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Biotechnical faculty
Univerzitet Crne Gore
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Bul. M. Lalića 1, 81000 Podgorica, Crna Gora (Montenegro), P.Box 97,
Tel.: +382 20 268434; +382 20 268437; Fax: +382 20 268432
Web: www.agricultforest.ac.me; E-mail: agricultforest@ac.me

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CONTENT

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Mohammed AMRAOUI[1], Larbi BOUABIDI[1], Mohamed El AMRANI[1], Hasan OUAKHIR[2], Branislav DUDIC[3], Tin LUKIĆ[4] and Velibor SPALEVIC [5]

LAND USE DYNAMICS AND SOIL CONSERVATION STRATEGIES IN THE EL KSSIBA REGION, ATLAS MOUNTAINS OF MOROCCO

SUMMARY

This study investigates the dynamics of land use and land cover (LULC) changes in the El Kssiba region of the Middle Atlas Mountains, Morocco, with a focus on soil conservation and environmental sustainability. Through a comprehensive examination of historical archives, climatic data, geological characteristics, and land use patterns, this research assesses the impacts of environmental factors and socio-economic changes on land utilization over time. The study area, covering 576.57 km², has experienced significant transformations in land use driven by government policies, population growth, land tenure, and climatic conditions. Findings reveal a notable decline in agricultural and olive cultivation, alongside a substantial increase in bare land and forest cover, suggesting both degradation and potential recovery of land. These changes highlight the urgent need for sustainable land management and soil conservation strategies to mitigate degradation and enhance land productivity. The conducted SWOT analysis highlights the intricate relationship between the traditional strengths of pastoral systems and the pronounced contemporary challenges, especially regarding environmental degradation, climate change, and socio-economic pressures. This study offers key insights for policy development to promote balanced conservation and sustainable resource management, highlighting the need for long-term strategies to ensure sustainable land use in fragile ecosystems.

Keywords: Pastoralism; Land use dynamics; Soil conservation; Land cover change; Sustainability; El Kssiba region; Atlas Mountains; Morocco.

[1] Mohammed Amraoui (corresponding author: mohammed.amraoui2@usmba.ac.ma), Larbi Bouabidi, Mohamed el Amrani, History Legal sciences and languages laboratory, Sidi Mohamed Ben Abdellah University, Fez, Faculty of Letters and Human Sciences Saïs, MOROCCO;

[2] Hasan Ouakhir, Landscape, Risks and Heritage laboratory, University Sultan Moulay Slimane, Beni Mellal, MOROCCO;

[3] Branislav Dudic, Faculty of Management, Comenius University Bratislava, Bratislava, SLOVAKIA;

[4] Tin Lukić, Department of Geography, Tourism and Hotel Management, Faculty of Sciences, University of Novi Sad, Novi Sad, SERBIA

[5]Velibor Spalevic, Biotechnical Faculty, University of Montenegro, Podgorica, MONTENEGRO.

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INTRODUCTION

Land use changes, particularly in regions characterized by traditional subsistence practices, have profound impacts on soil conservation and environmental sustainability (Spalevic et al., 2021; Palevic et al., 2021; Kader et al., 2022). In the Middle Atlas of Morocco, the transformation of land use and land cover (LULC) is shaped by both natural processes and human activities, including pastoralism, which has historically dominated the region. Leveraging Earth Observation Data (EO) and soil erosion modelling makes feasible the spatial and temporal monitoring of soil loss rates due to land cover change and implementation of mitigation strategies (Costea et al., 2022; Sestras et al., 2023; Stefanidis et al., 2024a). As land degradation, shifting vegetation cover, and socio-economic shifts continue to influence the landscape, understanding the relationship between land use dynamics and environmental conservation becomes critical for sustainable development. Particularly important for stakeholders and policy makers is the quantification of ecosystem services related to soil conservation and provided by naturally vegetated areas (Stefanidis et al., 2024b; Olivetti et al., 2015; Lense et al., 2020). This study explores the patterns and drivers of land use change in the El Kssiba region, assessing its implications for soil conservation and sustainable resource management.

Pastoralism is a form of agriculture or subsistence system where people primarily rely on the raising and herding of domesticated animals for their livelihood. It is often practiced in areas where crop farming is difficult due to harsh environmental conditions, such as deserts, mountains, or dry plains, where the climate or terrain makes large-scale agriculture unfeasible. Livestock such as cattle, sheep, goats, camels, yaks, or reindeer are the primary source of food, clothing, and materials. Pastoralists rely on these animals for meat, milk, hides, wool, and other resources. Some pastoralists are fully nomadic, moving with their herds in search of fresh pastures and water throughout the year. Others practice transhumance, a seasonal movement between fixed summer and winter pastures. Pastoralists adapt to their environment by moving their herds to find adequate food and water, often in response to seasonal changes or environmental factors like droughts. In pastoral societies, livestock often holds not just economic value but also social, cultural, and religious significance. Animals may serve as a form of wealth and status and play a role in social and ceremonial life.

According to Shikui et al. (2016), pastoralism is a production system and livelihood strategy based on extensive livestock grazing on rangelands or grasslands, often involving some form of herd mobility, and has been practiced in many regions of the world for centuries. Currently, extensive pastoralism occurs on about 25% of Earth's land area, mostly in the developing world, spanning from the drylands of Africa and the Arabian Peninsula to the highlands of Asia and Latin America, where intensive crop cultivation is physically not possible due to harsh environments and poor access (Freier et al., 2014). Pastoralism is globally important for the human population it supports, the food and ecological services it provides, the economic contributions it makes to some of the world's poorest regions, and the long-standing civilizations it helps to sustain (Fernández-Giménez et al., 2021; Milanovic et al., 2010).

Within the Atlas Mountains of Morocco, cold oases connected to rangelands and collective grazing areas have been created by the Amazigh people despite extreme climate conditions. Throughout centuries of isolation, they have maintained their cultural heritage, including agricultural, social, artisanal, and linguistic traditions (Gault & Saïdi, 2016; Bammouet al., 2024a; Bammou et al., 2024b). Today, these people and their culture are recognized by the Moroccan Constitution as an integral part of the national identity.

Consequently, the nomadic populations of the Moroccan Middle Atlas have undergone major territorial, political, and economic transformations since the beginning of the twentieth century. These changes, deeply embedded in the collective memory of informants, reflect a transition from a fully nomadic society, prior to French colonization, to one increasingly aligned with the neoliberal principles of postmodern society.

As a result, pastoralism, traditionally characterized by extensive livestock grazing practices, has experienced substantial transformations globally due to factors such as climate change, socio-economic shifts, and policy interventions (Spalevic et al., 2020; Ljavić et al., 2023; Zhang et al., 2023; Lukovic et al., 2024). These changes have led to altered land use patterns, including shifts in vegetation cover, soil degradation, and changes in biodiversity (Mohammadi et al., 2021; Kader & Jaufer, 2022; Spalevic et al., 2024; Sabljic et al., 2024).

While some argue that these transformations have increased productivity and resilience among pastoral communities, others highlight negative implications such as land degradation, loss of biodiversity, and conflicts over land resources. Understanding the multifaceted impacts of pastoralism transformation on land use requires an interdisciplinary approach, drawing insights from ecology, geography, anthropology, and socio-economics.

Through empirical studies and theoretical frameworks, researchers aim to unravel the complexities of these dynamics and inform sustainable land management strategies (Fernández-Giménez et al., 2017; Galvin et al., 2008; Turner et al., 2016). In this context, the present study aims to assess the changes in land due to pastoral activities in the Middle Atlas of Morocco, particularly within the Ait Werra Tribe.

MATERIAL AND METHODS

Study area. The Ait Werra tribe historically extended over the Atlas Mountains of Morocco, covering both the Middle and parts of the High Atlas, within an area of 576.57 km². This area is bounded by the coordinates 6°6′12″W, 6°6′24″W and 33°32′34″N, 33°33′14″N (Figure 1). The tribal lands are situated between 1,600 and 2,100 meters above sea level, with all sites equally accessible by road (Figure 1).

The region experiences significant variation in annual rainfall, ranging from a maximum of 635 mm in the upstream areas to a minimum of 42 mm in the downstream parts of the study area, with an average rainfall of 348 mm (Ouakhir et al., 2020). Additionally, there are, on average, 25 days of snowfall per year at elevations exceeding 800 meters above sea level.

Temperature fluctuations in the area are notable, ranging from 2°C to 45°C. The average minimum temperature occurs in January at 3.7°C, while the average maximum temperature reaches 38°C in August (USAID, 2010; Ennaji et al., 2024).

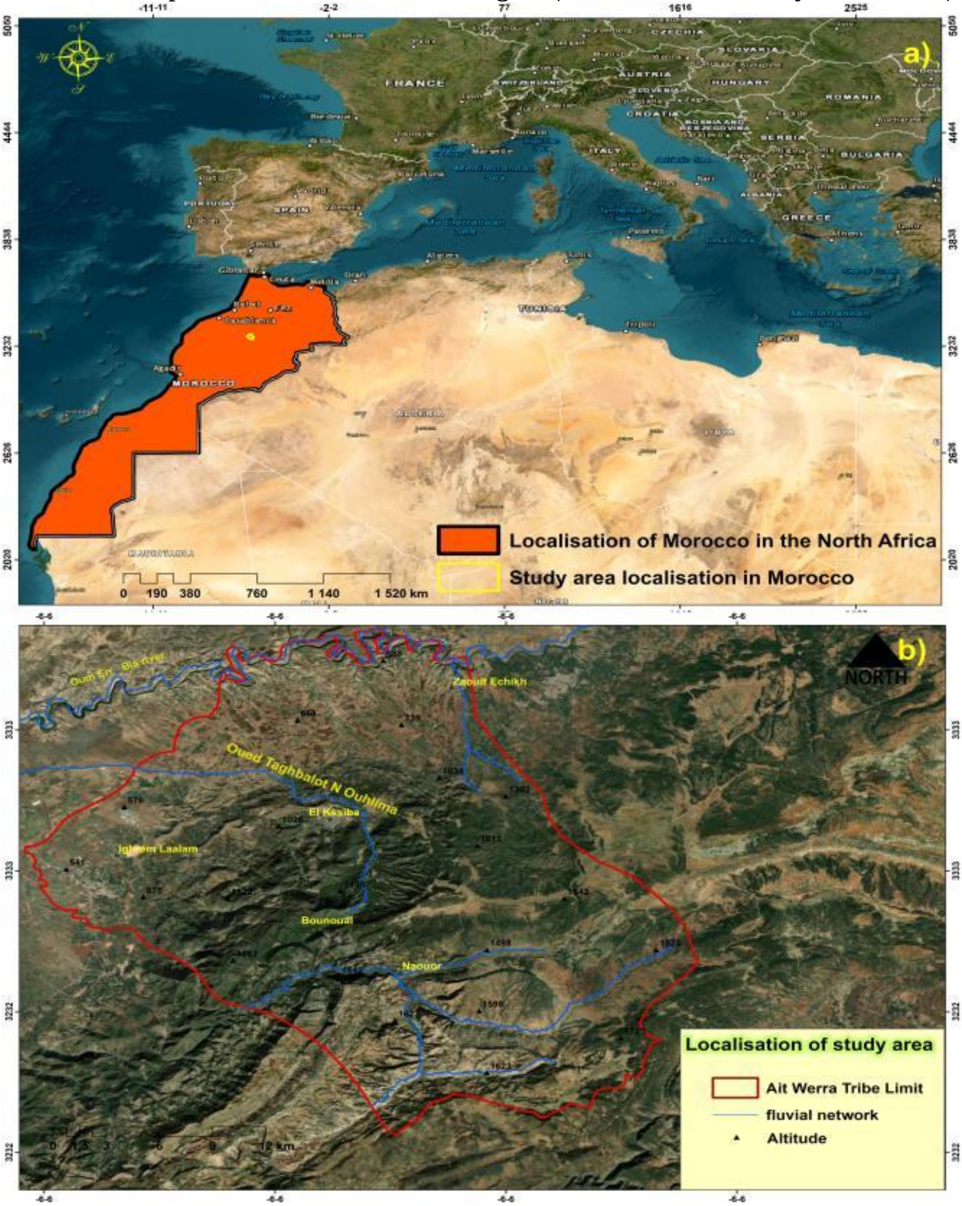

Figure 1: a) The geographical location of Morocco in North Africa, and b) the location of the study area within the Atlas Mountains of Morocco.

The study area is located within the Oum Err Bia Basin, which spans over 500 km in length, originating from the Middle Atlas at an elevation of 1,800 meters above sea level (Ouakhir & El Ghachi, 2023). The river traverses the Tadla plain and the coastal plateau before reaching the city of Azemmour, where it empties into the Atlantic Ocean (Barakat et al., 2016). The principal tributaries of the river, situated on the left bank, include the Derna, El Handek, Tessaout, Lakhdar, and El Abid rivers (Ennaji et al., 2022).

Numerous dams and reservoirs have been constructed along the Oum Er Rbia River to generate hydroelectric power, facilitate irrigation, and meet domestic and industrial water demands (Mosaid et al., 2022). The river and its tributaries pass through regions known for robust agricultural activities, featuring extensive irrigated areas and livestock farming, as well as industrial zones housing oil mills, phosphate extraction facilities, and sugar beet processing plants (Barakat et al., 2018).

The Aït Werra tribe is a significant and representative faction of the Aït Seri, located at the center of the Middle Atlas in Morocco, distant from major cities (Vaugien, 1950). Historically, the region's specific characteristics—such as its climate and location—made it ideal for livestock operations, with double transhumance being a traditional practice (Amraoui et al., 2023).

Fieldwork Methodology and Data Collection.

The main fieldwork, carried out in two phases December 2018 and in May-December of 2022, provided essential information on the impact of pastures in the field. Additionally, field surveys were conducted to gather comprehensive data on indigenous grazing practices, traditional pasture management, pastoral institutions, local perceptions, and responses to environmental changes. These surveys employed integrated methodologies such as participatory rural appraisal, open-ended questioning, and pretested questionnaires.

Supplementary data on challenges, opportunities, and changes in pastoral management systems, as well as external support and partnerships, were collected through group discussions and personal communications. The primary information gathered was documented through the transcription of audio or video recordings. Secondary information, including pasture management, pastoral development, and government policies, was collected and updated using various sources such as research publications, reports, newsletters, and yearbooks. Data quality was ensured through thorough investigation and cross-referencing with multiple sources. The analysis of all data was conducted using systematic qualitative techniques as recommended by Patton (1990) and Miles and Huberman (1994).

Hydrometeorological services provided rainfall and temperature data, allowing us to track the dynamics of the climatic situation in the study area during this period.

Data. The Table 1 presents the various types of data used and their sources, which include historical, environmental, and socio-cultural information, with a primary focus on El Kssiba. This includes rainfall data from the El Heri gauging station covering the period from 1975 to 2018, and French diplomatic archives extending from 1934 to 1956. Livestock and grazing data, spanning from 1953 to 2024, were also included, along with municipal and tribunal archives covering 1924 to 1956. Furthermore, river characteristics data from 1948 to 1952, as well as oral histories on water distribution traditions within the tribe during the same period, were collected.

These sources provide a comprehensive perspective on the tribe's development, environmental changes, legal frameworks, and cultural practices. They offer invaluable insights into the interplay between human activities, natural systems, and socio-cultural dynamics over time in El Kssiba.

Table 1: Sources and periods of data utilized in the study

Data type	Date	Source of data
Rainfall data (El Heri gauging station	1975-2018	HOBER 2024/ www.abhoer.ma
French diplomatic archive in Nantes (C.A.D.N)	1934-1956	French Foreign Affairs https://www.diplomatie.gouv.fr
Livestock and grazing data	1953-2024	The water and forest agency of El Kssiba http://www.eauxetforets.gov.ma/
Archive of El Kssiba Municipality	1924-1956	El Kssiba Morocco
Archive of El Kssiba Tribunal	1929-1956	El Kssiba Morocco
River's characteristics	1948-1952	Municipality of El Kssiba
Water distribution traditions in the tribe	1948-1952	Testimonial evidence
Land Use and Land Cover	2024	Google Earth, OziExplorer, and Fieldwork
Geology map	2024	Geological Maps of Beni Mellal, El Kssiba, and Imilchil (1:100,000 scale)
Pastures map	1953-2024	Centre des Archives Diplomatiques de Nantes https://www.diplomatie.gouv.fr

These sources provide a comprehensive perspective on the tribe's development, environmental changes, legal frameworks, and cultural practices. They offer invaluable insights into the interplay between human activities, natural systems, and socio-cultural dynamics over time in El Kssiba.

RESULTS AND DISCUSSION

Climate and Water Situation. In the studied region, there is significant variability in precipitation across the four seasons over the years. This seasonal rainfall variability is crucial for pastoralism, as it directly affects the availability of water and forage for livestock. While there are fluctuations from year to year, certain seasonal patterns emerge. For instance, winter and spring generally show higher precipitation levels compared to autumn and summer. These seasons may provide more favorable conditions for pasture growth, thereby supporting pastoral activities.

However, some years exhibit exceptionally low precipitation levels across all seasons, indicating potential drought conditions. Droughts can severely impact pastoralism by reducing grazing land and water availability for livestock. Despite the overall lower precipitation in summer, some years show significant rainfall during this season (Figure 2). Summer rains are particularly important for replenishing water sources and sustaining pasture growth during the dry season, which directly influences the viability of pastoralism in the region.

The considerable variability in rainfall from year to year suggests that pastoralists in the Atlas Mountains must adapt their grazing strategies and herd management practices to cope with changing environmental conditions. While specific trends may fluctuate, analyzing the data over several decades could reveal long-term trends in precipitation patterns, offering valuable insights into the effects of climate change on pastoralism in the region.

The seasonal precipitation data highlights the importance of monitoring weather patterns and adapting pastoral practices to ensure sustainable livestock management in the study area (Figure 2).

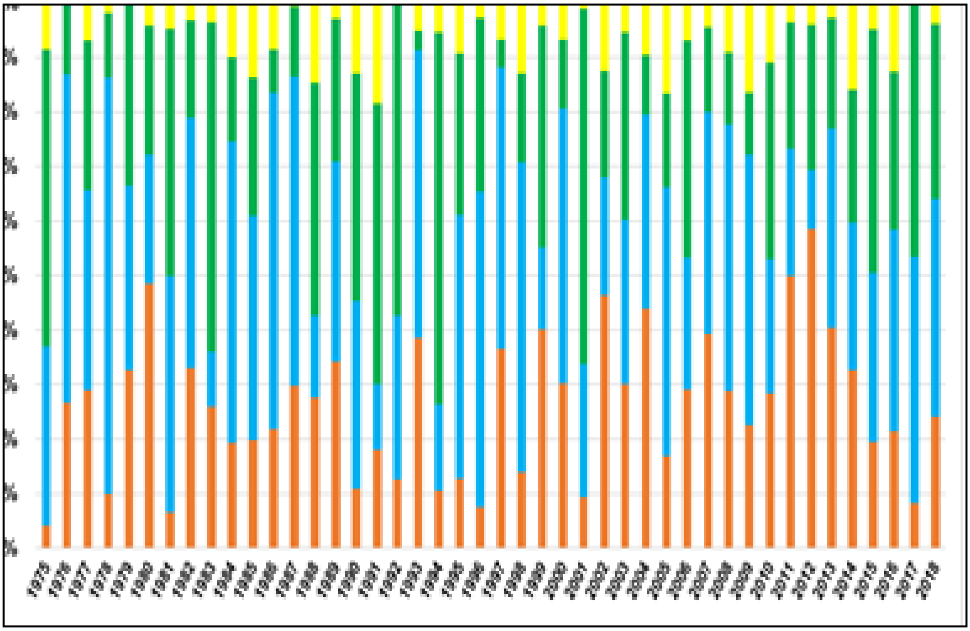

Figure 2: Seasonal rainfall distribution at the Tizi N'isli gauging station from 1975 to 2018.

Precipitation and Water Availability. Precipitation, especially during autumn, winter, and spring, plays a direct role in replenishing surface water sources such as rivers, streams, and lakes (Ouakhir et al., 2023). Higher rainfall during these seasons generally leads to increased water availability in these bodies of water, which is essential for both human consumption and supporting livestock during dry periods.

Geology and Lithology. According to the provided data, the lithology of the El Kssiba region is dominated by four main rock types: marl and limestone (31.74%), phosphate limestone (10.38%), marl and red clay (50.59%), and doleritic basalt (0.60%). Marl and limestone, as sedimentary rocks rich in calcium carbonate, tend to foster fertile soils by neutralizing acidity and improving soil structure. They also provide essential nutrients like magnesium, which benefit the overall soil fertility (Figure 3 and Table 2).

Table 2: Distribution of lithology percentage in the study area

Lithology type	Area (km^2)	Area (%)
Doleritic basalt	3.45	0.60
Marl and Limestone	182.83	31.74
Phosphate limestone	59.79	10.38
Marl and red clay	291.41	50.59
Deposit	38.55	6.69

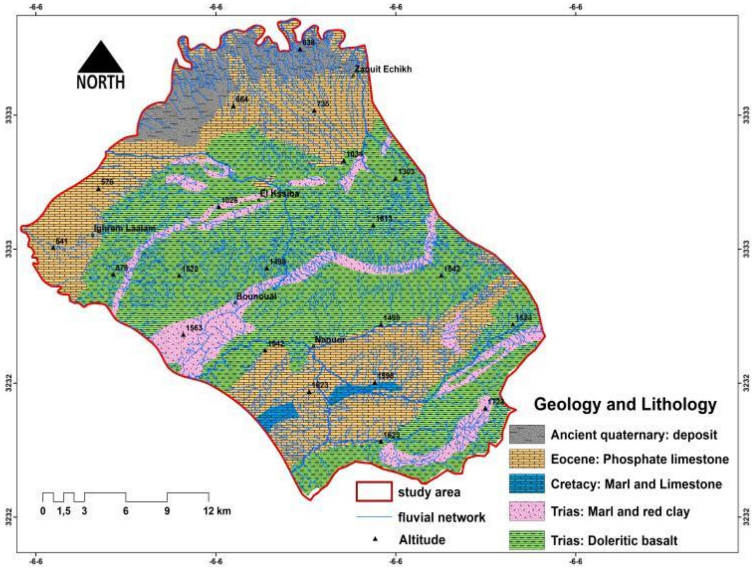

Figure 3: Geological and lithological characteristics of the study area.

