm

Два пута годишње. – Наставак публикације: Зборник за природне науке. – Текст на енг. језику, резимеи на енг. и на срп. језику.

ISSN 0352-4906

COBISS.SR-ID 5845250