Land Rights and Autonomy in the Ait Werra Region. The confederation of the Ait Werra settled in the Middle Atlas region between the seventeenth and nineteenth centuries, establishing their presence in territories such as El Kssiba and Ait Stri by the nineteenth century (Reifsteck, 1943). Over the centuries, they have accurately upheld their ancestral rights to the pastures within this critical area, rooted in age-old customs and traditions (El Bilali et al., 2020). These rights, established by historical precedent, granted them exclusive access to grazing lands through a system where precedence and defence were paramount.

Before the imposition of external governance in 1912, communities like the Ait Werra exercised autonomy, defining and enforcing their own imperatives. This autonomy was a testament to the sovereignty they once held over their territories (Tozy and Mahdi, 1990).

Figure 4: Partial view of the livestock fairground in Casablanca during the colonial period. Source: Velu, 1934.

Grazing Contracts During the Colonial Period. During the colonial period, grazing contracts replaced the pastoral systems that had characterized the region in the nineteenth century. Previously, most agreements were oral and based more on customs than on mutual understanding between the parties. Under these new contracts, shepherds were given the right to choose whether to receive their wages in cash or in kind. Additionally, they were able to obtain a share of the profits generated by the livestock. The contracts also specified the duration of the grazing (A.T.C.A.O, 1939).

For instance, in 1936, a grazing contract was agreed upon for a payment of 2,050 francs to a shepherd for grazing a flock of sheep for a year, without specifying the number of sheep. In the same year, another contract stipulated a payment of 1,042 francs for grazing twenty-five sheep annually. In the case of cattle, the shepherd's wage typically amounted to four hundred francs per head. When payment was made in kind, most contracts specified that the shepherd would receive a quarter of the profits as an annual wage in exchange for assuming all the responsibilities of caring for the livestock (A.T.C.A.O, 1936).

This shift can be attributed to the changes in the social system of the Ait Werra tribe. Grazing was no longer restricted to specific families but extended to various families that had lost their herds and lands due to the French invasion. The urgent need for cash to adapt to the developing economy, which integrated the local system into a capitalist framework, replaced traditional barter transactions with monetary ones. As many tribe members settled in the El Kssiba region and other urban centers, job opportunities in grazing became more abundant, allowing shepherds to choose their employers (Figure 4).

Consequently, shepherds gained the ability to impose their conditions in the grazing contracts, choosing between cash or in-kind payment. They also acquired the right to terminate the contract if the livestock owner failed to fulfill their obligations, with compensation for the time they worked. The role of the tribal community, which previously regulated the grazing process, became limited to documenting agreements between the contracting parties. Traditional customs for grazing were gradually reduced, persisting only in some mountainous areas that remained less affected by the capitalist colonial system (Figure 5).

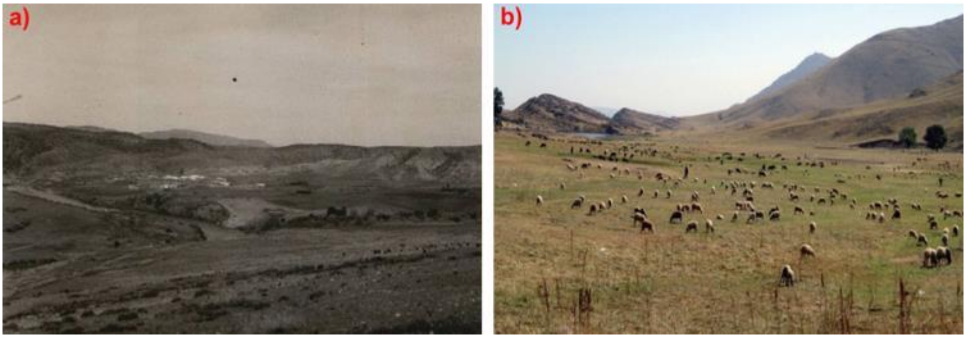

Figure 5: a) Examples of historical pastures (Velu, 1934.) and b) current pastures from the study area (Photo: Spalevic, 2018).

Distribution of Pasture Types in the Study Area. The need to adapt to evolving demands has led to a transition away from traditional nomadic lifestyles, necessitating a shift towards a more settled existence to maintain conventional livestock rearing practices. As a result, individuals who were once nomadic now establish fixed homes across different terrains within their tribal territories, adjusting their movements based on seasonal variations (Figure 6).

During the summer, they ascend to the mountainous regions, while in the winter and spring, they practice transhumance, moving towards lower, more hospitable pastures. As the seasons progress, they gradually migrate downstream, towards the banks of the Oum Err Bia River within the study area.

Changes in Land Use and Land Cover 1953 – 2024. The Atlas Mountains of Morocco have long supported transhumant pastoralism, a traditional practice characterized by the seasonal movement of livestock herds between lowland pastures in winter and highland pastures in summer. However, this age-old livelihood system has faced significant challenges due to changes in land use and land cover. Rapid urbanization, agricultural expansion, and infrastructure development have fragmented and degraded traditional grazing lands, reducing the availability of suitable pastures for transhumant herding (Figure 7).

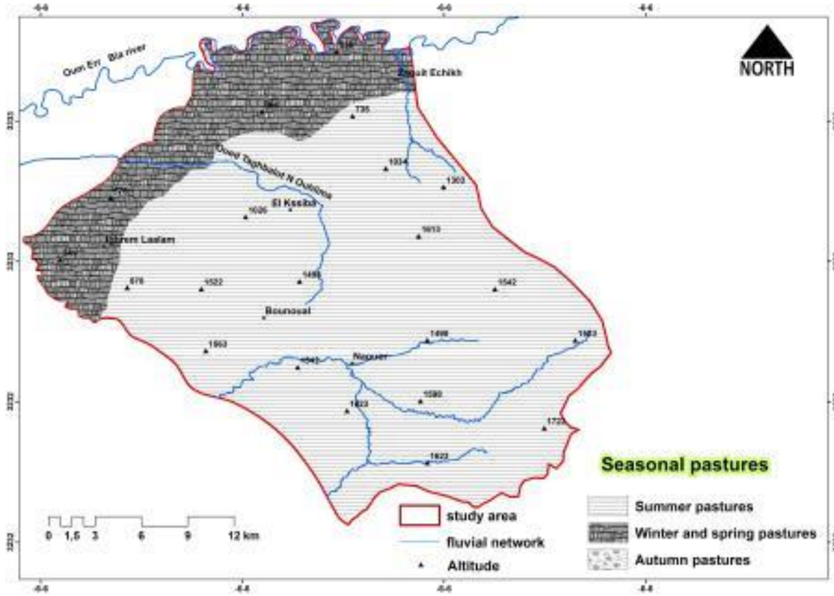

Figure 6: Distribution of seasonal pastures within the study area.

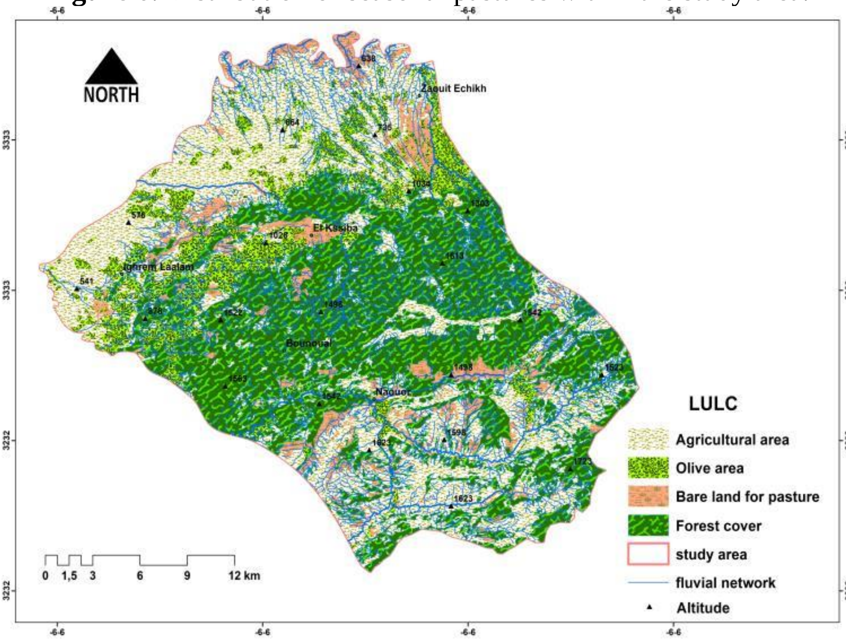

Figure 7: Land use and land cover in the study area.

Additionally, changes in land cover—such as deforestation and vegetation degradation—have diminished the quality and quantity of forage resources, further intensifying the difficulties faced by pastoralists. These pressures have forced many transhumant herders to adapt their traditional practices, including modifying migration routes, intensifying herd management, and seeking alternative livelihoods.

Table 3: Dynamics of Land Use and Land Cover 1953 - 2024 in Area

	1953		2024	
LULC type	area (km^2)	area (%)	area (km^2)	area (%)
Agricultural area	212.10	36.79	85.60	14.85
Olive area	77.21	13.39	4.20	0.73
Bare land	45.16	7.83	102.10	17.71
Forest cover	242.10	41.99	384.60	17.71
Total	576.57	100.00	576.50	51.00
Max	242.10	41.99	384.60	17.71
Min	45.16	7.83	4.20	0.73
SD	82.96	14.39	120.24	6.01

Source: The water and forest agency of El Kssiba and LULC map

Addressing the impacts of land use and land cover changes on transhumant pastoralism requires integrated land management approaches that balance conservation efforts with the needs of local communities, ensuring sustainable resource use practices (FAO, 2017; De Leeuw et al., 2020).

According to the inputs from table 3, significant changes in land use and land cover (LULC) have occurred in the study area between 1953 and 2024, reflecting both environmental and socio-economic dynamics. These transformations highlight key shifts in agricultural land, olive cultivation, bare land, and forest cover.

Agricultural Area. In 1953, agricultural land was a dominant feature, covering 212.10 km² or 36.79% of the total area. By 2024, this area had decreased sharply to 85.60 km², representing only 14.85% of the total. This reduction in agricultural land suggests a transition from farming to other land uses, in part related to industrial or urban development, but more likely a consequence of environmental changes such as land degradation and water scarcity. These factors may have made traditional farming practices unsustainable over time.

Olive Area. Olive cultivation also saw a dramatic decline. In 1953, olive groves covered 77.21 km² or 13.39% of the total area, but by 2024, this had dropped to only 4.20 km² or 0.73% of the total. The sharp reduction in olive cultivation could be due to several factors, including changing market demands, land degradation, and the repurposing of land for other uses such as residential or industrial zones. Additionally, challenges related to water availability and competition from other crops may have further contributed to this decline.

Bare Land. The area of bare land more than doubled between 1953 and 2024. In 1953, bare land accounted for 45.16 km² or 7.83% of the total area, but by 2024, it had expanded to 102.10 km² or 17.71%. This increase is indicative of environmental degradation, likely due to overgrazing, desertification, and land abandonment. The expansion of barren areas suggests a significant decline in land productivity, making these areas more vulnerable to erosion and other environmental stresses.

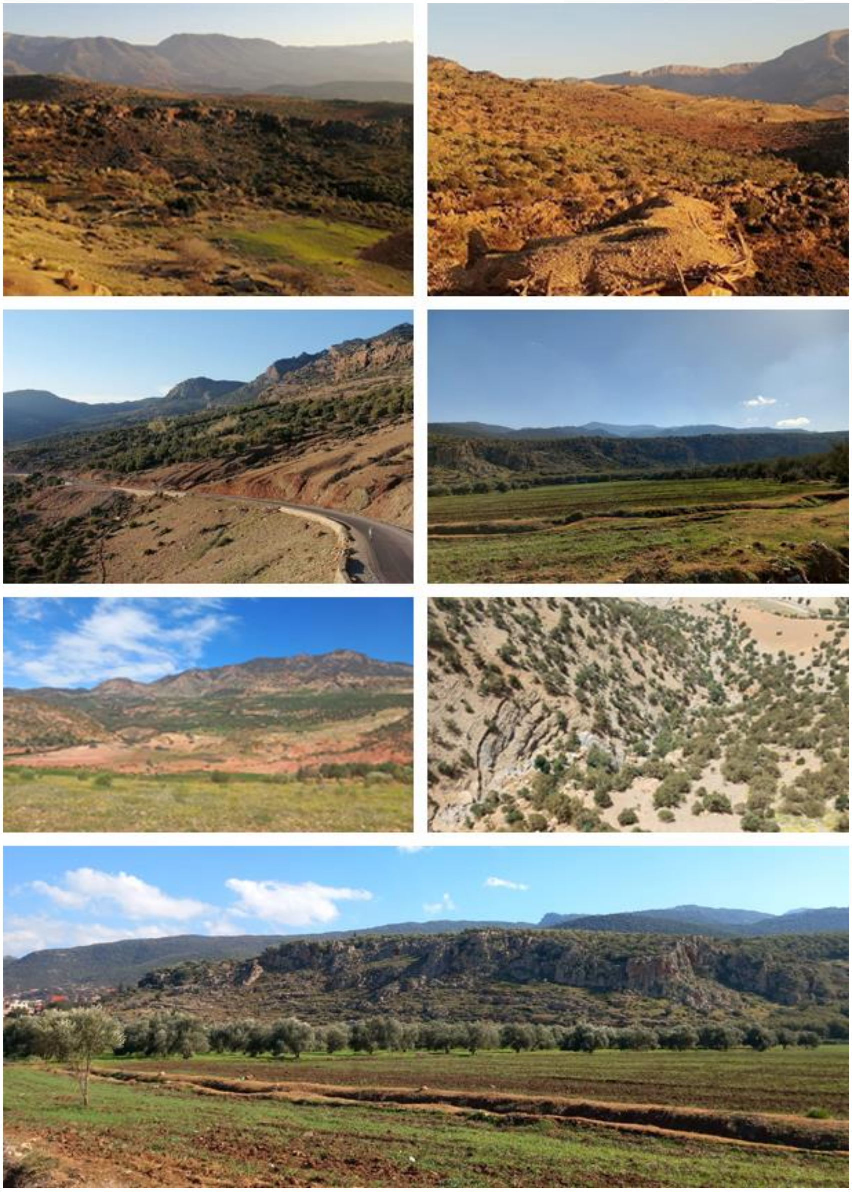

Figure 8: Landscape Dynamics in the El Kssiba Region: Pastoral and Agricultural Transformations (Photo: Spalevic, 2018; Amraoui 2024).

Forest Cover. While agricultural and olive areas have declined, forest cover has seen a positive trend. In 1953, forests covered 242.10 km² or 41.99% of the total area. By 2024, this had increased significantly to 384.60 km², accounting for

66.71% of the total land. The increase in forest cover could be attributed to the natural regeneration of vegetation as marginal agricultural land was abandoned. However, while this trend is encouraging, the quality of the forest, in terms of biodiversity and ecological health, requires further investigation to ensure the forests are thriving ecosystems and not just expanding in area.

Shift from Agriculture to Forests and Bare Land. The most prominent trend between 1953 and 2024 is the significant shift from agricultural and olive cultivation to forests and bare land. The decrease in agricultural land, along with the increase in bare land, suggests that large portions of land have been abandoned or degraded, likely due to unsustainable land management practices and environmental pressures. However, the increase in forest cover provides a sign of recovery and potential reforestation in the region, offering some optimism for the future of the landscape.

Environmental Concerns. The rise in bare land is concerning, as it signals ongoing environmental degradation driven by unsustainable land use practices, including overgrazing. This trend highlights the urgent need for sustainable land management strategies aimed at restoring degraded areas and preventing further environmental deterioration. The degradation of land is a critical issue that affects not only the environment but also the livelihoods of pastoralists and local communities.

Socio-Economic Shifts. The reduction in agricultural and olive cultivation could also reflect broader socio-economic changes. Urbanization, shifts in land ownership, and changing economic priorities have likely reduced the emphasis on traditional farming practices. As areas are repurposed for urban and industrial development, rural communities may be experiencing significant economic and lifestyle transformations. These socio-economic shifts are further evidenced by the changes in land use patterns over time.

Statistical Overview. Statistical analysis reveals increased variability in land use patterns between 1953 and 2024. The standard deviation values indicate greater fluctuations, particularly in forest cover and bare land. Maximum values suggest that forest cover became the dominant land use type in 2024, while bare land experienced the greatest increase in both area and percentage, highlighting major transitions in land cover and use.

The data reveal substantial changes in land use and cover between 1953 and 2024, reflecting both environmental and socio-economic shifts. The decline in agricultural areas and olive groves, coupled with the rise in forest cover and bare land, underscores the complex interaction between land degradation, reforestation efforts, and economic factors. These trends emphasize the importance of implementing integrated land management practices that balance conservation efforts with sustainable agricultural and socio-economic development in the region.

Overall, pastoralism has played a significant role in shaping the LULC dynamics in the Ait Werra tribe area. This traditional practice has influenced both landscape and resource utilization patterns over the years, contributing to the transformations observed in the region.

Exploitation of Resources and Colonial Impact. During the French colonial period, exploitation of the region’s resources—particularly the wood industry, firewood, and charcoal for export to Casablanca—had a significant impact on the local landscape. Despite efforts by the Water and Forest Service to protect and replant pine forests, the Ait Werra tribe continued to view the forest as communal property, exploiting it for their needs and resisting colonial authority over their lands.

While foreign exploiters dominated during the colonial period, the local population primarily used the forest for firewood and heating during the winter. The colonial authorities acknowledged the difficulty of strictly enforcing forest protection laws, especially given the Ait Werra tribe's dependence on the forest for survival (C.A.D.N, 1953). This dynamic reflects the ongoing tension between resource conservation efforts and the livelihood needs of local communities, a challenge that persists even today.

SWOT Analysis of the transformation of Land Use in the Ait Werra Tribe. The following Table 4 summarizes the key strengths, weaknesses, opportunities, and threats related to the transformation of land use within the Ait Werra Tribe.

The SWOT analysis (Table 4) reveals a complex interplay between the historical strengths of pastoral systems in the Ait Werra tribe and the modern challenges they face, particularly in terms of environmental degradation, climate change, and socio-economic pressures. While significant opportunities exist for sustainable land management and climate adaptation, addressing weaknesses like bare land expansion and declining agricultural productivity will require coordinated efforts involving both government policies and local community engagement. Sustainable practices, such as reforestation and improved grazing management, are essential for balancing conservation efforts with pastoral and agricultural livelihoods.

Recommendations to transform Threats into Opportunities. Implementing innovative water conservation techniques, such as rainwater harvesting and advanced irrigation systems, can help mitigate the effects of drought. Introducing more resilient crop varieties and livestock breeds can also support pastoral communities in adapting to changing climate conditions.

Enforcing stricter regulations on urban and industrial development can help preserve traditional grazing lands. Creating protected grazing areas and developing infrastructure solutions that minimize land fragmentation can ensure long-term resource availability for pastoralism.

Providing education and training for pastoral communities on sustainable practices and resource management can help them better integrate into modern economic systems, reducing reliance on unstable traditional methods. Developing alternative income sources through eco-tourism and geo-tourism or creating markets for pastoral products (such as organic meat or dairy) can reduce economic pressure on these communities.

Table 4: SWOT Analysis of the transformation of Land Use in the Tribe

Strengths:	**Weaknesses:**
Historical Heritage and Knowledge: The Ait Werra tribe's rich history and cultural heritage in pastoralism have provided sustainable land-use practices that have helped manage the region's resources over centuries. Their knowledge of seasonal migration and pasture management ensures the efficient use of the land. **Natural Resource Regeneration**: The increase in forest cover since 1953, attributed to natural regeneration and possible reforestation efforts, shows a positive environmental trend that could enhance biodiversity and improve ecosystem services. **Adaptability of Pastoral Communities**: The ability of pastoralists to adapt their transhumance routes and grazing practices in response to environmental and socio-economic changes highlights resilience in managing their livelihoods amid external pressures such as climate change and policy changes.	**Environmental Degradation**: The significant increase in bare land, indicating environmental degradation, desertification, and deforestation, poses a major threat to the sustainability of pastoralism and agricultural practices. Overgrazing, land abandonment, and soil erosion are critical issues that have not been adequately managed. **Decline in Agricultural and Olive Areas**: The reduction in agricultural and olive cultivation areas is a sign of declining land productivity and could indicate socio-economic struggles for communities dependent on farming as part of their livelihood mix. The shrinking olive cultivation area, in particular, is alarming given its importance for both local economies and ecosystems. **Lack of Sustainable Land Management**: Historical and ongoing challenges in implementing effective sustainable land management practices have led to over-exploitation of resources, as evident in the colonial and post-colonial periods. Resource management struggles continue to exacerbate the vulnerability of ecosystems and communities.
Opportunities:	**Threats:**
Sustainable Land Management Initiatives: There is potential for implementing integrated land management strategies, which would balance conservation efforts with sustainable land use. This includes improving forest management, promoting sustainable agriculture, and addressing land degradation issues through reforestation and soil restoration programs. **Policy Reforms and Community Involvement**: Strengthening government policies that prioritize the restoration of degraded lands, alongside community-driven initiatives, could foster more sustainable resource management. Recognizing indigenous knowledge and giving local communities greater authority over land-use decisions can empower better stewardship of the environment. **Climate Adaptation Strategies**: The ongoing climate variability presents an opportunity to introduce climate-resilient agricultural practices and innovative water management techniques that could mitigate the effects of droughts and improve pastoralists' livelihoods.	**Climate Change**: Increasing climate variability, including unpredictable rainfall patterns and extended droughts, threatens the delicate balance required for pastoralism and agriculture. This exacerbates water scarcity and reduces forage availability, making it difficult for traditional systems to thrive. **Land Fragmentation and Urbanization**: Rapid urbanization, infrastructure development, and agricultural expansion have led to the fragmentation of grazing lands, which could result in the further degradation of the pastoral system. Reduced pasture availability may force pastoralists to either abandon their traditional practices or overexploit remaining lands. **Socio-Economic Pressures**: Economic shifts, including increased reliance on cash-based economies and the decline in traditional barter systems, are putting pressure on pastoral communities. The move away from traditional pastoral livelihoods could result in the erosion of cultural heritage and the loss of land-use knowledge.

Recommendations to Transform Weaknesses to Strengths. Implementing programs for land conservation through agroforestry, reforestation, and soil conservation techniques can help reduce erosion and restore land fertility. These practices would increase the availability of grazing land and improve sustainable food production in the long run.

Developing irrigation infrastructure, including technologies for efficient water use, can help revitalize agricultural and olive-growing areas. Promoting organic and sustainable farming can increase the market value of these products, improving the economic sustainability of communities that rely on agriculture.

Strengthening the capacity of local communities to manage natural resources through training and policy support can establish effective practices that balance ecosystem conservation and agriculture. In this way, traditional knowledge and practices, which were once seen as weaknesses due to lack of modernization, can be transformed into strengths for preserving local resources.

In the long term, these steps can help transform threats into opportunities and weaknesses into strengths, enabling communities to pursue sustainable development and resilience against future challenges.

CONCLUSIONS

The study highlights significant transformations in land use and land cover (LULC) within the Ait Werra Tribe's region in the Middle Atlas of Morocco, driven by both environmental and socio-economic changes. The transition from agricultural and olive-growing lands toward more bare land and forest cover reflects broader issues of land abandonment, environmental stress, and socio-economic shifts. However, this transition also provides a window for potential recovery through reforestation and land rehabilitation. This underscores the need for concerted efforts from farmers, local communities, and policymakers to secure the future of pastoralism and land use in the region.

Recommendations for Farmers and Local Communities are to develop and adopt in communication with national services Sustainable Grazing Practices. Farmers and pastoralists should transition to more sustainable grazing techniques to prevent further land degradation. Rotational grazing and controlled stocking rates can help restore vegetation and reduce soil erosion, ensuring long-term productivity of the grazing lands.Given the vulnerability of pastoralism to climate change and land degradation, local communities should explore alternative income sources such as eco-tourism, organic farming, and value-added agricultural products (e.g., dairy and wool). This diversification would provide economic resilience and reduce pressure on land resources. With the increasing scarcity of water due to fluctuating rainfall, it is essential for local farmers to adopt water conservation methods. Techniques such as rainwater harvesting, the use of efficient irrigation systems, and drought-resistant crops should be encouraged to sustain agricultural activities during dry periods.

Community-based reforestation projects can help rehabilitate degraded lands and increase forest cover. By planting native tree species and restoring ecosystems, farmers can not only protect their land but also contribute to mitigating the effects of climate change.

Local Authorities and Decision Makers should strive towards implementation of Integrated Land Management Policies. Decision-makers should establish comprehensive land management frameworks that integrate sustainable agriculture, conservation, and pastoralism. This includes setting aside protected grazing areas, encouraging reforestation programs, and providing incentives for sustainable land use practices. Local authorities should invest in infrastructure that supports sustainable farming, such as improving irrigation systems and offering training programs for farmers on organic and regenerative agriculture. Providing access to markets for sustainably produced goods will help farmers make the transition to greener practices.

Strengthen Climate Adaptation Strategies is of crucial importance. Policymakers should prioritize climate adaptation initiatives by promoting research and development of resilient agricultural technologies, including the introduction of drought-tolerant crop varieties and the creation of early warning systems for extreme weather events. Financial support, such as grants or subsidies, should be made available to farmers adopting these strategies.

Traditional knowledge and practices of pastoralists, such as transhumance, should be documented and integrated into modern resource management frameworks. Decision-makers should work with local communities to preserve these practices and incorporate them into policies aimed at sustainable land use and environmental conservation. Urban expansion and infrastructure projects must be carefully regulated to prevent further fragmentation of grazing lands and agricultural areas. Zoning laws and land-use planning should take into account the needs of pastoralists and farmers, ensuring that development does not compromise the sustainability of rural livelihoods.

Local communities, particularly pastoralists and farmers, should have a seat at the table in discussions about land use policy. Establishing participatory governance structures where local stakeholders can voice their concerns and contribute to decision-making will lead to more effective and equitable outcomes.

The challenges faced by pastoralism and land use in the Ait Werra region reflect larger global issues concerning the sustainability of traditional livelihoods in the face of climate change and socio-economic development. However, these challenges also present opportunities for transformation. By fostering collaboration between local communities and policymakers, it is possible to create sustainable solutions that ensure both the conservation of the environment and the long-term viability of pastoralism and agriculture.

Sustainable development in the Middle Atlas region will require balancing conservation efforts with the economic needs of local populations. With the right strategies and commitment from all stakeholders—farmers, local communities, and decision-makers alike—it is possible to achieve a future where the environment is preserved and livelihoods are secure.

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Mykola NAZARENKO[1], Rostyslav SHYTIKOV [2]

BIOCHEMICAL VALUE OF STRAWBERRY VARIETIES GROWN UNDER THE CONDITIONS OF THE NORTHERN STEPPE OF UKRAINE

SUMMARY

The study of new agents for indicating practical biodiversity in local varieties of strawberry is a promising area in terms of obtaining both new variant for health diet and culture in general for local consumption. The purpose of the study was to demonstrate the possibilities of variability of different varieties of strawberry (*Fragaria ananassa* Duch) in terms of the content of valuable food elements people consumption. Five varieties of strawberry Honey, Rusanivka, Asia, Alba, Clary were studied by biochemical analysis methods by contents of macro-, micro- and biologically-active elements. Comprehensively, the variety Clary prevailed in terms of the content of valuable food substances, which is recommended for more intensive production implementation due to its high value in satisfying nutritional needs. Local diversity of older varieties is a good source of hidden polymorphism for use in improving glucose and dietary fiber content. Local varieties also have a high level of content of relevant valuable minerals and vitamins for use in schemes for the genetic improvement of modern varieties of this crop. These characteristics are determined mainly genetically and are weakly dependent on environmental conditions in their formation. In the future, it is planned to study the variability in biochemical parameters of berries for more modern varieties.

Keywords: strawberry, variety, nutritional value, microelements, product quality, biologically active substances.

INTRODUCTION

Ecologically variety exam is a long process that takes at least about ten years from the initial stages to the introduction of a promising adapted genotype (Nazarenko, 2015). The main trends are the introduction of varieties that simplify

[1] Mykola Nazarenko (corresponding author: nazarenko.m.m@dsau.dp.ua), Department of Plant Breeding and Seeds Production, Dnipro State Agrarian and Economic University, 49600, Dnipro, UKRAINE.

[2]Rostyslav ShytikoV, Department of Plant Breeding and Seeds Production, Dnipro State Agrarian and Economic University, 49600, Dnipro, UKRAINE.

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the cultivation technology, have increased resistance to biotic and abiotic stresses, expand the adaptation zones of culture, create new types of fruits, primarily more attractive in shape and color, growing strawberry varieties with increased health benefits and ensuring consistent high quality (Goldberger et al, 2019).

According to the Food and Agriculture Organization of the United Nations (FAO), global strawberry production was over 9.56 million tons in 2022 (Hernández-Martínez et al, 2023). China is the world’s largest producer of strawberries, accounting for one-third of the world’s production and more than three times that of the United States, the world’s second-largest strawberry producer (Simpson, 2018).

Breeders in the fruit industry must anticipate the need for varieties at least 10 years into the future, as this is the minimum time that the most strawberry varieties require for the establishment process from first pollination to introduction in farming (Kadir et al, 2006). This aspect looks at general trends in our lives, such as environmental issues, healthy whole foods, consumer lifestyle trends (Farvid et al, 2021), as well as the expectations and needs of farmers (Zhou et al, 2015; Nazarenko, 2016).

Global warming significantly effects on the development strategy of agriculture (Nazarenko et al, 2019; Nazarenko et al, 2022), as this sector negatively affects the intensity of forest plantations and significantly increases the carbon footprint created during the fruits cultivation and processing (Andrés et al, 2021). Long-term fruit production system is more sustainable than an annual cropping system, which may be true, but in both cases the natural vegetation is replaced by cultivated plants, greatly impoverishing biodiversity (Zacharaki et al, 2024).

As we learn more about the health benefits of eating fruit, the demand for more whole foods is increasing (Granatstein et al, 2013). These products can take the form of fresh fruit with a high content of health-promoting substances or other natural products, such as fruit concentrates of natural sources of antioxidants, antimicrobial agents or food colors for the health and food industry (Giampieri et al, 2015). The concern for biochemically complete nutrition is one of the main driving forces of the global food market for the global community, although it depends on the region, it is one of the first issues in importance for consumers (Hernández-Martínez et al, 2023). Consumers see the connection between nutrition and health and associate their diet with prevention of cardiovascular disease, vision problems, lack of energy, obesity, arthritis/joint pain and high cholesterol (Zhou et al, 2015).

Fruit production should be at the forefront of the movement for a healthy diet with the spread of so-called "superfruits", which have proven to be a source of extremely beneficial components for health (Ahmad et al, 2024). Although the most famous are blueberries, pomegranates and a few exotic foods such as acai, noni fruit and mangosteen, many fruits from temperate climates are also considered "superfruits", according to the recommendations of scientists. These include fruits such as apple, plum, prune, blackberry, raspberry, strawberry, grape, black currant (Simpson, 2018).

The purpose of the investigation is to identify the limits of variability of different varieties of strawberry (*Fragaria ananassa* Duch) in terms of the content of valuable food elements that determine the consumer quality of products, under the condition of wide utilization of these varieties in the Steppe region agriculture, preferably at the level of small farms, to identify genotypic and conditions of cultivation variance, their interaction. These varietal resources are a local source of biodiversity and enrichment of the human diet.

MATERIAL AND METHODS

The research was carried out under the conditions of LLC Agrosilprom, village Znamenivka, Novomoskovsk district, Dnipropetrovsk region, in 2021-2023 (48°62′05″ n. l. 35°48′20″ e. l.). Five varieties of strawberry Honey, Rusanivka, Asia, Alba, Clary were studied by biochemical analysis methods (average sample of 1 kg from each plot, in three replications for each variety). The cultivation technology was trivial for the region, with drip irrigation.

The content of such valuable elements as calcium, sulfur, magnesium, potassium, zinc, boron, copper, molybdenum, manganese were studied in the laboratory of the research center for biosafety and ecological control of agricultural resources of the Dnipro State Agrarian and Economic University.

The samples preparation protocol involved mineralization using the Multiwave GO Plus microwave decomposition system (Anton Paar (Austria), adding 0.5 g of 10 ml of 65% nitric acid and 1 ml of concentrated hydrochloric acid (Sigma-Aldrich). The mineralization time (together with the cooling time) was 45 min at a temperature of 185 °C.

The study of the mineral substances content was carried out using an atomic emission spectrometer with an Agilent 5110 inductively coupled plasma at the emission intensity of the light flux at the wavelengths corresponding to each element. Multi-element solutions produced by Agilent (Ca, S, Mg, K, B, Zn, Mo, Mn, Cu) were used as standards during the study.

The content (per 100 g) of such substances as glucose, dietary fiber in berries, vitamins A, E, C, PP was analyzed in the laboratory of biochemistry and plant physiology of the Department of Plant Physiology and Introduction of Dnipro national university named by O. Honchar.

To determine the glucose content, extraction was performed and a VPCH-17 sugar meter (Elcantr (Spain) was used. Extraction of the solution to determine the percentage of glucose content was carried out by a standard method. Dietary fibers content was analyzed by enzymatic and gravimetric methods.

The contents of A, E (tocopherol), PP (nicotinamide) in the samples was investigated by a standardized fluorometric method at the appropriate wavelengths of light using a ULAB 102UV spectrophotometer, vitamin C was detected by a titrometric method due to oxidation in dehydroascorbic acid in grape samples (5 g sample).

Statistic analyze of data was performed by ANOVA-analysis, grouping and estimation of data was provided by discriminant and cluster analysis (Euclidian distance, single linkage) (Statistic 10.0, multivariant module, TIBCO, Palo Alto, USA). The normality of the data distribution was examined using the Shapiro–Wilk W-test. Differences between samples were assessed by Tukey HSD test.

RESULTS AND DISCUSSION

The investigation of the obtained material was performed in several stages. During the first, the content of key valuable macroelements calcium, sulfur, magnesium, potassium was determined. Usually, low availability of sulfur and magnesium can cause problems, sources of other elements are usually sufficient. The fact that the content of these elements may depend on the variety and/or growing conditions (year) was also significant.

The results are presented in Table 1. A total of three samples per variant (varieties) were examined during three years of cultivation. According to the results of the factor analysis, both the variety factor ($F = 147.17$; $F_{0.05} = 2.66$; $P = 3.27*10^{-18}$) and the year factor ($F = 3.62$; $F_{0.05} = 2.44$; $P = 0.03$), although with a highly significant difference. The interaction of factors was not significant ($F = 3.34$; $F_{0.05} = 3.50$; $P = 0.06$). According to a pairwise comparison (Tukey's test), Clary was ahead with a high calcium content, followed by Asia, followed by Honey and Alba, Rusanivka (were on the same level). The trait varies significantly between varieties, but in limits of each variety it is low-variable (less than 5%).

Table 1. The content of macronutrients in strawberry berries depending on the genotype (2021 – 2023), (x=27, ±SD).

Parameters	Honey	Rusanivka	Asia	Alba	Clary
Calcium, mg/kg	41.30±1.56[a]	39.00±1.79[a]	45.20±1.61[b]	40.40±1.41[a]	53.20±1,98[c]
Sulfur, mg/kg	12.00±1.04[a]	17.20±1.12[b]	16.00±1.14[b]	15.10±1.15[bc]	19.90±1,27[d]
Magnesium, g/kg	18.10±0.86[a]	17.25±0.77[a]	26.87±1.25[b]	30.11±1.34[c]	34.76±1,37[d]
Potassium, mg/kg	162.00±1.09[a]	169.00±1.05[b]	151.00±1.01[c]	149.00±1.00[c]	215.00±1,17[d]

Note: indicate significant differences at $P < 0.05$ by ANOVA-analyze with Bonferroni amendment.

Sulfur content is especially interesting, since the sources of this element in the diet are more limited. According to the results of the factor analysis, the variety factor had a significant effect ($F = 24.92$; $F_{0.05} = 2.66$; $P = 4.18*10^{-5}$), the year factor was unreliable ($F = 2.04$; $F_{0.05} = 2.44$; $P = 0.08$). The genotype-environment interaction was also unreliable ($F = 2.01$; $F_{0.05} = 3.50$; $P = 0.09$). According to a pairwise comparison (Tukey's test), variety Clary was ahead with a high sulfur content, then Rusanivka, on the same level as variety Asia, variety Alba was on the same level as the Asia, but statistically significantly inferior to variety Rusanivka in terms of sulfur content. The worst was variety Honey. The trait varies less significantly between varieties, but in limits of each variety trait is slightly variable (at the level of 3-5%). The investigated varieties demonstrate the absence of significant polymorphism for this trait, but it is quite difficult to manifest.

For the magnesium content, according to the results of the factor analysis, the variety factor had a significant effect ($F = 779.19$; $F_{0.05} = 2.66$; $P = 3.22*10^{-45}$), but not the year factor ($F = 2.33$; $F_{0.05} = 2.44$; $P = 0.06$). The interaction of factors by influence was also unreliable ($F = 2.85$; $F_{0.05} = 3.50$; $P = 0.07$). According to a pairwise comparison (Tukey's test), variety Clary was ahead with a high magnesium content, followed by Alba, Asia (with significant differences between the varieties), significantly lower content in varieties Honey and Rusanivka. The trait varies significantly between varieties, but in limits of each variety trait is slightly variable (at the level of 3-4%). Thus, the set of varieties does not have significant polymorphism for this trait.

According to the factor analysis, potassium content depended significantly on the variety factor ($F = 133.10$; $F_{0.05} = 2.66$; $P = 5.34*10^{-14}$), but not on the year factor ($F = 2.30$; $F_{0.05} = 2.44$; $P = 0.06$). The interaction of factors by influence was also unreliable ($F = 2.10$; $F_{0.05} = 3.50$; $P = 0.09$). According to a pairwise comparison (Tukey's test), variety Clary was ahead with a high potassium content, followed by variety Rusanivka, variety Honey was significantly different from the previous one, and the Alba and Asia varieties had a significantly lower content (they were on the same level according to the trait). The trait varies significantly between varieties, but in limits of each variety trait is low-variable (at the level of 2-3%). Thus, the set of varieties does not show significant polymorphism for this trait.

The results of the conducted discriminant analysis showed (Table 2) that the content of sulfur is less variable in terms of the component determined by the variety, and the variability in the content of potassium, calcium, and magnesium is much more determined.

Table 2. The results of the discriminant analysis of the investigation parameters reliability according to the content of macroelements.

Trait	Wilks' Lambda	Partial	F	p-level
By variety ($F_{critical}$ =4.37)				
Calcium	0.01	0.06	136.60	< 0.01
Sulfur	0.01	0.27	24.36	< 0.01
Magnesium	0.01	0.01	769.71	< 0.01
Potassium	0.01	0.08	105.59	< 0.01
By years ($F_{critical}$ =2.39)				
Calcium	0.09	0.75	3.18	0.04
Sulfur	0.97	0.98	0.04	0.96
Magnesium	0.98	0.99	0.19	0.82
Potassium	0.98	0.99	0.8	0.82

The factor of year, i.e. environment, was significant only for calcium content. It is possible to conclude that the content of the relevant substances is actually mediated only by genotypic features, given that according to the results of the calculated centroid distances (Fig. 1), the years were quite contrasting in terms of their conditions. At the same time, the obtained characteristics of the clusters in the factor space according to the canonical functions show a clear differentiation and separation of each of the varieties, much lower variability in the group than between genotypes. Each variety had its own significant features in the complex, only Rusanivka and Alba form a group with an unreliable difference between the varieties.

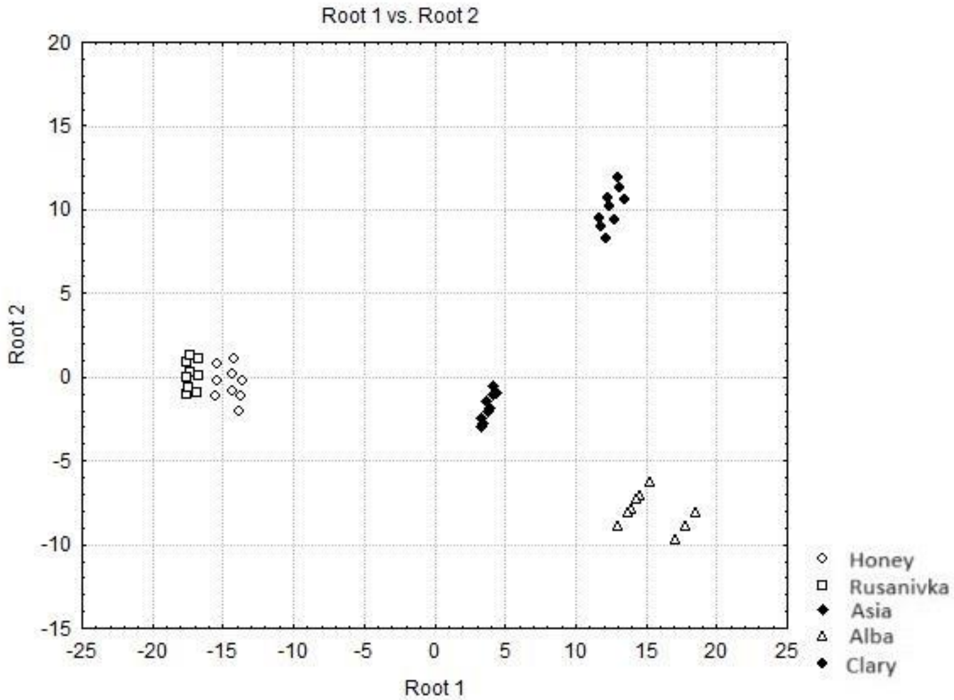

Figure 1. Results of discriminant analysis by macroelements content.

Thus, the studied traits are mainly weakly variable, which indicates the significant homogeneity of the studied material. In terms of the content of calcium, magnesium, sulfur, potassium, variety Clary clearly prevailed, the variety Honey was the worst overall, which showed the lowest indicators in most parameters (except for the potassium content), Rusanivka and Alba groups are in second place and variety Asia is the third. According to the factor analysis, the variety factor was always significant, the year factor only for calcium content, the genotype-environment interaction was always unreliable.

At the next stage (Table 3), the content of valuable trace elements boron, zinc, copper, molybdenum, and manganese was analyzed for all five strawberry varieties. According to the results of the factor analysis, the boron content was significantly influenced by the variety factor ($F = 95{,}45$; $F_{0.05} = 2{,}66$; $P = 2{,}62 \cdot 10^{-11}$), year factor was not significant ($F = 1{,}64$; $F_{0.05} = 2{,}44$; $P = 0{,}09$). The interaction of factors by influence was not significant ($F = 1{,}10$; $F_{0.05} = 3{,}50$; P =

0,11). According to a pairwise comparison (Tukey's test), variety Clary was ahead with a high boron content, then Honey and Alba (on the same level), then Rusanivka (on the same level as variety Alba), variety Asia was worse. The trait varies significantly between varieties, in terms of one variety trait is low-variable (at the level of 2-5%).

Table 3. The content of micronutrients in strawberry berries depending on the genotype (2021 – 2023), (x=27, ±SD).

Parameter	Honey	Rusanivka	Asia	Alba	Clary
Boron, mg/kg	190.00±1.74[a]	185.00±1.69[b]	179.00±1.43[c]	188.00±1.71[ab]	213.00±2.12[d]
Zinc, mg/kg	0.09±0.01[a]	0.11±0.01[a]	0.17±0.02[b]	0.07±0.01[ac]	0.19±0.02[b]
Copper, mkg/kg	120.00±1.24[a]	112.00±1.12[b]	112.00±1.11[b]	102.00±1.05[c]	121.00±1.25[a]
Molybdenum, mkg/kg	10.20±0.22[a]	11.00±0.33[b]	10.00±0.23[a]	9.20±0.17[c]	12.80±0.35[d]
Manganese, mg/kg	0.21±0.02[a]	0.22±0.03[a]	0.28±0.03[b]	0.22±0.03[a]	0.31±0.03[b]

Note: indicate significant differences at P < 0.05 by ANOVA-analyze with Bonferroni amendment.

According to the results of the factor analysis, zinc content was significantly influenced by the variety factor (F = 84.56; $F_{0.05}$ = 2.66; P = $2.64*10^{-10}$), year factor was not significant (F = 1.15; $F_{0.05}$ = 2.44; P = 0.10). The interaction of factors by influence was unreliable (F = 1.00; $F_{0.05}$ = 3.50; P = 0.12). According to a pairwise comparison (Tukey's test), varieties Clary and Honey were ahead with a high zinc content, then Rusanivka and Asia (were on the same level), then Alba. The trait varies significantly between varieties, in terms of one variety trait is low-variable (at the level of 2-4%).

As for the copper content, according to the results of the factor analysis, this trait was significantly influenced by the variety factor (F = 59.34; $F_{0.05}$ = 2.66; P = $5.95*10^{-9}$), year factor was not significant (F = 1.85; $F_{0.05}$ = 2.44; P = 0.08). The interaction of factors by influence was unreliable (F = 1.41; $F_{0.05}$ = 3.50; P = 0.09). According to a pairwise comparison (Tukey's test), variety Clary was first with a high copper content, followed by Honey, then Rusanivka and Asia (were on the same level), variety Alba was significantly worse. The trait varies lower significantly between varieties, in terms of one variety trait is low-variable (at the level 2-3 %).

According to the results of the factor analysis, the molybdenum content was significantly influenced by the variety factor (F = 6.77; $F_{0.05}$ = 2.66; P = 0.01), year factor was not significant (F = 1.90; $F_{0.05}$ = 2.44; P = 0.08). The interaction of factors by influence was unreliable (F = 1.41; $F_{0.05}$ = 3.50; P = 0.09). According to a pairwise comparison (Tukey's test), variety Clary was first with a high molybdenum content, variety Rusanivka was second, then varieties Honey and Asia (were on the same level), variety Alba was significantly inferior to all other. The trait varies slightly between varieties, in terms of one variety trait is low-variable (at the level of 2-3%). It can be concluded that there is little polymorphism in the varieties.

According to the results of the factor analysis, the manganese content was significantly influenced by the variety factor (F = 60.16; $F_{0.05}$ = 2.66; P = $1.43*10^{-9}$), year factor was not significant (F = 1.72; $F_{0.05}$ = 2.44; P = 0.09). The interaction of factors by influence was unreliable (F = 1.11; $F_{0.05}$ = 3.50; P = 0.11). According to a pairwise comparison (Tukey's test), varieties Clary and Asia (at the same level) with a high manganese content were ahead, followed by a group of varieties Rusanivka, Honey and Alba. The trait varies slightly between varieties, in terms of one variety trait is low-variable (at the level of 2-3%).

The results of the conducted discriminant analysis showed (Table 4) that the content of molybdenum is less variable in terms of the component determined by the variety, and the variability in the content of zinc and boron is much more determined.

The factor of year, i.e. environment, was not significant for any of the traits. It is possible to conclude that the content of the relevant substances is actually depended on only genotypic features, given that according to the results of the calculated centroid distances (Fig. 2), the years were quite contrasting in terms of their conditions.

Table 4. The results of the discriminant analysis of the investigation parameters reliability according to the content of microelements.

Trait	Wilks' Lambda	Partial	F	p-level
By variety ($F_{critical}$ =4.37)				
Boron	0.01	0.09	91.51	< 0.01
Zinc	0.01	0.10	81.68	< 0.01
Copper	0.01	0.13	58.20	< 0.01
Molybdenum	0.07	0.57	6.77	0.01
Manganese	0.01	0.12	59.17	< 0.01
By years ($F_{critical}$ =2.39)				
Boron	0.98	0.99	0.02	0.98
Zinc	0.99	0.98	0.23	0.79
Copper	0.98	0.99	0.01	0.99
Molybdenum	0.98	0.99	0.01	0.98
Manganese	0.98	0.99	0.02	0.98

At the same time, the received characteristics of the clusters in the factor space according to the canonical functions show clear differentiation and separation of Clary, Alba and Asia, quite significant variability in the group, varieties Rusanivka and Honey form one group. Clusters of varieties, unlike the previous group of traits, are quite sparse.

In this way, the investigated traits are mainly weakly variable, which indicates the significant homogeneity of the studied material. Comprehensively, variety Clary prevailed in terms of the best content of microelements. According to the factor analysis, the variety factor was always significant, the year factor was never and the genotype-environment interaction was always unreliable. The group of traits is characterized by a significantly lower differentiating ability.

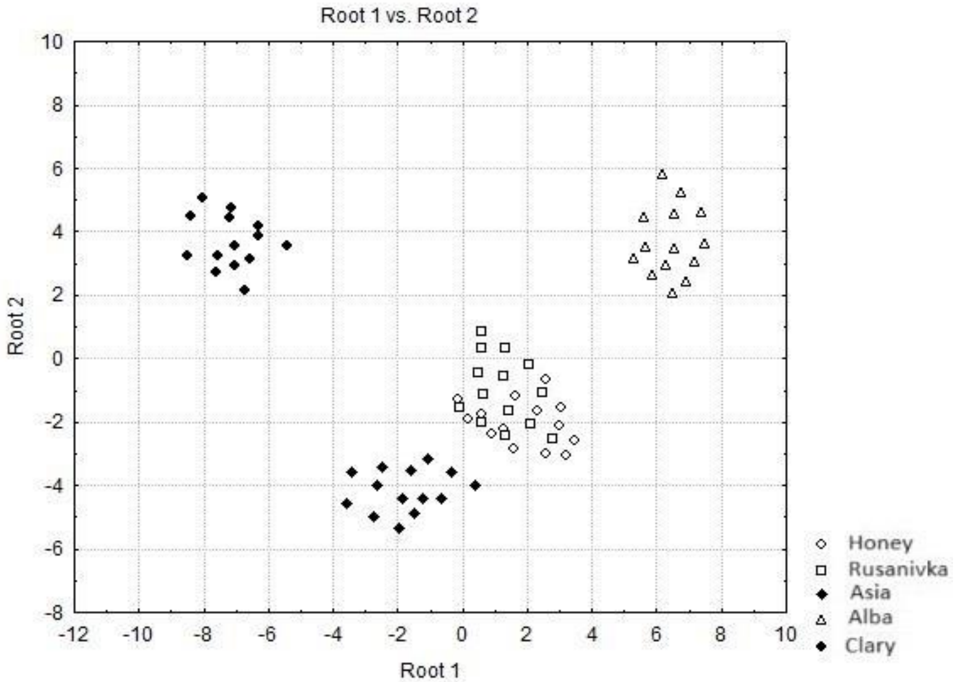

Figure 2. Results of discriminant analysis by microelements content.

At the last stage (Table 5), the content of biologically active components of glucose, dietary fiber, vitamins A, E, C and PP was analyzed for all five strawberry varieties. According to the results of the factor analysis, the glucose content was significantly influenced by the variety factor ($F = 16.15$; $F_{0.05} = 2.66$; $P = 1.62*10^{-5}$), year factor was not significant ($F = 1.15$; $F_{0.05} = 2.44$; $P = 0.11$). The interaction of factors by influence was not significant too ($F = 0.34$; $F_{0.05} = 3.50$; $P = 0.14$). According to a pairwise comparison (Tukey's test), varieties Clary, Alba and Asia were on the first place with a high glucose content, followed by Rusanivka and Honey. The trait varies quite significantly between varieties; in terms of one variety this trait is moderately variable (at the level of 7-8%)

Table 5. The content of biologically-active components in strawberry berries depending on the genotype (2021 – 2023), conversion per 100 g (x=27, ±SD).

Parameter	Honey	Rusanivka	Asia	Alba	Clary
Glucose, g	7.34 ± 0.25^{a}	7.45 ± 0.29^{a}	8.32 ± 0.23^{b}	8.43 ± 0.21^{b}	8.61 ± 0.22^{b}
Dietary fibers, g	$2.17 \pm 0{,}14^{a}$	$2.17 \pm 0{,}32^{a}$	$1.98 \pm 0{,}10^{a}$	$1.94 \pm 0{,}15^{a}$	$2.45 \pm 0{,}15^{b}$
Vitamin A, mkg	5.17 ± 0.11^{a}	5.34 ± 0.12^{a}	5.39 ± 0.12^{a}	5.45 ± 0.13^{ab}	6.12 ± 0.14^{c}
Vitamin E, mg	$0.57 \pm 0{,}02^{a}$	$0.61 \pm 0{,}02^{a}$	$0.55 \pm 0{,}03^{a}$	$0.53 \pm 0{,}01^{ab}$	$0.66 \pm 0{,}02^{ac}$
Vitamin C, mg	$60.10 \pm 0{,}29^{a}$	$63.20 \pm 0{,}31^{b}$	$68.10 \pm 0{,}35^{c}$	$69.70 \pm 0{,}32^{d}$	$69.20 \pm 0{,}37^{d}$
Vitamin PP, mg	0.310 ± 0.14^{a}	0.340 ± 0.13^{a}	0.320 ± 0.13^{a}	0.300 ± 0.14^{ab}	0.380 ± 0.15^{c}

Note: indicate significant differences at $P < 0.05$ by ANOVA-analyze with Bonferroni amendment.

According to the results of the factor analysis, the dietary fiber content was also significantly influenced by the variety factor (F = 51.95; $F_{0.05}$ = 2.66; P = $1.62*10^{-11}$), year factor was not significant (F = 2.26; $F_{0.05}$ = 2.44; P = 0.06). The interaction of factors by influence was not significant (F = 1.35; $F_{0.05}$ = 3.50; P = 0.11). According to a pairwise comparison (Tukey's test), variety Clary was ahead with a higher content of dietary fibers, the difference between the others was not statistically significant. The trait varies quite significantly between varieties; in terms of one variety this trait is moderately variable (at the level of 7-10%, which indicates significant hidden polymorphism in this parameter).

According to the results of the factor analysis, the vitamin A content was also significantly influenced by the variety factor (F = 28.17; $F_{0.05}$ = 2.66; P = $3.45*10^{-8}$), year factor was not significant (F = 0.15; $F_{0.05}$ = 2.44; P = 0.16). The interaction of factors by influence was not reliable (F = 0.10; $F_{0.05}$ = 3.50; P = 0.22). According to a pairwise comparison (Tukey's test), variety Clary was ahead with a higher content of vitamin A, the difference between the others was not statistically significant. The trait varies slightly between varieties, in terms of one variety this trait is slightly variable (at the level of 2-4%, which indicates the absence of hidden polymorphism in this parameter).

Table 6. The results of the discriminant analysis of the investigation parameters reliability according to the content of biologically-active components.

Trait	Wilks' Lambda	Partial	F	p-level
By variety ($F_{critical}$ =4.37)				
Glucose	0.01	0.35	15.85	< 0.01
Dietary fibers	0.01	0.14	51.24	< 0.01
Vitamin A	0.01	0.23	28.79	< 0.01
Vitamin E	0.32	0.84	1.56	0.20
Vitamin C	0.01	0.15	46.79	< 0.01
Vitamin PP	0.01	0.45	10.45	< 0.01
By years ($F_{critical}$ =2.39)				
Glucose	0.85	0.99	0.08	0.92
Dietary fibers	0.90	0.94	1.12	0.33
Vitamin A	0.86	0.99	0.18	0.83
Vitamin E	0.90	0.94	1.12	0.33
Vitamin C	0.86	0.99	0.12	0.88
Vitamin PP	0.85	0.99	0.08	0.92

The variety factor did not affect vitamin E content (F = 2.47; $F_{0.05}$ = 2.66; P = 0.06), year factor was not significant (F = 1.01; $F_{0.05}$ = 2.44; P = 0.12). The interaction of factors by influence was also not significant (F = 0.70; $F_{0.05}$ = 3.50; P = 0.14). According to a pairwise comparison (Tukey's test), variety Clary was ahead with a higher content of vitamin E, but on the same level as variety Rusanivka. The trait varies slightly between varieties, in terms of one variety this trait is slightly variable (at the level of 2-3%, which indicates the absence of hidden polymorphism in this parameter).

The vitamin C content was significantly influenced by the variety factor

($F = 45.07$; $F_{0.05} = 2.66$; $P = 1.12*10^{-10}$), year factor was not significant ($F = 1.10$; $F_{0.05} = 2.44$; $P = 0.11$). The interaction of factors by influence was also reliable ($F = 0.12$; $F_{0.05} = 3.50$; $P = 0.24$). According to a pairwise comparison (Tukey's test), varieties Clary and Alba were on the first place with a higher content of vitamin C, followed by variety Asia, followed by variety Rusanivka, variety Honey was the worst. The trait varies slightly between varieties, in terms of one variety this trait is slightly variable (at the level of 4-5%, which indicates the absence of hidden polymorphism in this parameter).

The content of vitamin PP was significantly affected by the variety factor ($F = 9.07$; $F_{0.05} = 2.66$; $P = 0.002$), year factor was not significant ($F = 0.80$; $F_{0.05} = 2.44$; $P = 0.12$). The interaction of factors by influence was not significant ($F = 0.92$; $F_{0.05} = 3.50$; $P = 0.11$). According to a pairwise comparison (Tukey's test), variety Clary was ahead with a higher content of vitamin PP. The trait varies slightly between varieties, in terms of one variety this trait is slightly variable (at the level of 1-3%, which indicates the absence of hidden polymorphism in this parameter).

The results of the conducted discriminant analysis showed (Table 6) that the content of vitamins (except vitamin E) is less variable in terms of the component determined by the variety, and the variability in the content of vitamin C and dietary fibers is much more determined.

The factor of year, i.e. environment, was not significant for the indicators. It is possible to conclude that the content of the relevant substances is influenced only by varietal diversity, not and by the environmental effect for all cases, especially for the content of vitamin C and dietary fibers, taking into account that according to the results of the calculated centroid distances (Fig. 3), the years were quite contrasting in terms of their conditions.

At the same time, the received characteristics of the clusters in the factor space according to the canonical functions show a clear differentiation and separation of the variety Clary; varieties Rusanivka and Honey, Alba and Asia form a group. Clusters of varieties of this traits group are quite rare, which indicates a significant polymorphism of traits.

In this way, the investigated vitamin content traits are mainly low-variable, which indicates the significant homogeneity of the studied material, the traits of glucose and dietary fiber content are moderately variable (significant varietal polymorphism, which is characteristic of older varieties). The variety Clary prevailed comprehensively in terms of the best content of glucose, dietary fiber and vitamins. According to the factor analysis, the variety factor was mostly significant (except for the vitamin E content); the year factor was not significant in any case. The group of traits is characterized by a significantly intermediate differentiating ability between the first and second. The best practice option would be to plant variety Clary.

The problem of preserving the appropriate biodiversity of the main cultivated plants is largely based on the possibility of using local varietal resources (Nazarenko et al, 2022), in this case, traditional strawberry varieties for growing in farms of the region, which mainly, considering the conservative trends in viticulture, can be created tens of years ago (Galli et al, 2016; Andrés et al, 2021). According to research, these varieties can nevertheless be the basis for improving the nutritional quality of the diet and contain a high level of relevant valuable substances for consumption (Skrovankova et al, 2015; Nazarenko and Simchenko, 2023). Moreover, as already partially noted earlier, such an advantage can be both complex and tied to the use of several varieties in production (Liu et al, 2023a), which is all the more relevant considering the need for strawberries, as for a culture, to form according to ripening periods and external features (color and shape of the berry) of its kind on the conveyor during the relevant marketing period (Durán-Soria et al, 2020; Zacharaki et al, 2024).

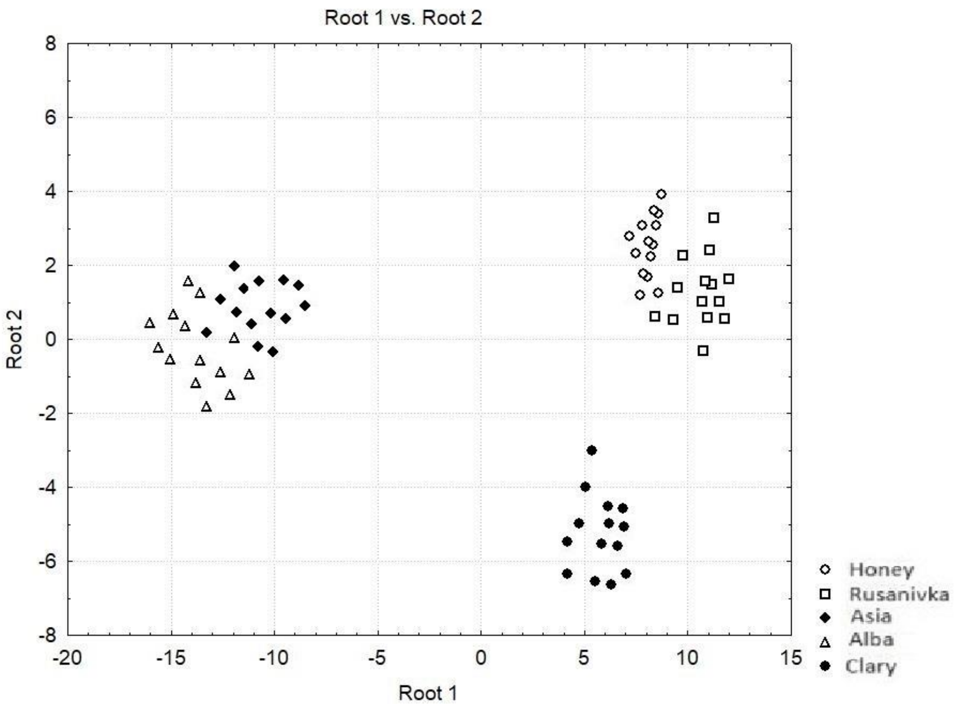

Figure 3. Results of discriminant analysis by biologically-active components content.

We find that local material may also have a sufficient level of hidden polymorphism to be used as a source of primary selection for the improvement of certain traits (Badenes and Byrne, 2012), which was also partly noted earlier (mainly the first and second groups, glucose and dietary fiber traits) (Nazarenko and Simchenko, 2023). High varietal differentiation (the first group of traits) indicates the possibility of biodiversity impoverishment with careful selection of forms more positive for complete nutrition, while lower (the second-third group of traits) to a significant complication in the selection of more useful varieties

(Liu et al, 2023b), although in these studies it can be considered quite optimal for the second group of traits.

In turn, a higher variability in the variety itself by trait can lead to a more significant variation of the parameter depending on environmental conditions, which is not always positive for the regular practice. But, taking into account the recent trends of cultural advancement to the south due to global climate changes (Hernández-Martínez et al, 2023), this problem can be neglected for some of the studied traits (Badenes and Byrne, 2012; Fan et al, 2024). At the same time, this study shows that traditional strawberry varieties, which have been tested by a long period of production use, are in principle enough to ensure proper level in diet (Petranet al, 2017). The possibility of finding a variety with a high level of key characteristics (Sakamoto et al, 2016; Raj et al, 2022).

At the same time, less valuable varieties are also available in production, which may be appropriate to use due to marketing preferences, but they are able to decrease the level of consumption of valuable food elements (Cockerton et al, 2021). But any final recommendation needs clarification from the point of view of such important characteristics for strawberry as taste qualities, preferences for the shape and color of the berry (Petranet al, 2017), possibilities for processing and technological qualities of products (Űnal and Okatan, 2023; Jiang et al, 2024).

In the future, it is planned to conduct a comparative study with more modern and intensive strawberry varieties according to this set of characteristics, in order to establish the variability of these forms in comparison and the level of necessary provision of the completeness of the diet by varieties that are more traditional for growing by small agrofirms and farms in the region, which mainly provide strawberry production for the region.

CONCLUSIONS

Except for the content of glucose and dietary fiber, the studied characteristics are mainly low-variable, which indicates the significant homogeneity of the varietal set. That is, primary selection is possible for improving the source material based on only two moderately variable traits; other traits are stable in term of variety variability and do not show significant polymorphism. Taking into account the generally high level of variability for local germplasm, this trend is significant. The variety Clary prevailed comprehensively in terms of the content of valuable food substances, it can be considered the unequivocal leader in terms of nutritional value. The conducted classification shows the uniqueness of variety Clary according to the studied parameters, which indicates the complex problems of the majority of local varieties with regard to the content of necessary substances. The variety factor has always been significant with one exception for vitamin E content, which indicates, firstly, about the need in find new sources to improve this characteristic and secondly, the importance of introducing a new source of complete nutrition of the variety itself, which is a prerequisite for the success of this strategy. The year factor is important for the calcium content, i.e. environmental characteristics were significant for this characteristic. Considering that the conditions of environment were contrasting by results of discriminant analysis, it should be noted that for these traits are not depend on this factor as morphometric or yield traits (except E content).Genotype-environment interaction was actually derived for traits where the effect of environment was significant.

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Miroslav NEDELJKOVIĆ[1], Adis PUŠKA[2], Dragan PAMUČAR[3,4,5], Dragan MARINKOVIĆ[6]

SELECTION OF AGRICULTURAL PRODUCT SALES CHANNELS USING FUZZY DOUBLE MEREC AND FUZZY RAWEC METHOD

SUMMARY

When selling food products, it's important to choose the appropriate sales channel. These channels connect producers with consumers. The aim of this study was to select a channel for the sale of cabbage to end customers. In this paper, six different sales channels that are used in the Semberija region for the sale of cabbage were observed. These sales channels were evaluated using 11 different criteria. In order to choose the sales channel that best meets the set objectives, a fuzzy set approach was used. This approach was chosen because qualitative criteria were used and expert ratings were in the form of linguistic values. Based on the input of seven experts who are professors at agricultural faculties in Bijeljina, it was found that consumer habits were the most important criterion, followed by the criterion compliance with environmental standards, while the smallest weight value was given to the criterion delivery method. Using the RAWEC (Ranking of Alternatives with Weights of Criterion) method, it was shown that online sales yield the best results, after that follows Producer-sales agent-consumer, while according to experts, the sales channel is the best rated Producer-wholesaler-retailer-consumer. This is because various tools can be utilized on the Internet for selling agricultural products. Based on the conducted research, the contribution of this study lies in the selection of sales channels using the integration of the MEREC and RAWEC methods.

Keywords: Sales Channels; Agricultural Products; fuzzy set; RAWEC method; MEREC method.

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[1]Institute of Agricultural Economics, Volgina Street no. 15, 11060 Belgrade, SERBIA

[2]Government of Brčko District of Bosnia and Herzegovina, Department of Public Safety, Bulevara Mira no. 1, 76100 Brčko, BOSNIA AND HERZEGOVINA

[3]Széchenyi István University, Győr, HUNGARY

[4] Faculty of Organizational Sciences, Department of Operations Research and Statistics, University of Belgrade, 11010 Belgrade, SERBIA

[5] Department of Mechanics and Mathematics, Western Caspian University, Baku, AZERBAIJAN

[6]Technische Universität Berlin; Institut für Mechanik, FG Strukturmechanik und Strukturberechnung, Straße des 17. Juni 135, 10623 Berlin, GERMANY

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INTRODUCTION

Agricultural products are food products and have a limited shelf life (Jiuhardi, et al., 2022). In order to maintain their nutritional values, it is necessary to deliver these products to end customers as soon as possible (Ndori Queku, et al., 2024). This is done using sales channels. These sales channels can be different and include all intermediaries from the producer to the buyer (Milford et al., 2021). However, it is also possible to sell products directly from the manufacturer to the customer (Podlevska, and Podlevskyi 2023). With the application of new technologies, the number of these sales channels is increasing (Hartmann and Lussier, 2020). Due to the diversity of sales channels, it is necessary to choose the one that can deliver products to customers in the best way (Volvach, 2023).

To address this decision-making problem, the example of selling cabbage in the Semberija region in North-eastern Bosnia and Herzegovina was used. In this region, agricultural activity constitutes a key element of its development (Nedeljković et al., 2023). In addition, this region is known for the production of cabbage throughout Bosnia and Herzegovina, so it is necessary to develop an efficient system of distribution of these products to final customers. Choosing an adequate sales channel helps farmers to achieve higher revenues and maintain economic stability in their operations (Khan et al., 2022). These channels facilitate connecting agricultural producers with customers, generating revenue through sales, and contributing to the overall development of the region.

The motivation behind this research is to provide suggestions to cabbage farmers regarding which sales channels to use in order to improve their businesses. This is because there are fewer and fewer agricultural producers in Semberija, so it's necessary to enhance this production to improve agricultural output and raise the standard of living for farmers. In this way, the number of agricultural producers will also increase. Solving the choice of sales channels can help in this regard, but it's necessary to strategically work on improving the conditions prevailing in agriculture.

The choice of sales channel falls under the realm of decision-making problems (Zheng et al., 2021), where it's necessary to select from available alternatives the one that best satisfies the established criteria. In this decision-making problem, there are several alternatives considered against multiple criteria, making it a multi-criteria decision-making (MCDM) issue (Rahman, 2023). These decision-making problems are practically addressed using methods for multi-criteria decision-making (MCDM) (Tešić et al., 2024). Based on the foregoing, this study aims to use multi-criteria analysis methods to select a sales channel that will help agricultural producers achieve higher revenues. Additionally, auxiliary objectives of the study are as follows:

- Evaluate various sales channels for agricultural products.
- Examine how specific sales channels can be utilized in selling cabbage to end consumers.
- Assess sales channels based on expert ratings.

- Select a sales channel that will help cabbage producers in Semberija achieve higher revenues.

Based on these set objectives, the following contributions are achieved in this study:

- Providing guidance to cabbage producers on which sales channels to use in order to achieve higher revenues.
- Developing fuzzy RAWEC (Ranking of Alternatives with Weights of Criterion) methods.
- Developing double fuzzy MEREC (Method based on the Removal Effects of Criteria) methods for objectively determining the weight of criteria.
- Developing methodologies based on double normalization.

To achieve the goals of this study, expert decision-making based on linguistic values will be utilized. These values will be employed due to the fact that qualitative criteria have been utilized (Wang et al., 2024; Sahoo et al., 2024). Assessment of these criteria is conducted through the application of linguistic values, as they are more adaptable to human thinking compared to numerical values (Phulara et al., 2024). It is easier to assess whether something is good or not rather than determining the precise value of that alternative (Kannan et al, 2024; Kizielewicz and Sałabun, 2024).

This paper also develops a new decision-making model based on double normalization. The RAWEC method, in its original form, employs two normalizations to obtain the ranking order of alternatives: normalization where all criteria are transformed into maximum criteria and normalization where all criteria are transformed into minimum criteria. The MEREC method utilizes minimum normalization, transforming all criteria into minimum criteria. Therefore, in this study, the fuzzy MEREC method will be applied twice, using both of these normalizations. This approach will involve employing two different sets of criteria weights in ranking the alternatives.

MATERIAL AND METHODS

When evaluating different cabbage sales channels, using the example of Semberija, the methodology presented in Figure 1 will be applied. In practice, there are numerous criteria and alternatives, so the study encompasses 6 alternatives and 11criteria.

When selecting alternatives, the focus was on identifying cabbage sales channels currently available in the Semberija region. Therefore, six different sales channels were considered:

- Producer-consumer (SC1). Represents a direct sales format where there are no intermediaries involved in the sale.
- Producer-wholesaler-retailer-consumer (SC2). This sales channel involves the insertion of a wholesaler between the producer and the consumer.

- Producer-wholesaler-retailer-consumer (SC3). In this sales channel format, there are two intermediaries involved: the wholesaler and the retailer.
- Producer-sales agent-consumer (SC4). This sales channel involves a sales agent who acts as an intermediary between the producer and the consumer.
- Online sales (SC5). This channel involves various online tools to connect producers with consumers.
- Sales through brokers (SC6). Involves a broker as an intermediary who sells products from the producer to the consumers.

To evaluate these sales channels, the following criteria are used:

- Product characteristics (C1). Represent the basic properties of the product being sold (Nedeljković et al., 2023; Thilmany et al., 2021).
- Sales reliability (C2). Reflects the consistency and reliability in fulfilling orders (Simms et al., 2022).
- Financial situation (C3). Reflects the liquidity and profitability of a specific sales channel (Nedeljković et al., 2023; Đalić et al., 2020).
- Consumer habits (C4). Relates to preferences and patterns in purchasing agricultural products (Đalić et al., 2020; Pang and Chen, 2024).
- Sales costs/commissions (C5). This criterion pertains to all costs associated with product distribution and promotion, which may include transportation, storage, marketing, and sales activities (Dong et al., 2021; Wang et al., 2024).
- Geographic concentration (C6). This criterion relates to focusing sales activities on a specific geographical area to sell products more efficiently and reduce logistics costs (Nedeljković et al., 2023; Đalić et al., 2020).
- Product range (C7). This criterion pertains to the ability to sell a variety of products through sales channels, allowing the producer to adapt to customer needs (Milford et al., 2021; Fałkowski and Chlebicka, 2021).
- Delivery method (C8). Refers to how products are delivered from the producer to the consumer, including the choice of transportation, delivery routes, and logistics management (Prashar et al., 2020; Markowska et al., 2023).
- Delivery volume (C9). This criterion concerns the quantity of products that can be conveyed through a specific sales channel within a certain time frame. The greater the quantity, the more efficient the sales channel (Tadić and Veljović, 2021; Erfurth and Bendul, 2018).
- Sales sustainability (C10). Relates to the application of sustainability strategies in the sales channel, considering ecology and future generations (Raimbekov et al., 2023; Mannarelli Filho, 2020).
- Compliance with environmental standards (C11). This criterion pertains to the adoption of ecological practices to preserve the environment and reduce the negative impacts of sales channels (Mihailović et al., 2017; Millet et al., 2020).

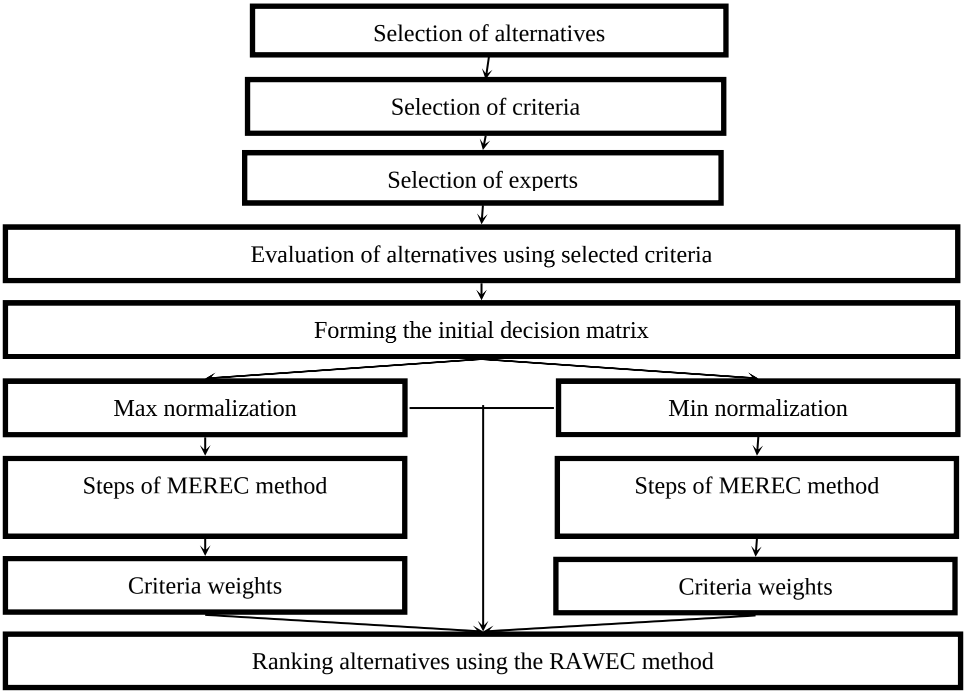

Figure 1. Research Methodology

After certain alternatives and criteria have been determined, it is necessary to evaluate these channels using these criteria. A total of 15 professors from agricultural faculties in Bijeljina were contacted to participate in this research. Out of these 15, seven professors agreed to participate.

After selecting the experts, the next step is to assess the listed sales channels based on the selected criteria. This assessment is done using linguistic values. It is easier to use linguistic values (good, bad, few, many) when evaluating rather than precisely determining how well a sales channel meets the set criteria. Based on this, they will provide ratings ranging from "very bad" to "very good" with seven levels of values (Table 1). To use these values in the evaluation of sales channels, fuzzy numbers need to be used. These numbers are formed by assigning certain fuzzy numbers to certain linguistic values using a membership function (Stević et al., 2022).

Table 1. Linguistic values and membership functions

Linguistic values	Membership function
Very bad (VB)	(0, 0, 1)
Bad (B)	(0, 1, 3)
Medium bad (MB)	(1, 3, 5)
Medium (M)	(3, 5, 7)
Medium good (MG)	(5, 7, 9)
Good (G)	(7, 9, 10)
Very good (VG)	(9, 10, 10)

Next follows the formation of the initial decision matrix. This matrix is the basis for conducting the steps of the MCDM method. It is formed by experts providing ratings for sales channels based on the established criteria. Since the experts' ratings are in the form of linguistic values, an initial linguistic decision matrix is formed, which is then transformed into a fuzzy initial decision matrix.

This research aims to reduce the subjective influence of experts on the importance of criteria, so the fuzzy MEREC method is used to objectively determine the criterion weights. The ranking of alternatives will be performed using the fuzzy RAWEC method, which employs two normalizations: maximum normalization and minimum normalization. Therefore, the next step after forming the fuzzy initial decision matrix is normalization. In this case, two normalizations will be performed, and for each of these normalizations, the fuzzy MEREC method will determine the criterion weights. Using this approach, two different criterion weights will be used in the fuzzy RAWEC method, representing an innovation in the previous application of MCDM methods.

The fuzzy MEREC method

The MEREC method was developed by Keshavarz-Ghorabaee et al., (2021). The steps of this method are as follows:

Step 1. Formation of the initial decision matrix.

Step 2. Normalization of the initial decision matrix.

Step 3. Calculation of the overall performance of alternatives. (S_i) (Ristić et al., 2024):

$$\tilde{S}_i = \ln\left(1 + \left(\frac{1}{m}\sum_j \left|\ln\left(\tilde{n}_{ij}^x\right)\right|\right)\right) \quad (1)$$

Step 4. Calculating the effects of alternatives for each criterion.

$$\tilde{S}'_{ij} = \ln\left(1 + \left(\frac{1}{m}\sum_{k,k\neq j} \left|\ln\left(\tilde{n}_{ik}^x\right)\right|\right)\right) \quad (2)$$

Step 5. Calculating the sum of absolute deviation values.

$$\tilde{E}_j = \sum_i \left|\tilde{S}'_{ij} - \tilde{S}_i\right| \quad (3)$$

Step 6. Calculating the final weights of criteria.

$$\widetilde{w}_j = \frac{\tilde{E}_j}{\sum_k \tilde{E}_k} \quad (4)$$

The fuzzy RAWEC method

The RAWEC method was developed to facilitate decision-making, as in its original form, this method consists of only four steps (Puška et al., 2024). The steps of this method are as follows:

Step 1. Formation of the linguistic decision matrix. It is necessary to transform linguistic values into fuzzy numbers (l, m, u) in order to proceed with the other steps of the fuzzy RAWEC method.

Step 2. Normalization of the decision matrix. When using normalization, two types of normalization are employed:

Maximum normalization

$$n_{ij} = \frac{x_{ij}^{l}}{\max x_j^{u}}, \frac{x_{ij}^{m}}{\max x_j^{u}}, \frac{x_{ij}^{u}}{\max x_j^{u}} \text{ ; for benefit criteria} \tag{5}$$

$$n_{ij} = \frac{\min x_j^{l}}{x_{ij}^{u}}, \frac{\min x_j^{l}}{x_{ij}^{m}}, \frac{\min x_j^{l}}{x_{ij}^{l}} \text{; for cost criteria} \tag{6}$$

Minimum normalization

$$n'_{ij} = \frac{\min x_j^{l}}{x_{ij}^{u}}, \frac{\min x_j^{l}}{x_{ij}^{m}}, \frac{\min x_j^{l}}{x_{ij}^{l}} \text{; for benefit criteria} \tag{7}$$

$$n'_{ij} = \frac{x_{ij}^{l}}{\max x_j^{u}}, \frac{x_{ij}^{m}}{\max x_j^{u}}, \frac{x_{ij}^{u}}{\max x_j^{u}} \text{ ; for cost criteria} \tag{8}$$

Where $x_{j\,min}$ – minimal value of each criterion, and $x_{j\,max}$ – maximum value of each criterion.

Step 3. Calculating the deviation from the criterion weight.

$$\tilde{v}_{ij} = \sum_{i=1}^{n} \tilde{w}_j \cdot \left(1 - \tilde{n}_{ij}\right) \tag{9}$$

$$\tilde{v}'_{ij} = \sum_{i=1}^{n} \tilde{w}_j \cdot \left(1 - \tilde{n}'_{ij}\right) \tag{10}$$

Where $\widetilde{w}_j$ represents the weights obtained using the fuzzy MEREC method.

Step 4. Defuzzification of deviation from the criterion weight

$$v_{ij\,def} = \frac{v_i^{l} + 4v_i^{m} + v_i^{u}}{6} \tag{11}$$

$$v'_{ij\,def} = \frac{v'^{l}_i + 4v'^{m}_i + v'^{u}_i}{6} \tag{12}$$

Step 5. Calculating the value of the RAWEC method.

$$Q_i = \frac{v'_{ij} - v_{ij}}{v'_{ij} + v_{ij}} \tag{13}$$

Further details about the steps of these methods will be explained in the results of this research.

RESULTS AND DISCUSSION

The first step in both methods involves determining the initial decision matrix. This matrix is formed by evaluating the observed channels based on set criteria according to expert opinions. Experts have provided ratings in the form of linguistic values, thus forming the initial linguistic decision matrix (Table 2).

The next step is to transform these linguistic values into fuzzy numbers. This is done by applying the membership function (Table 1). Each linguistic value is thus transformed into the corresponding fuzzy number. By transforming linguistic values into fuzzy numbers, the initial fuzzy decision matrix is formed. Since seven initial decision matrices have been formed, one for each expert, it is necessary to form an aggregate decision matrix. This decision matrix is formed by calculating the average value. This way, each expert is given equal importance in the choice of sales channels.

Table 2. The initial linguistic decision matrix

E1	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
SC1	M	MB	MB	M	MB	B	MB	VB	B	MB	MB
SC2	MB	MB	MB	M	MB	MB	MB	MB	M	MB	M
SC3	MB	B	MB	M	VB	B	M	B	MB	MB	M
SC4	B	B	VB	MB	VB	M	MB	MB	MB	MB	M
SC5	VB	VB	B	B	B	M	MB	M	MB	MB	MB
SC6	MB	M	MB	M	M	MB	MB	B	M	B	MB
E2	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
SC1	M	MB	MB	MB	MB	B	MB	VB	VB	MB	MB
SC2	M	MB	MB	M	M	MB	B	MB	M	MB	MG
SC3	MB	B	M	MG	B	MB	M	MB	B	B	M
SC4	M	MB	VB	MB	MB	M	M	MB	MB	MB	MB
SC5	VB	VB	MB	B	B	MB	MB	MB	B	B	MB
SC6	MB	M	M	M	M	MB	MB	B	M	B	MB
⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮	⋮
E7	C1	C2	C3	C4	C5	C6	C7	C8	C9	C10	C11
SC1	MB	MB	MB	MB	MB	B	MB	B	MB	M	MB
SC2	B	M	B	G	M	M	B	VB	M	VB	MB
SC3	B	MB	B	M	VB	B	B	VB	B	MB	B
SC4	M	MB	VB	B	MB	MB	VB	M	M	MB	B
SC5	VB	VB	B	B	VB	M	MB	MB	VB	MB	B
SC6	MB	M	M	M	B	MG	VB	B	M	B	MB

The next step is the normalization of the aggregate decision matrix. This normalization will be applied to both methods. Since the linguistic values are formed from 'very bad' to 'very good' and all criteria used are in the form of benefit criteria, expressions 5 and 7 are applied. For example, for the first criterion and the first alternative and the fourth alternative, the calculation of the normalized decision matrix is done as follows:

$$n_{11} = \frac{3.29}{10} = 0.33; \ \frac{5.29}{10} = 0.53; \ \frac{7.29}{10} = 0.73$$
$$n'_{11} = \frac{3.29}{7.29} = 0.45; \ \frac{3.29}{5.29} = 0.62; \ \frac{3.29}{3.29} = 1.00$$
$$n_{41} = \frac{5.29}{10} = 0.53; \ \frac{7.00}{10} = 0.70; \ \frac{8.29}{10} = 0.83$$
$$n'_{41} = \frac{3.29}{8.29} = 0.40; \ \frac{3.29}{7.00} = 0.47; \ \frac{3.29}{5.29} = 0.62$$

In maximum normalization of fuzzy values, they are divided by the highest value of the fuzzy number of the given criterion, while in minimum normalization; all fuzzy numbers are divided by the lowest value of the fuzzy number of the given criterion. It is necessary to emphasize that care must be taken so that the first fuzzy number is less than or equal to the second fuzzy number, while the second fuzzy number must be less than or equal to the third fuzzy number. Therefore, in minimum normalization, for the first fuzzy normalized number, the lowest value is divided by the third fuzzy number, for the second fuzzy normalized number, the lowest value is divided by the second fuzzy number, while for the third fuzzy normalized number, the lowest value is divided by the first fuzzy number (expression 7). In this way, the normalized decision matrix is formed, and the steps of the selected methods are applied.

Since it is necessary to possess criterion weights for ranking, the calculation of criterion weights using the fuzzy MEREC method is performed first. Since this method has already been applied in many studies (Narang et al., 2023; Kousar et al., 2024; Wan et al., 2023), the steps of this method will not be further elaborated.

The next step of this method is to calculate the total performance of alternatives ($\tilde{S}_i$). This is done by first calculating the natural logarithms (*ln*) for all values, then computing the absolute values of these numbers, summing these values for the corresponding fuzzy numbers, dividing the obtained value by the number of criteria, adding one (1) to that value, and finally taking the natural logarithm of the resulting value. The calculation of the effects of alternative ($\tilde{S}'_{ij}$) is done in the same way, except that the value of the alternative for which this indicator is calculated is not included. This way, the effects are calculated without that value. Then, the sum of the deviations from the absolute values is calculated, where the values of the total performance of alternatives are subtracted from the values of the effects of alternatives, forming the absolute value of that value. Finally, the criterion weights are calculated. This procedure is applied to both normalized decision matrices, resulting in two weight values (Table 3). Based on the results of the fuzzy MEREC method, criterion C4 has the highest weight, indicating the highest importance in both calculations, while criterion C8 has the lowest weight. Based on these weight values, it can be said that the importance of criteria remains the same but the weight values have changed.

Table 3. The values of criterion weights

	w_j	w'_j
C1	(0.03, 0.09, 0.31)	(0.07, 0.15, 0.37)
C2	(0.03, 0.09, 0.30)	(0.08, 0.17, 0.41)
C3	(0.02, 0.08, 0.29)	(0.07, 0.16, 0.38)
C4	(0.06, 0.16, 0.52)	(0.14, 0.28, 0.68)
C5	(0.02, 0.08, 0.27)	(0.05, 0.13, 0.32)
C6	(0.03, 0.10, 0.33)	(0.06, 0.15, 0.37)
C7	(0.02, 0.08, 0.30)	(0.08, 0.16, 0.38)
C8	(0.01, 0.06, 0.23)	(0.04, 0.11, 0.27)
C9	(0.03, 0.09, 0.30)	(0.08, 0.17, 0.41)
C10	(0.02, 0.07, 0.28)	(0.07, 0.15, 0.36)
C11	(0.02, 0.10, 0.36)	(0.14, 0.26, 0.62)

After calculating the values of the weights, the selected sales channels are ranked using the fuzzy RAWEC method. After calculating the normalized decision matrices, the deviations from the values of the criterion weights are calculated. For example, for the first alternative, this is done as follows:

$v_1 = [0.03 \cdot (1-0.73) + 0.03 \cdot (1-0.90) + 0.02 \cdot (1-0.91) + \cdots + 0.02 \cdot (1-0.95)] = 0.03;\ [0.09 \cdot (1-0.53) + 0.09 \cdot (1-0.70) + 0.08 \cdot (1-0.71) + \cdots + 0.10 \cdot (1-0.74)] = 0.47;\ [0.31 \cdot (1-0.33) + 0.30 \cdot (1-0.50) + 0.29 \cdot (1-0.51) + \cdots + 0.36 \cdot (1-0.53)] = 1.64$

$v'_1 = [0.07 \cdot (1-1.00) + 0.08 \cdot (1-0.60) + 0.07 \cdot (1-0.66) + \cdots + 0.14 \cdot (1-0.34)] = 0.37;\ [0.15 \cdot (1-0.62) + 0.17 \cdot (1-0.43) + 0.16 \cdot (1-0.47) + \cdots + 0.26 \cdot (1-0.24)] = 1.11;\ [0.37 \cdot (1-0.45) + 0.41 \cdot (1-0.33) + 0.38 \cdot (1-0.37) + \cdots + 0.62 \cdot (1-0.19)] = 3.08$

This way, values are calculated for all sales channels (Table 4). After calculating the deviations from the criterion weights, defuzzification of these values is performed to determine the final value of the fuzzy RAWEC method. For example, for the first sales channel, this is calculated as follows:

$$\mathrm{def}\, v_1 = \frac{0.03 + 4 \cdot 0.47 + 1.64}{6} = 0.592$$

$$\mathrm{def}\, v'_1 = \frac{0.37 + 4 \cdot 1.11 + 3.08}{6} = 1.313$$

By calculating the defuzzified value, the value of the fuzzy RAWEC method is computed. For example, for the first alternative, this is done as follows:

$$Q_1 = \frac{1.313 - 0.592}{1.313 + 0.592} = 0.379$$

Based on these results, according to expert opinions, the distribution channel SC5 (Online Sales) demonstrates the best performance, followed by SC4 (Manufacturer-Agent-Consumer), while SC2 (Manufacturer-Retailer-Consumer) achieved the poorest results.

Table 4. The results of the fuzzy RAWEC method

Id	$\tilde{v}$	*def v*	$\tilde{v}'$	*def v′*	Q_i	Rank
SC1	(0.03, 0.47, 1.64)	0.592	(0.37, 1.11, 3.08)	1.313	0.379	4
SC2	(0.06, 0.55, 1.89)	0.690	(0.22, 1.01, 2.95)	1.200	0.270	6
SC3	(0.04, 0.47, 1.60)	0.585	(0.39, 1.14, 3.09)	1.338	0.392	3
SC4	(0.03, 0.45, 1.55)	0.560	(0.42, 1.14, 3.09)	1.341	0.411	2
SC5	(0.02, 0.34, 1.21)	0.432	(0.46, 1.19, 3.14)	1.395	0.527	1
SC6	(0.05, 0.54, 1.86)	0.678	(0.30, 1.04, 2.98)	1.242	0.294	5

The obtained results show that online sales are the best channel for selling cabbage, and they confirm the results of Sheng and Lu (2020), who found that rural settlements must use Internet connectivity more in order to have better sales channels. However, Smoluk-Sikorska (2019) says in her research that online sales are only in fourth place among the sales of organic agricultural products, and that direct sales are the most widely used. This is because these products are more prone to rotting and must be stored in a prescribed manner in order to stay fresh longer, while this is not the case with cabbage. Cabbage is less susceptible to rotting than any other agricultural product, so it is possible to find buyers through online sales.

Also, Milford et al. (2021) considered the problem of selling organic production, and found that the use of specialized stores is most common in Norway. Nedeljković et al. (2023) said that the best sales channel for the agri-food sector in Semberija is producer - seller (retailer) - consumer, but they did not consider the online sales channel in their work. However, online sales can include different types of sales channels, i.e. cabbage is sold directly to consumers or feeders or even agents, and that is why it is the most expedient and that is why it was rated the best by experts.

CONCLUSIONS

This study focused on selecting sales channels for cabbage sales in the Semberija region. Expert opinions and fuzzy set theory were used in the process, yielding the following results:

- The most significant criterion was consumer habits.
- Online sales showed the best results.

Furthermore, the contributions of this research are as follows:

- Providing guidelines to cabbage producers regarding which sales channels to use to increase revenue
- Integrating the fuzzy MEREC and RAWEC methods into a unified methodology for selecting sales channels.
- Applying double normalization in the MEREC method.
- Developing the fuzzy RAWEC method which hasn’t been used in the previous research.

In addition to these contributions, this research also has certain limitations. These limitations include the selection of experts, as there could be other experts whose knowledge could further enhance agricultural product sales. However, efforts were made to avoid subjectivity among agricultural producers by selecting professors from agricultural faculties in Bijeljina.

Furthermore, future research could involve interviewing these producers to understand why they use specific sales channels and the advantages they derive from them. Another limitation is the number of experts involved in the research. Increasing the number of experts complicates the decision-making process, so it's essential to maintain a balance in the number of experts involved. Additionally, the criteria used for ranking sales channels could be a limitation. While other criteria could be considered, it's not feasible to include all possible criteria in the ranking process. Therefore, future research should explore other criteria and compare the results with those obtained in this study.

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Radena NENOVA[1], Yordanka ILIEVA,[1]
Ivan FASULKOV[2], Manol KARADAEV[2],
Nasko VASILEV[2], Pencho PENCHEV[1*]

EFFECT OF DIFFERENT HORMONAL PROTOCOLS ON CLINICAL SIGNS OF ESTRUS AND CONCEPTION RATES IN BUFFALOES

SUMMARY

The protocols PreSynch/OvSynch, OvSynch and OvSynch+PRID were applied in breeding season (IBS) and out of it (OBS) in 133 buffalo cows and OBS in 75 heifers. Gestation was diagnosed sonographically 45 days after timed artificial insemination (TAI). Dispersion analysis was conducted, including the factors: protocol; clinical signs of estrus (without CSE; patency of cervix; mucous discharge); age. Another 3-factor analysis solely on buffalo cows included the effect of season. The results show that the factor protocol has the most pronounced effect on TAI success ($P \leq 0.01$), significantly lowest being the pregnancy rate (p_i values) under PreSynch/OvSynch protocol – only 23.9%, compared to 40.7 and 47.4% under OvSynch and OvSynch+PRID, respectively. Although the effect of age is non-significant, in the heifers OvSynch+PRID and OvSynch show markedly higher results (50.0 and 52.9% respectively), while in the buffalo cows the differences are smaller, the OvSynch protocol having a relatively low pregnancy rate (38.3%), OvSynch+PRID – highest (45.0%), and PreSynch/OvSynch – lowest (30.4%). Season has non-significant effect, but the conception rate from OvSynch+PRID applied OBS is higher than IBS, so it can be used for overcoming the problematic seasonal anestrus. CSE is a significant source of variance of conception rates ($P \leq 0.05$), predictably the highest *pi* value belonging to the cases with mucus. The superiority of the OvSynch+PRID protocol finds expression in the highest incidence of full estrus (with mucus) in the lactating buffaloes (70%) and even more in the heifers (82%), as compared to PreSynch/OvSynch (56.5 and 52.2% respectively) and OvSynch (51.1 and 50.0%).

Keywords: buffaloes, conception rate, estrus signs, PRID, prostaglandin, gonadotropin

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[1]Radena Nenova, Yordanka Ilieva, Pencho Penchev (*corresponding author: pen.penchev@gmail.com), Agricultural Institute – Shumen, Agricultural Academy, Sofia, BULGARIA

[2] Ivan Fasulkov, Manol Karadaev, Nasko Vasilev, Department of Obstetrics, Reproduction and Reproductive Disorders, Faculty of Veterinary Medicine, Trakia University, Stara Zagora, BULGARIA

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INTRODUCTION

Management of herd replacement and profitability in buffalo farming are majorly based on age of first calving and calving interval, rather than on milk yield. This is proved with the economic weights of the traits in the Bulgarian Murrah (Peeva, 2000) and in the Italian Mediterranean breed (Barile, 2005). In particular, breeding efficiency of buffaloes is affected by late puberty, seasonality of calving, prolonged anestrus after calving, irregular cyclicity, low conception rates, etc. (Madan 1990). The great variability of estrus and ovulation is a challenge to AI, as well (Ohashi, 1994; Perera, 2011). Moreover, the reproductive process in the buffalo farms is specifically characterized by vaguely expressed signs of estrus and hence by poor timing and low success of artificial insemination (Alonso et al., 1992; Barkawi et al., 1993). In addition, the portion of animals in long anestrus is great, especially in the non-breeding season (El-Wishy, 2007). It is a characteristic of the species that high sexual activity comes in response to the reducing day light in late summer and early autumn in association with melatonin secretion. In the rest of the year buffaloes often exhibit anestrus which, on one hand, reduces reproductive efficiency and, on the other, results in a misbalanced milk production throughout the year (Zicarelli, 1997, 2007; Neglia et al., 2004).

Efficiency of protocols for estrus and ovulation synchronization has been established to increase the percentage of pregnant animals (Baruselli & Carvalho, 2005; Balamurugan et al., 2017). Further, combined administration of gonadotropins and prostaglandins has been introduced with the purpose to optimize the effect of these protocols by the inclusion of timed artificial insemination (De Rensis & López-Gatius, 2007; Hammam et al., 2009). Consequently, certain success has been achieved in eliminating the effect of breeding season (Carvalho et al., 2013; Baruselli et al., 2013). In buffalo breeding, the applied synchronization schemes (Paul & Prakash, 2005; Carvalho et al., 2007; Warriach et al., 2008) are of even greater importance, compared to cattle, in view of the above-mentioned species-specific peculiarities of reproduction. Namely, the new follicular wave they induce affords circumventing the species-problematic visual heat detection for AI timing (Barile, 2005). There are only few research works studying the presence of clinical signs of full estrus in synchronized buffaloes on global (Neglia et al., 2003) and national (Atanasov et al., 2012) scale, suggesting that mucous discharge can be used as an indicator for good conception rates. All this is especially important for the buffalo population of Bulgaria in view of the considerable weights of the reproduction traits, mentioned above (Peeva, 2000).

The objective of the presented field experiment was to study the efficacy of the application of three different protocols for estrus synchronization in buffalo heifers and buffalo cows of the Bulgarian Murrah breed expressed in manifestation of clinical signs of estrus and conception rates.

MATERIAL AND METHODS

The field experiment was carried out in the years 2021 and 2022 and included 75 buffalo heifers and 133 buffalo cows of the Bulgarian Murrah breed bred on the farm of Agricultural Institute – Shumen. The two categories of buffaloes are kept in tie-stall housing with exercise yards.

The diet of the buffalo cows includes 22 kg of maize silage, 3 kg of leguminous hay, 4 kg of cereal straw, and 5 kg of compound feed (*TopMix – 15% protein*) per capita per day. The diet of the heifers consists of 18 kg of silage, 3 kg of hay and 4 kg of feed. The buffaloes were selected to be up to sixth lactation and more than 60 days postpartum, and the heifers – to be roughly 22 months old with a live weight of about 380 kg. All included animals were in good body condition (BCS= 3–4) and free of obstetrical disorders and abnormalities in their prior parities (if any). On day 0, ovarian structures were examined sonographically and for treatment were chosen individuals with diameter of the follicles over 8 mm and no *corpus luteum*.

Three protocols of hormonal treatment for estrus induction/synchronization with timed artificial insemination (TAI) were applied in heifers and lactating buffaloes, as presented in Table 1. In the lactating buffalo cows, each of the protocols was applied both in breeding and non-breeding season, while in the heifers – out of season only. Gestation was also diagnosed sonographically on the 45th day after TAI. The echographic examinations were performed with a SonoScape A2 Vet (SonoScape Co. LTD, Shenzhen, China), a multi-frequency (7-12 MHz) linear probe and transrectal approach.

Table 1. Timing of hormonal administration within the three tested protocols

Day	hour	PreSynch/OvSynch (n_H= 46; n_c= 46)	OvSynch (n_H= 12; n_c= 47)	OvSynch+PRID (n_H= 17; n_c= 40)
1	8 a.m.	500 UI Synchrostim[1] i.m. + 5 ml Enzaprost T[2] i.m.	2 ml Ovarelin i.m.	PRID DELTA[4] vaginally inserted
4	8 a.m.	2 ml Ovarelin[3] i.m.		
8	8 a.m.		5 ml Enzaprost i.m.	PRID DELTA removal + 5 ml Enzaprost i.m.
10	4 p.m.		2 ml Ovarelin i.m.	2ml Ovarelin i.m.
11	8 a.m.	5ml Enzaprost T i.m.	TAI	TAI
	4 p.m.			TAI
13	4 p.m.	2 ml Ovarelin i.m.		
14	8 a.m.	TAI		

i.m. – intramuscular injection; n_H – number of buffalo heifers, n_c – number of buffalo cows; [1]Equine Serum Gonadotropin, *Ceva Sante Animale, France*; [2]Dinoprost Trometamol, *Ceva Sante Animale, France*; [3]Gonadorelin, *Ceva Sante Animale, France;* [4]Intrauterine device for cattle PRID delta 1.55 g Progesterone, *Ceva Sante Animale, France*

The criteria for the diagnosis of pregnancy were an enlarged uterine lumen, visualization of the embryonic vesicle, and foetal cardiac activity, as described by Fricke et al. (2016).

The artificial insemination and evaluation of the clinical signs of the genital apparatus associated with estrus were performed by an AI technician. The presence/absence of a clear cervical-vaginal mucous discharge was detected, as well as the passability of the cervical canal with a pipette (patency). Cryopreserved semen from tested buffalo bulls was used.

Examinations and treatments were carried out in compliance with the requirements and regulations of the Animal Welfare Act (AWA).

The set of data registered sonographically were processed via dispersion analysis of a non-orthogonal set of qualitative traits (Model-1), including the following factors and respective classes:

- Age – two classes: heifers; lactating buffaloes
- Protocol – three classes: PreSynch/OvSynch; OvSynch; OvSynch+PRID (as in Table 1)
- Clinical signs of estrus (CSE) as detected during TAI application – three classes: without CSE (lack of vaginal mucous discharge and low patency of the cervix); patency (good patency but lack of mucus); mucus (both presence of mucous discharge and good patency, herein referred to as expression of full estrus)

Another 3-factor analysis was conducted on buffalo cows only. Instead of age, Model-2 included the factor: season – two classes: in season (high breeding season); out of season (low breeding season). In-season TAI breeding was applied in the period from August 10th to November 21st. The rest of the year was considered out of season.

In the linear model, the gradations are presented as p_i values, resulting from the number of the individuals characterized by the qualitative trait pregnancy as diagnosed 45 days post TAI (Σm_x) out of the total number of individuals in the respective class (Σn_x). The effects of the singular factors included in the ANOVA, their co-effects and the all-factors effect (x) are expressed in coefficients of impact (η^2) and coefficients of significance (F), and the significance of the differences within gradations – in F_d-values.

RESULTS

Table 2 shows the raw data from the ultrasound examinations for the diagnosis of pregnancy 45 days after TAI, as well as the visual detection of the presence of vaginal mucus and the patency of the cervical canal during TAI. It is noteworthy that in TAI after application of the PreSynch/OvSynch protocol, 17.4% of the heifers have shown no visual CSE, but still one animal conceived (12.5%).

This indicates that this protocol had low success in the heifers, while under the OvSynch and OvSynch+PRID scheme all young animals manifested at least patency of cervical canal.

Accordingly, as indicated by the data in Figure 1, the pregnancy rate from the PreSynch/OvSynch protocol in the heifers was markedly lower as compared to the OvSynch and OvSynch+PRID synchronization schemes.

Table 2. Results from day 45 post TAI by protocols and by CSE and distribution by high or low season of breeding

Protocols within age groups	**CSE**	**n_x**	**m_x**	**Season, n_x/m_x**	
				High	**Low**
Buffalo heifers					
PreSynch/OvSynch	No CSE	8	1	-	46/8
	Patency	14	2		
	Mucus	24	5		
OvSynch	No CSE	0	0	-	12/6
	Patency	6	4		
	Mucus	6	2		
OvSynch+PRID	No CSE	0	0	-	17/9
	Patency	3	1		
	Mucus	14	8		
Lactating buffaloes					
PreSynch/OvSynch	No CSE	1	0	38/11	8/3
	Patency	19	4		
	Mucus	26	10		
OvSynch	No CSE	3	1	25/9	22/9
	Patency	20	5		
	Mucus	24	12		
OvSynch+PRID	No CSE	5	1	16/6	24/12
	Patency	7	2		
	Mucus	28	15		

n_x – number of individuals hormonally treated in the respective class;
m_x – number of the individuals characterized by the qualitative trait pregnancy 45 days post TAI out of n_x

In the buffalo cows under the PreSynch/OvSynch protocol, conception rate was also lowest, but with less pronounced differences with OvSynch and OvSynch+PRID (Figure 1). It is noteworthy that while in the heifers OvSynch+PRID and OvSynch lead to high results (conception rates of 50.0 and 52.9% respectively), in the buffalo cows OvSynch has lower success (38.3%).

With no CSE in the PreSynch/OvSynch protocol was only one buffalo cow (2.2%) and she did not conceive; in each of OvSynch and OvSynch+PRID scheme there was one animal, which constitutes respectively 6.4 and 12.5%, with pregnancy rates of 33.3 and 20.0 % (Table 2).

It is noteworthy that in OvSynch the results when patency is the only CSE detected are even lower, while when mucous discharge is present the conception rate is markedly higher.

The differences in the manifestation of full estrus are remarkable. Figure 1 indicates that the heifers with mucus constitute as much as 82.4% of all OvSynch+PRID treated animals, compared to 52.2 and 50.0% in the other protocols. Similar but slightly less-expressed dependency is observed in the buffalo cows – 70.0% in OvSynch+PRID, 56.5% in PreSynch/OvSynch and 51.1% in OvSynch.

Obviously, as Table 2 shows, the PreSynch/OvSynch protocol results in relatively good percentage of heifers with mucous discharge but also in poor follicular wave associated with poor conception rates (Figure 1). Figure 1 also shows that in both age groups the conception rates from full estrus are highest after application of TAI within the OvSynch+PRID protocol (over 50%), but in the lactating buffaloes OvSynch also has a high result (50.0%).

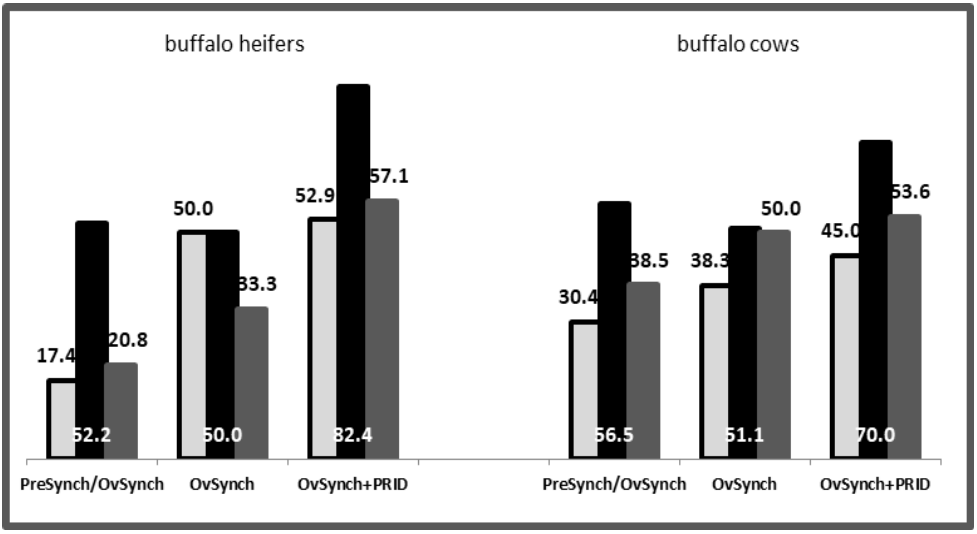

Figure1. Results based on raw data about overall conception rates (framed grey), incidence of full estrus (black) and conception rates from full estrus (dark grey) per protocol within age group, %

It is noteworthy that in the OvSynch scheme the heifers show good conception rates when their cervix has good patency and no mucus. Pregnancies in the buffalo heifers under the OvSynch+PRID are achieved majorly in the presence of mucous discharge (i.e., of both clinical signs), which applies also to the buffalo cows treated under the all three protocolsANOVA from the dispersion analysis in Model-1 indicates that the factor protocol has the most pronounced effect on conception rates – $P \leq 0.01$ (Table 3). The significantly lowest p_i-estimate belongs to the animals treated with PreSynch/OvSynch (Table 4), which is commensurate with the information in Figure 1 within age groups. CSE also has significant effect on the success of TAI ($P \leq 0.05$, Table 3), the highest p_i-value observed in the cases with mucous discharge during insemination.

Table 3. ANOVA from the dispersion analysis of conception rates, whole dataset – Model-1

Sources of variance	df	η^2	F	P
Age	1	0.0049	1.027	P> 0.05
Protocol	2	0.0463	4.894	**P≤ 0.01**
Clinical signs of estrus (CSE)	2	0.0373	3.948	**P≤ 0.05**
Age × Protocol	2	0.0505	5.340	**P≤ 0.01**
CSE × Protocol	4	0.0180	0.953	P> 0.05
CSE × Age	2	0.0595	6.287	**P≤ 0.001**
x	17	0.1016	1.265	P> 0.05
z	190	0.8984		
y	207	1.0000		

Table 4. Effect of the significant factors from ANOVA on conception rates, whole dataset – Model-1

Classes	**Σn_x**	**Σm_x**	**p_i**	**F_d**
Protocol				
1. PreSynch/OvSynch	92	22	0.239	1-[2, 3]*
2. OvSynch	59	24	0.407	
3. OvSynch+PRID	57	27	0.474	
Clinical signs of estrus (CSE)				
4. Without CSE	17	3	0.177	6-[4, 5]*
5. Patency	69	18	0.261	
6. Mucus	122	52	0.426	
Σ / Mean	208	73	0.351	

Σn_x – number of individuals in the class/gradation; Σm_x – number of individuals echographically diagnosed pregnant on day 45 post TAI out of Σn_x; Significance of differences among $\boldsymbol{p_i}$ values within gradation: 1-[2, 3]* and 6-[4, 5] at $P \leq 0.05$

Although Figure 1 suggested some differences between heifers and cows, the dependence of conception rates on protocol and CSE have similar trends in the two categories of buffaloes. In this context are the data in the ANOVA (Table 3), showing that age is not a significant source of variance of pregnancy rates.

On the other hand, the co-effects of age with the factors protocol (P≤ 0.01) and CSE (P≤ 0.001) are significant. The co-effect of protocol with CSE and the all-factors co-effect, expressed as *x*-value, are not significant.

The ANOVA under Model-2 (Table 5) shows that the factor protocol is not a significant source of variation of conception rates in the lactating buffaloes, which corresponds with the above observed smaller differences in conception rates between high and low breeding season in the buffalo cows than in the heifers (Figure 1).

Table 5. ANOVA from the dispersion analysis of conception rates in lactating buffaloes – Model-2

Sources of variance	df	η^2	F	P
Season	1	0.0137	1.794	P> 0.05
Protocol	2	0.0147	0.962	P> 0.05
Clinical signs of estrus (CSE)	2	0.0586	3.855	**P≤ 0.05**
Season × Protocol	2	0.0953	6.256	**P≤ 0.001**
CSE × Protocol	4	0.0504	1.653	P> 0.05
CSE × Season	2	0.0514	3.371	**P≤ 0.05**
x	17	0.1237	0.955	P> 0.05
z	115	0.8763		
y	132	1.0000		

This implies that the above established differences among protocols depend mostly on those in the heifers. More importantly, the analysis of variance indicates that season does not affect pregnancy rates significantly, as well. Table 2 also shows the timing of the protocols as per season. It is noteworthy that the conception rate after out-of-season TAI under the OvSynch+PRID protocol is higher (50.0%) than in the main breeding season (37.5%). The differences between the seasons in the protocols PreSynch/OvSynch and OvSynch are smaller, implying a tendency to diminish the impact of season on buffalo reproduction.

DISCUSSION

On the basis of the observation in the presented field trial that high conception rates are achieved in the cases with both clinical signs (full estrus) only, we share the opinion in other works with the OvSynch protocol in the Bulgarian Murrah that the presence of cervical mucus during AI can be used as an indicator for good conception rates (Atanasov et al., 2012), all the more that in our study the incidence of this clinical sign is much higher. Moreover, this rationale applies also to the OvSynch+PRID protocol where the proportion of buffalo cows and heifers with induced full estrus is definitely highest.

As in the breeds Murrah (Ghumen et a., 2014), Surti (Patel et al., 2022), Jafarabadi (Raval et al., 2021) and Italian Mediterranean (Presicce et al., 2004; De Rensis et al., 2005), field experiments on buffalo heifers and cows have resulted in very good estrus-inducing effect of the OvSynch protocol (under the same GnRH-

$PGF_{2\alpha}$-GnRH scheme), an additive favorable effect on conception rates afforded by progesterone treatment in anestrus in high and low breeding season.

Applying out-of-season treatment with PreSynch/OvSynch, in buffalo heifers was achieved high estrus induction and conception rate which were close to the results from the other two protocols (Sing et al., 2010). However, according to these and to other Indian authors, the sole application of this protocol does not provide good outcome, while in combination with progesterone inserts or prostaglandins it is much more effective (Saini et al., 1988; Andurkar et al., 1995; Kumar H. et al., 2010). Italian researchers came to the same conclusion, emphasizing the major economic impact of such protocols in buffalo heifers and pointing out that at earlier age the effect is relatively better due the usual lack of cyclicity at that stage of individual development (Barile et al., 2001). All these effects are implied also in the results from the Bulgarian Murrah heifers and buffalo cows from the present experiment.

The integral role of the timed components of the OvSynch+PRID protocol is described by the experiment of McDougall and co-researchers in dairy cows with the observation that increased concentrations of progesterone during the anestrous are important for incidence of estrus and for the luteal phase afterwards, hypothetically contributing for maturation of a dominant follicle and hence for prostaglandins secretion and ovulation (McDougall et al., 1992). The combination with prostaglandins at the beginning of the protocol was established to induce follicular atresia and a new follicular wave also in buffaloes (Baruselli et al., 2007).

When buffalo reproduction is concerned, season should be always taken in consideration. Having been included in the linear model with classes of the factor specific for the bubaline species within the conditions of Bulgaria, the effect of season is commensurate with the experience in the Italian Mediterranean breed, where especially in the recent decade the experiments with gonadotropins, progesterone and other exogenous hormones have demonstrated similar pregnancy rates after out-of-season breeding as in the high breeding season (Carvalho et al., 2013, 2016; Baruselli et al., 2013); and also with the experience in Murrah breed in India (Kumar P. et al., 2016). The lack of significant differences in the success of TAI by season is actually good news for the conditions in Bulgaria. It implies that the use of these protocols, and especially OvSynch+PRID, affords manipulation of the breeding season for better reproductive efficiency, hence profitability, of buffalo farming. It also contributes for better distribution of bulk milk throughout the year, in case there is misbalance between annual dynamics of production and prices of raw milk, as it is in Italy.

CONCLUSIONS

All three protocols tested in the present study show capacity to mitigate the impact of season on reproduction, having practical importance for overcoming the problematic seasonal anestrus, especially OvSynch+PRID. It can be summarized that in the Bulgarian Murrah buffaloes the follicular wave in response to the hormonal protocols is similar to that in other breeds in different conditions.

Namely, a favorable effect from the application of OvSynch+PRID in both age groups was established, and also of OvSynch in heifers, as well as of poor results from PreSynch/OvSynch. In our study, the superiority of the OvSynch+PRID protocol – as compared to the other protocols and also to other studies – finds expression in the higher incidence of full estrus especially in the heifers, which predetermines the high conception rate, despite the relatively high percentage of animals without CSE. In this way, its use in field conditions finds justification despite its labor-intensive application in practice. In this context, the presence of cervical mucus during AI can be confidently used as an indicator for high pregnancy rates under the protocols OvSynch and OvSynch+PRID.

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Mehmed ČILAŠ[1], Ćemal VIŠNJIĆ

RECENT STRUCTRUAL CHANGES, SPECIES COMPOSITION AND INTERACTION IN AN OLD-GROWTH FOREST – AN EXAMPLE FROM BJELAŠNICA MT.

SUMMARY

This study examines the structural dynamics and species composition of the "Ravna Vala" old-growth forest on Bjelašnica Mountain, focusing on neighborhood effects on tree growth. Data from a permanent 1-hectare plot, measured 2012 and 2023, revealed that silver fir (*Abies alba* Mill.) constitutes nearly 73% of the total volume, while beech (*Fagus sylvatica* L.) dominates in tree numbers. The forest displays a reverse J-curve pattern, with significant mortality in smaller diameter classes. Using indices for species intermingling, aggregation, and competition, the study found that diameter at breast height (dbh) and the aggregation index significantly influenced growth, especially for silver fir and beech. Additional factors like the mixing index and competition index were significant for beech growth. These results underscore the complexity of species interactions and the importance of tailored management strategies. Future research should explore factors limiting fir regeneration and the link between disturbances and beech recruitment. Management practices that mimic natural disturbances, such as creating larger gaps, are recommended to enhance species diversity and forest resilience.

Keywords: Old-growth forest, species composition, tree growth, beech-fir, competition

INTRODUCTION

Old-growth forests can be defined as naturally regenerated stands composed of native tree species with minimal human impact, where ecological processes operate dynamically (Barton and Keeton, 2018; Sabatini et al., 2020). Currently, less than 1.5 million hectares are considered old-growth forests, predominantly located in the boreal zone (Sabatini et al., 2018). In the temperate zone of Europe, old-growth forests are exceedingly rare, covering less than 0.1% of the forest area, and are nearly non-existent for many forest types within the EU (Meyer et al., 2021). The Dinaric Alps is one of the European regions that still hosts old-growth forest stands (Motta

[1] Mehmed ČILAŠ (corresponding author: m.cilas@sfsa.unsa.ba), Ćemal VIŠNJIĆ, University of Sarajevo, Faculty of Forestry, Sarajevo, BOSNIA AND HERZEGOVINA

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et al., 2024). Historically, this mountain range served as a border between European Kingdoms and the Ottoman Empire, resulting in relatively low population densities over centuries. This low human density led to less intensive and less widespread deforestation and land use compared to other European mountain regions (Kaplan et al., 2009; Cagliero et al., 2023). One of the most prominent forest communities in this mountainous region consists of mixed stands of European beech (*Fagus sylvatica* L.), Norway spruce (*Picea abies* Karst.), and silver fir (*Abies alba* Mill.), which dominate not only managed forests that follow "close-to-nature" principles due to the long-term application of the selection (plenter) management system but also old-growth forests (Keren et al., 2014; Diaci et al., 2011; Čilaš et al., 2023).

Consequently, large, well-preserved forest patches have been conserved, attracting the attention of European foresters, ecologists, and botanists since the early 20th century (Motta et al., 2024). Studying old-growth forests is crucial for understanding forest ecosystem dynamics and developing effective forest management strategies. In central Europe, the current forest structure and composition are largely the result of human activities, whereas old-growth forests are shaped by infrequent, stand-replacing disturbances in the absence of human interference (Nagel et al., 2010). Excluding human activities provides valuable insights into trends in tree species occurrence and changes linked to factors such as climate and air pollution. For instance, an increase in beech in old-growth forests began 60 years ago (Šafar, 1951) and has become more evident in recent decades. Conversely, a decline in fir has been observed in some old-growth forests of the Dinaric region (Diaci et al., 2022; Keren et al., 2014; Klopcic et al., 2010).The coexistence of beech, fir, and spruce is influenced by many factors, complicating the study of these dynamics. The spatial and temporal dynamics of some forest types are significantly affected by neighbourhood effects (Frelich and Reich, 1999). The neighbourhood effect can be measured using various competition indices, with growth response yield or increment as a function of density being common measures (Balandier et al., 2006). However, this method does not fully capture competition due to spatial and temporal variation, plant immobility, and phenotypic plasticity. An alternative approach involves studying plants individually, focusing on their interactions with specific neighbours rather than overall population density, with diameter at breast height (dbh) serving as a key indicator of growth and resource utilization (Antúnez et al., 2023; Stoll and Weiner, 2000).

The aim of this study was to investigate the recent structural dynamics and tree species composition resulting from past disturbances and to assess the impact of neighbourhood factors on tree growth. Specifically, we aimed to:

(I) Evaluate the recent structural dynamics and compare it to other old-growth forests in the Dinaric region.

(II) Analyse tree distribution within the stand as a consequence of nonhuman activities

(III) Determine the influence of neighbourhood parameters (e.g. neighbour dbh, tree species, distance, etc.) on tree growth and identify the key drivers.

MATERIAL AND METHODS

The research was conducted on a permament 1 ha plot within a pristine forest reserve „Ravna vala“ on Bjelašnica mountain. The forest reserve „Ravna vala“ is located around 30 km south west from Sarajevo, with an altitude range from 1280 to 1450 m. The total area is around 45 ha. „Ravna vala“ belongs to *Abieti – Fagetum illyricum* community with Beech and Fir as dominant tree species and Norway spruce (*Picea abies* Karst) and Sycamore (*Acer pseudoplatanus* L.) can also occur. The dominant parent substrate is limestone and dolomite, where moraine deposits are also present. This results in soils with variable depth. Most common soil types are rendzina, calcomelanosol and calcocambisol. Climate is continental with strong mountain influence (Lučić, 1966). Average annual temperature is 6°C, and annual precipitation sum is 1600 mm. The data was collected on a permanent 1 ha plot within the reserve. The plot was futher divided into 10 x 10m grid were for all trees above 5 cm following attributes were recorded: tree species, diameter at the breast height (dbh), height, height to the live crown, x and y coordinates using the pollar method (later recalculated on whole plot). For purpose of this study we used the data from the last two measurments in 2012 and 2023. To asses the tree species composition and interaction Fuldner's intermingling index (Fuldner 1995) and Clark-Evans aggregation index (Clark and Evans 1954) were calculated. To asses the influence of competition Hegyis index was also calculated using the *pairwise* function from the *siplab* R package (Garcia 2014). Trees close to the edge were excluded using the *edge* function from the same package. As influence radii 6 meters was chosen since smaler radii tend to spatial autocorrelation (Pedersen et al. 2013) and with larger radii its difficult to spot direct competitive differences. In our analysis we used the competition values from the end of period since Wilcoxon signed rank test showed no stastical differences. Additionaly, we identified for each tree his closest neighbour. All these factors were used in analsys of variance (ANOVA) to asses the tree species interaction. The interaction was represented throug dbh increment which was calculated as the difference of dbh between two periods (2023 and 2012).

RESULTS

The total volume in 2023 was approximately 746 m³/ha, with silver fir contributing the majority share at 72%, followed by beech at 20%, sycamore at 6%, and spruce at 2%. The total number of trees was 746, with beech trees constituting more than 70% of the total tree count. Compared to the data from 2012, there is a notable reduction in both the total volume and the number of trees. This reduction is particularly pronounced for silver fir in terms of volume and for beech in terms of tree numbers (table 1).

The forest structure illustrated in Figure 1 exhibits a reverse J-curve pattern, characteristic of an uneven-aged forest stand. Comparative analysis between the years 2012 and 2023 reveals minimal variation in forest composition over this period. Notably, there is a significant mortality rate observed among the smallest diameter class (dbh < 10 cm). As tree diameter increases, mortality rates decrease progressively across the dbh classes.

Table 1. Comparison between the last two measurements

	2012				2023			
Tree species	dbh	G (m^2/ha)	V (m^3/ha)	n	dbh	G (m^2/ha)	V (m^3/ha)	n
Silver fir	51.21	42.6	594.1	163	50.98	39.58	548.05	154
Beech	11.83	12.91	157.14	612	12.41	12.6	156.12	543
Spruce	22.83	1.26	13.52	21	23,.2	1.25	13.5	20
Sycamore	23.23	3.02	45.87	36	27.33	3	45.79	29
Total	27.28	59.79	810.63	832	28.53	56.43	763.46	746

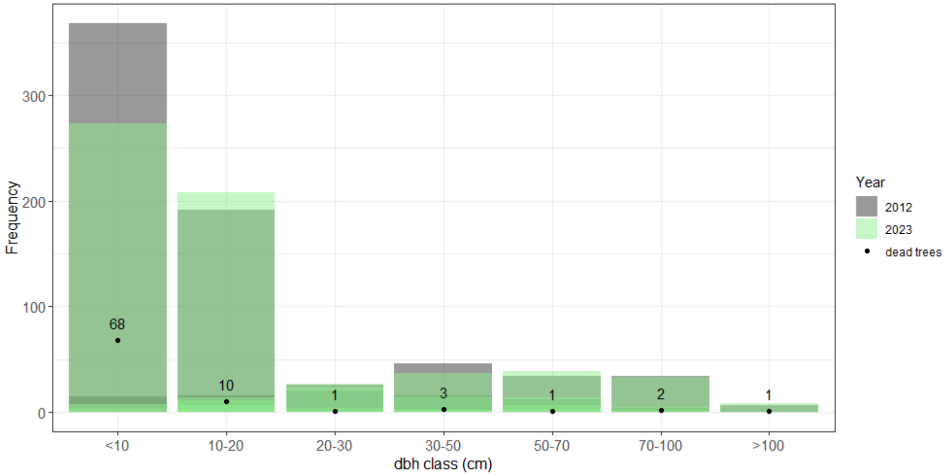

Figure 1. A comparison of dbh structure between two measurements in 2012 and 2023

Figure 2 illustrates a significant contrast between beech and silver fir, the two most prominent species. The dbh distribution of beech exhibits a characteristic reverse J-shaped curve, typical of uneven-aged or mixed-species forest stands. In contrast, silver fir demonstrates a more normal distribution, akin to even-aged stands. Notably, there were no significant differences observed in the dbh distribution of beech between the two measurements except a decrease in numbers in the lowest dbh class. However, for silver fir, a discernible shift to the right, indicative of negative skewness, suggests a decline in older silver fir trees over time. Moreover, sycamore and spruce exhibit distinct patterns across dbh classes. Sycamore distribution appears bimodal, indicating the presence of two distinct age cohorts. In contrast, spruce demonstrates a positivie skewness. But the total number of spruce and sycamore trees is not enough to have concrete conclusions.

Relative risk, a common term used in medical studies such as epidemiology represents the ratio of the outcome among the exposed group to the risk of the outcome among the unexposed group. In the context of spatial data analysis, relative risk can be interpreted as the probability of occurrence of one type, while considering the occurrence of other types. Specifically, it measures the likelihood of one tree species occurring relative to the occurrence of another species within the same spatial context. Higher values of relative risk indicate 'hot spots' where the occurrence of a particular tree species is more probable, suggesting favorable conditions for its growth and development. Conversely, lower values indicate less favorable conditions.

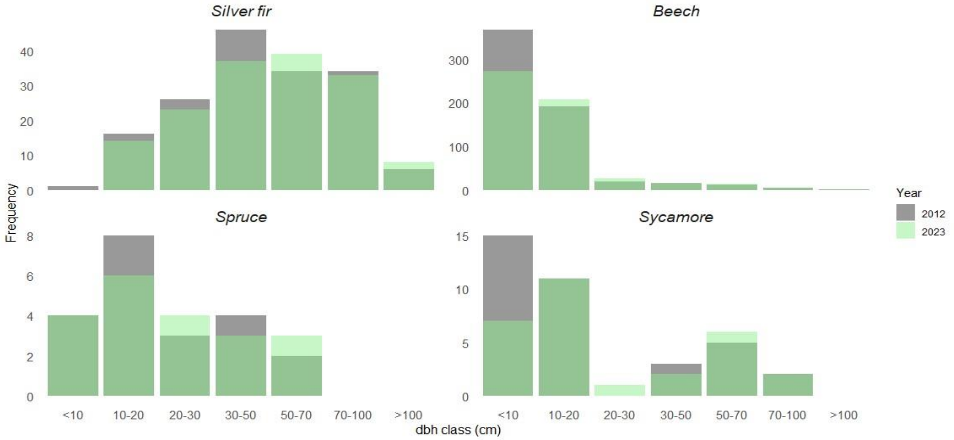

Figure 2. dbh distribution per tree species

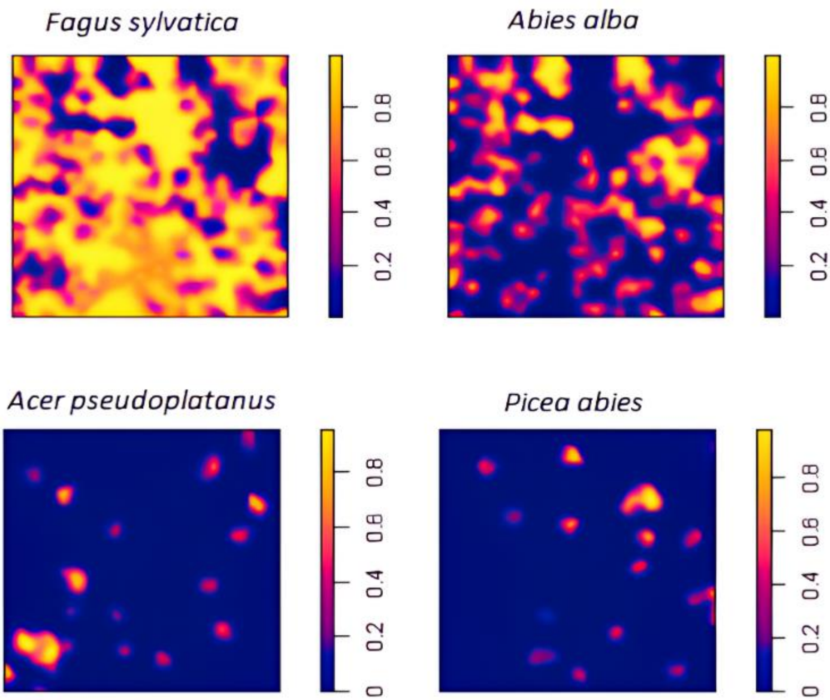

Figure 3. Relative risk analysis as heat map

The analysis reveals that beach trees are the most favored species, occupying a substantial portion of the plot. Favorable conditions for silver fir are primarily observed in clustered groups, while spruce and sycamore tend to thrive in smaller groups or as individual specimens. Interestingly, there are distinct 'hot spots' for spruce and sycamore, particularly in areas of the plot that are less suitable for beech. The analysis of Fuldner's index revealed notable variations in species aggregation among the studied tree species. Spruce exhibited the highest index value at 0.94, followed by silver fir at 0.70 and sycamore at 0.66. Conversely, beech demonstrated the lowest index value at just 0.24. Similar trends were observed for the Clark-Evans aggregation index, although silver fir showed slightly higher values. When examining the index values per dbh class, a clear pattern was not evident, with Pearson correlation coefficients (PCC) consistently below 0.3, except for sycamore in both cases. Notably, for spruce, there was a slight negative trend observed with increasing dbh. However, upon closer examination of the data, it becomes apparent that while values were lower at smaller dbh classes, the increase did not follow a linear trend with increasing dbh.

Table 2. Tree species intermingling and aggregation per tree species, where M – Fuldners index, R – aggregation index according to Clark – Evans, n – number of trees, PCC- Pearson corelation coefficient

	Tree species							
	Beech		Fir		Norway spruce		Sycamore	
Measure	mean	PCC	mean	PCC	mean	PCC	mean	PCC
M	0,24	0,155	0,7	0.144	0,94	-0,079	0,66	0,641
R	0,91	0,279	1,3	0.062	1,24	0,274	0,96	0,457

Additionally for each tree species his closest neighbour was identified as shown on the Sankey diagram (fig 4). The Sankey diagram provides a visual representation of the spatial distribution and proximity relationships between different tree species within the study area. Our analysis revealed a predominant clustering of beech trees, with the majority having other beech trees as their nearest neighbors, followed by silver fir. Silver fir and sycamore trees exhibited mixed proximity patterns, with flows indicating both intra-species and inter-species clustering, highlighting a more heterogeneous distribution within the stand.

Multifactorial ANOVA was employed to investigate the influence of various factors and their interactions on dbh increment. Overall, the analysis identified dbh as the primary determinant of tree growth, exhibiting substantial effects, along with the aggregation index and its interaction with dbh, both of which were statistically significant ($p < 0.01$). Further, significant interactions were observed between dbh and the competition index ($p = 0.006$), suggesting a notable modulation of growth by competitive dynamics. Moreover, complex interactions involving multiple factors, such as dbh, neighbour tree species, and neighbour dbh, were significant ($p = 0.048$), as was the interaction that additionally included the aggregation index ($p = 0.048$). For silver fir only dbh and aggregation index were found to have a significant impact on tree growth ($p < 0.01$). For beech multiple individual factors affected the growth significantly: dbh, aggregation, mixing ($p < 0.01$) and competition index ($p = 0.02$) and also the interaction between dbh:competition, aggregation: competition, mixing:competition and dbh:aggregation:mixing had a significant impact ($p< 0.05$). For spruce dbh and mixing index were significant where for sycamore only dbh was significant ($p=0.02$).

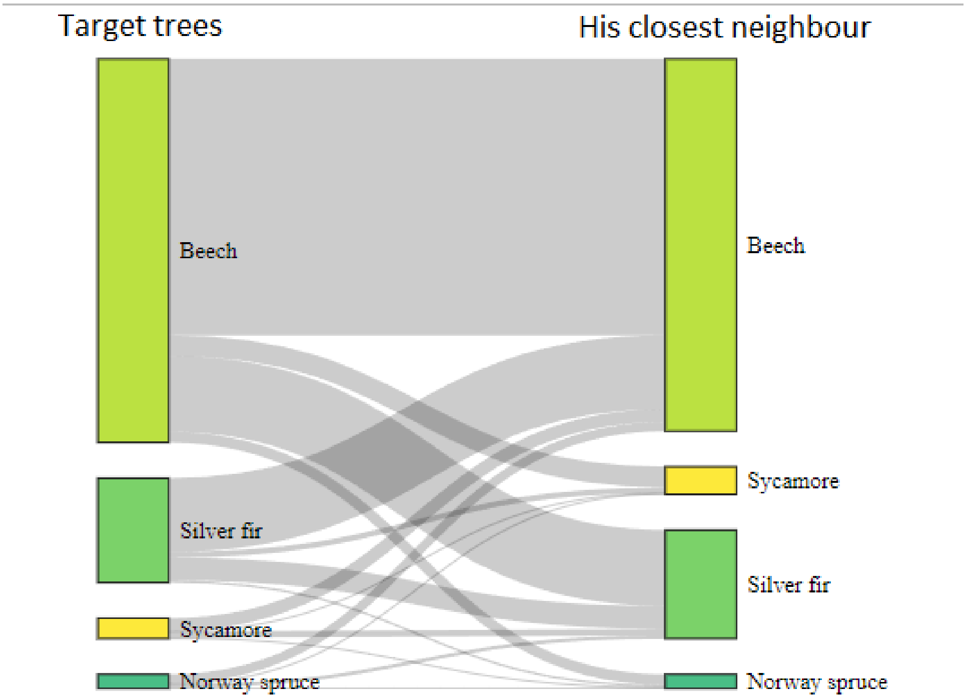

Figure 4. Sankey diagram representing the relationship between each tree and theirs neighbour

DISCUSSION

The total volume was estimated at approximately 746 m³/ha, which is comparable to the estimates for Lom (Motta et al., 2024) and Čorkova Uvala (Saniga et al., 2011) old-growth forests. However, it is lower than in Janj, Perućica, and Biogradska Gora (Motta et al., 2024), where the total volume exceeds 1000 m³/ha. The only old-growth forest with a lower total volume than this study was Plješevica (Višnjić et al., 2009). Tree species composition according to growing stock comprised one of the highest shares of silver fir (73%) compared to other old growth forest in this region (Anić and Mikac, 2008; Keren et al., 2017; Motta et al., 2015, 2014, 2011; Višnjić et al., 2017, Curovic et al., 2020). Low percentage of beech was also found in the old growth forest Janj (cca 20%) and Biogradska gora (cca 25%). The share of spruce was the lowest compared to other old growth forest, where the share of sycamore was similar. In terms of tree density, it had the highest number of trees per hectare compared to other old growth forest (Kral et al. 2010, Kucbel et al. 2010, Saniga et al. 2011, Parabekova et al. 2018, Motta et al., 2024). Beech was found to be the dominant tree species with 73% similar to Janj (63%) and Lom (60%). The contrast in fir and beech share in terms of volume and number is related to the structure of each tree species as shown in figure 2. Beech is dominant in lower dbh classes where the tree distribution of fir can be described as a negative skewed also dominantly present in higher dbh classes. Similar distribution between beech and fir have been found also in other old-growth forests of this region: Janj and Lom (Govedar et al., 2021), Plješevica (Višnjić et al., 2009), Peručica (Palandrani et al., 2021) and Biogradska gora (Čurović et al. 2011).

Compared to the last measurement in 2012, a slightly reduction in volume and tree number was evident. The reduction in volume was due to mortality of fir trees. High mean dbh and the negative skewed distribution indicate a late optimal or early decay phase for fir where a reduction in numbers for beech is due to competition and light availability. A long-term decay of coniferous and progression of beech in understory was recorded in other old growth forest of this region. For instance, the study by Keren et al. (2014) showed a decrease in spruce and fir tree density across all three analyzed reserves (Janj, Lom, and Perućica), while the proportion of beech increased. In contrast, the share of fir in basal area increased in Janj, remained stable in Lom, and decreased in Perućica. The proportion of beech in basal area also increaseds. To explain these changes in species composition within spruce, fir and beech stands is difficult (Šafar, 1951.; Nagel et al., 2010) since it depends on many factors. These factors can be related to human activities or natural disturbances. For deeper understanding long-term data is required. An increase of beech on plot is also to be expected in future as reveald by relatve risk analysis. Most parts of the plot were identified as the most suitable growing conditions for beech. Similar prediction have arised in some early studies of beech – fir old growth forests (Šafar 1951, Pintarić 1982) and the issue about the regeneration of fir came up. The study of Ivojević et al., (2018) reveald a dyeback of fir samplings with the increase of its size. The highest number was present at the smallest height class and lowest in the heighest class, which was contrary to the managed stand. This implies deeper studies into the regenarition of fir to identify the obstacles of fir which promotes beech regeneration. Fir had the heighest value of Clark-Evans aggregation index (1.3) indicating a tendency to a regular distribution on the plot, followed by spruce, sycamore and beech (see table 2). The lowest values were in the lower dbh classes, where trees are more clumped and with increase of dbh trees are more regulary distributed. Altough, the correlation coefficients are not high, similar observations were found in other old growth forests (Bilek et al., 2011; Parobeková et al., 2018; Wang et al., 2020). This is also in accordance with the classical Janzen-Connell spacing effect predictions which states that the spatial distributions of adult trees would become more regular than those of juveniles because of the differential attack rates between adults and juveniles by pathogens or herbivores (Janzen 1970, Connel 1971). The aggregation index was statisticaly correlated with mingling index showing a moderate positive correlation (0.37). In lower dbh classes trees were more clumped sourounded with same tree species resulting in lower values of Fuldners mingling indices. With a more random distribution the probability of different neighbour species is higher. Similar results were also found by (Li and Wei, 2024).

The key driver for dbh increment was dbh, aggregation index and its intereraction, all with high statistical significance. Additionaly, dbh and competittion interaction and the interaction of dbh with multiple factors showed to be statisticaly significant. Thus, the main driver is the dbh of target tree. Thats why many dbh increment model are using dbh as the main predictor (Bayat et al., 2013; Ciceu et al., 2022; Manso et al., 2022; Schelhaas et al., 2018) where some additional predictors can be used. Aggregation value which on tree level is the distance to the closest neighbour showed to have a high impact on tree growth for booth beech and fir. The results of this study are contrary to the results of Antúnez et al., (2023) where distance of neighbors had a significant effect only when the dimension of those neighbors were larger than those of the refenece tree. The neighbour tree species and their dbh showed no significant impact on dbh increment. These findings doesnot align with the theory of Forrester and Bauhus, (2016) and similar studies (Chen et al., 2016; Di Maurizio et al., 2023; Fichtner et al., 2017; Searle and Chen, 2020). But even in these studies the efffect varried depending on tree species and also on competition meassure. In the study of Di Maurizio et al., (2023) growth response of most species to neighborhood dissimilarity was negatively influenced by increased competition, but on the other side the study of Searle and Chen, (2020) showed better growth with surrounded by dissimilar neighbors than those surrounded by similar neighbors under high competition intesity. This indicates additional considerations are required to describe the connection between tree growth, dissimilarity and competition. The contrast in findings between this study and previous mentioned can be due to different study plots. While previous studies were conducted in visiual homogenous plots, our study was conducted in a heterogenous stand where different tree species occure in different sizes and distribution. In homogenous plots the effect of reference tree size may be equal, in a heterogenous stands the effect of reference tree size may be dominant, masking the effect of other factors. For istance the effect of hegyi compettition index was significant for beech while for silver fir showed no significance. Overall, the compettition index was only significant in interaction with the dbh of the reference tree, which indicates that the competition index was only relevant for smaller beech trees. Different indices affect the tree growth differently depending on the tree size. Therefore, its important to take into account the tree size distribution when analysing species mixing and interaction on tree growth. Similar suggestions can be found in the study of Madrigal-González et al., (2016).

CONCLUSIONS

The total volume was similar to Lom and Čorkova uvala but much lower than Janj, Peručica or Biogradska gora. Contrary, tree species compositions in this study are consistent with patterns observed in other old-growth forests in the region, with fir dominating the basal area and beech increasing in density. Fir's late-optimal or early-decay phase, alongside beech's competitive advantage, suggests a gradual shift in species composition. The results suggest that beech will continue to dominate in the future, especially in conditions that favor its growth, as indicated by relative risk analysis. Fir regeneration remains a concern, as seen in lack of fir trees in lower dbh classes. The competition dynamics and tree size distribution were found to significantly influence tree growth, with dbh emerging as a key predictor. The lack of significant impact from neighboring species suggests that intra-species competition may be more critical in this context. Addressing the factors limiting fir regeneration is also crucial for maintaining species diversity and health and can give valuable insights for forest managers. On the other hand, management approaches that mimic disturbances, such as creating larger gaps, can promote beech regeneration. However, further investigations are needed to understand the relationship between these disturbances and beech recruitment fully.

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Jelena GOLIJAN PANTOVIĆ [1], Vera POPOVIĆ[2,3], Mile SEČANSKI[4], Aleksandar POPOVIĆ[4], Olivera ĐORĐEVIĆ MELNIK[4], Ljubica ŠARČEVIĆ TODOSIJEVIĆ[5]

FACTORS AFFECTING SEED VIGOUR

SUMMARY

The seed industry is essentially based on the production of quality seeds with high germination and vigour percentages and for that reason the aim of this study was to point out the most important factors that affect seed vigour. This study shows an overview of the development of seed vigour tests throughout history. Seed vigour is affected by many environmental factors, as well as genetic factors, of which the chemical composition of seeds, hardheadedness and resistance to disease-producing agents express positive effects. Special attention is paid to the role of phytohormones, such as abscisic acid (ABA) and gibberellin (GA), in regulating seed dormancy and their interaction with environmental factors, including temperature, light and moisture. The research results reveal a complex network of interactions among genetic factors, phytohormones and environmental conditions that mutually modulate the degree of seed dormancy and its impact on germination and seed vigour. Experimental data show that the application of certain agrochemical treatments can change the level of phytohormones and consequently alter the rate of seed dormancy and germination. Further research of these mechanisms will reveal prospects for the development of innovative technologies for the improvement of the seed industry as an inseparable part of agricultural production. This study provides a deeper understanding of the regulation of seed vigour and, also identifies new approaches to the improvement of plant production in accordance with the demands of contemporary agriculture.

Key words: seed, vigour, germination, dormancy, environmental factors

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[1]Jelena Golijan Pantović, (Corresponding author: golijan.j@agrif.bg.ac.rs) University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, REPUBLIC OF SERBIA
[2]Vera Popović, (Corresponding author: vera.popovic@ifvcns.ns.ac.rs; drvvpopovic@gmail.com), Institute of Field and Vegetable Crops, Maxim Gorky 30, 21000 Novi Sad, REPUBLIC OF SERBIA, and [3]University of Bijeljina, Faculty of Agriculture, Pavlovića put bb, Bijeljina, BOSNIA AND HERZEGOVINA,
[4]Mile Sečanski, Aleksandar Popović; Olivera Đorđević Melnik, Maize Research Institute, “Zemun Polje”, Belgrade, REPUBLIC OF SERBIA,
[5]Ljubica Šarčević-Todosijević, Sanitary Medical School of Applied Sciences “Visan”, Belgrade, REPUBLIC OF SERBIA

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INTRODUCTION

Seed is one of the most important links in agricultural production. It has an important role in the cycle of production of safe and healthy food of high quality. In terms of agriculture, seed means the renewal of plant production, i.e. its continuity, and is one of the most important factors of successful production (Popović, 2010; Ilin, 2014). That is why ensuring high seed quality is a priority of current seed production and a prerequisite for high yields of all plant species (Poštić et al., 2014). Seed production encompasses the establishment and cultivation of crops developed from all categories of seeds, control of this production, drying, processing, packaging, sampling, testing and certification (declaration) of seeds, their trade, storage, transportation and distribution or conservation until sowing (Ilin, 2014; Popović *et al*., 2010; 2020a-b; 2021; 2022; Trung and McMillan, 2021; Zhang and Colak, 2022; Milunović *et al.*, 2022; Lakić *et al.*, 2022; Burić *et al.*, 2023; Stevanović *et al.*, 2023; Sekulić *et al.*, 2023; Stupar *et al.*, 2023; Miladinović et al., 2024). Production of field crops is of great economic importance, and seed quality is also of high significance, because errors following sowing can no longer be corrected (Velijević *et al.*, 2016; Filipović *et al*., 2021; 2023; Rajičić *et al.*, 2023; Dimitrijević *et al.*, 2023; Vasileva *et al.*, 2023; Kosev *et al.*, 2023a-b; 2024). The task of seed production is to provide sufficient quantities of quality seeds of the appropriate assortment for all production regions. Therefore, all countries strive to achieve the highest degree of self-sufficiency in their own markets and create surpluses for export (Popović, 2010). Humans, since the beginning of their activities in crop growing approximately 10 millennia B.C. and especially since their recognition of the importance of seeds in plant multiplication have been interested in the physiology of seed, particularly germination and its effects on crop traits (Marcos-Filho, 2015).

The determination of seed quality is done by the application of standardised methods. These methods have been altered in accordance with scientific knowledge within the field of seed physiology. In addition, new technological procedures of seed processing can also influence the change of methods, in accordance with the requirements of certain plant species (Ilin, 2014).

Seed quality testing includes the determination of seed purity, germination, moisture and health condition and for certain plant species, the first count, 1000-seed weight and some other properties. This testing is performed in the manner and in accordance with the procedures established in seed testing methods. Seed purity, first count, germination, count of other species of cultivated plants and weeds in seed samples have to be within the tolerance limits (Regulation on Seed Testing Quality of Agricultural Crops).

Germination and seedling vigour are the most important parameters of seed viability, on which seed usability depends. Germination is expressed as the percentage of seeds capable of germinating into healthy, strong, undamaged seedlings, which can develop into normal plants under normal field conditions. In the case of a seed crop, effects of seed vigour are reflected on the initial growth, uniformity of the crop, uneven maturation and the level of seed yield (Knežević *et* *al*., 2014). High vigour seeds produce seedlings that will establish the best stand in the field, uniform emergence of crops, which will provide stable yields of outstanding quality (Poštić *et al.*, 2014). Since high-quality seed production is based on high-quality seeds, and production of such seeds depends on seed vigour, the aim of this study was to review the relevant literature in order to indicate the most important factors that affect seed vigour.

The aim of this study was to point out the most important factors that affect seed vigour, ie. production of quality seeds with high germination and vigour percentage.

MATERIAL AND METHODS

The aim of this study was to point out the most important factors that affect seed vigour, i.e. production of quality seeds with high germination and vigour percentage. This study shows an overview of the development of seed vigour tests throughout history. According to numerous studies, seed vigour is affected by many environmental factors, as well as genetic factors, of which the chemical composition of seeds, hard seededness and resistance to disease-producing agents express positive effects. This study presents mechanisms of seed deterioration and effects on seed vigour with the aim to comprehend internal and external factors affecting this process.

RESULTS AND DISCUSSION

Chronology of seed vigour testing

According to McDonald (1993) the first law on seed was enacted in Berne, Switzerland in 1816. Fifty three years later, the first seed testing laboratory was founded by Frederick Nobbe in Tharandt, Germany. In the same year (1876), Edward Hopkins Jenkins, agricultural chemists, established the first seed testing laboratory in the United States in Connecticut. The first "Rules for Testing Seeds" were written by Jenkins in 1917. The expression "seed vigour" was for the first time used by Nobbe in his “Handbuch der Samenkunde” in 1876. Furthermore, the same author suggested germination test procedures (Marcos-Filho *et al.*, 2015). The term *triebkraft* with the meaning "driving force" and "shooting strength" related to germinating seedlings was introduced by Hiltner and Ihssen in 1911. These authors wanted to draw attention to seeds that had produced seedlings with longer roots in contrast to seedlings developed from "weaker" seeds drawn from the same lot. In the 1930s, the US term "germination energy" related to the germination rate was broadly accepted. However, scientists and seed technologists of that time showed little or no interest in improving such studies. The lack of interest continued throughout the first half of the 20th century (Marcos-Filho *et al.*, 2015). The International Seed Testing Association (ISTA) was founded in 1924 with the idea to promote standard methods in seed testing all over the world. In 1931, ISTA adopted the first "International Rules for Seed Testing" (ISTA Rules) (Milivojević *et al*., 2018). During the 1940s, the tetrazolium (TZ) test, as a fast and reliable test, was developed for the determination of seed viability. This test is still one of the most commonly used seed tests in Brazil (França-Neto *et al.*, 1998). On the other hand, germination and TZ viability tests differ in relation to the emergence of seedlings in the field under unfavourable environmental conditions. Therefore, results obtained by these two tests have to be supported by results gained by more precise determinations. In order to be included into the ISTA Rules, the majority of test methods have to undergo interlaboratory test comparisons, because these comparisons provide reliable and reproducible results (Milivojević *et al.*, 2018).

In 1950, the ISTA Congress was held in Washington DC, USA, the first time outside Europe, emphasising the contribution of the US seed technologists. The then ISTA President W.J. Franck, being aware of rising international seed trade, pointed out the differences in results of germination tests between the US and the European seed testing laboratories. As stated by Franck, the main difference between the US and the European laboratories was in the purpose of the standard germination test. In the European laboratories, technologists believed that the most important point for the export of seed lots was reproducibility of test results, while the US technologist thought that the capability of seeds of a certain seed lot to produce plants was the essential aim of the germination test. Franck suggested that germination should be determined under favourable conditions in order to gain uniform test results. Furthermore, it was accepted that capability of seeds to produce plants under the field conditions should be defined by a new expression: vigour. During the same year, the ISTA Biochemical and Seedling Vigour Committee was founded and two primary goals were set up: 1) to define seed vigour, and 2) to develop standardised vigour test methods (McDonald, 1993). Standardisation of methods, also recognised as validation of methods, was implemented and developed at the end of 19th century. Unfortunately, the system was not reliable until the end of the 20th century. At that time, the procedure of method validation for seed testing was set up (Steiner et al., 2008; Milivojević *et al.*, 2018). The development of the ISTA Rules is a permanent procedure. ISTA members are involved in the constant process of developing methods for seed sampling and testing. The methods undergo appropriate validation to ensure that the test procedures provide reliable and reproducible results (Milivojević *et al.*, 2018).

During the 1960s, studies on the estimation and determination of seed vigour and its effects on seed traits were broadened, which resulted in important studies of seed physiology (Marcos-Filho *et al.*, 2015). The following year, the Vigour Test Committee was founded by the Association of Official Seed Analysts (AOSA) (McDonald, 1993).

The alteration of the ISTA Rules format started at the beginning of the 21st century (Jones and Kahlert, 2005). In 2001, yearly editions of the ISTA Rules were initially presented as replaceable pages (Jones and Allen, 2017) and then, the electronic annual version was introduced in 2015. ISTA accredited laboratories have to revise their technical knowledge and practice every year in accordance with the authorised edition of the ISTA Rules. This is assessed and verified by

Proficiency tests, so that laboratory analysts keep up with currently valid versions (Milivojević *et al*., 2018).

Concept of the term vigour

Seed vigour is the most important biological trait of seed. In the broadest sense, it encompasses several inter-related traits (germination, viability, seed vigour, energy of growing, vitality, emergence force, longevity, germination capacity), i.e. properties determining the activity and performance of a seed lot of commercially acceptable seed germination under diverse environmental conditions (Lekić, 2009). The term vigour covers traits that are related to the following aspects of the performance of the seed lot (ISTA, 2014): rate and uniformity of seed germination and seedling growth, the ability of seeds to germinate under unfavurable environmental conditions, seed condition after storage, primarily retention of germination capacity. Seed vigour is defined as "the sum total of those properties of the seed which determine the level of activity and performance of the seed lots of acceptable germination in a wide range of environments" (ISTA, 2015).

Seed vigour does not only indicate the percentage of viable seeds in a sample, but reflects seed ability to produce normal seedlings under unfavourable growing conditions that may occur in the filed. Seed vigour is most simply evaluated through seed germination (Lekić, 2003). Vigour cannot be determined individually such as it is possible with seed germination. Van de Venter (2000) explained the phenomenon of vigour through a practical example of seed vigour differences (Table 1).

He hypothetically observed two seed lots (A and B) and their germination percentage and field emergence percentages on three farms. Germination percentages of these two lots were identical according to the standard germination test. Seedbed conditions (temperature, moisture, etc.) in the farm 1 were favourable and the stands of A and B were similar to their laboratory germination percentages. On the other hand, soil conditions in the farm 2 were slightly unfavourable (possibly cold and wet to some extent) and the seed lot B produced a stand which was less than that of the seed lot A. Conditions in the farm 3 were exceedingly unfavourable and the stand produced by the seed lot B was much less than that of the seed lot A.

The percentage of emergence was, in both cases, much lower than emergence percentage obtained in the laboratory. What can be a cause for the stand differences in the farms 2 and 3 regardless of the similarities between the two seed lots in the farm 1 and in the standard germination test? The answer can be found in different vigour of seeds: vigour of the seed lot A was higher than vigour of the seed lot B. Seed vigour is affected by many physiological and biochemical traits, including their complex interactions. Scientists are not able yet to entirely comprehend effects of these traits and interactions among them, thus it is not fully clear what seed vigour actually is.

Table 1. Hypothetical example of germination and emergence of two seed lots.

Seed lot	Germination (%)	Field emergence (%)		
		Farm 1 (favourable conditions)	Farm 2 (slightly unfavourable)	Farm 3 (very unfavourable)
A	90	88	80	70
B	90	87	60	40

Source: Van de Venter (2000)

Factors affecting seed vigour

It is known that genetic factors strongly affect seed vigour: chemical composition of seeds, hardseededness and resistance to disease-producing agents (pathogens) have positive effects on seed vigour (AOSA, 2002). Vigour is a promising trait of viable seeds in cropping practices and is affected by the complex interrelation between genetic and environmental factors (Figure 1.). Why properties of vigour vary it is insufficiently comprehended (Finch-Savage and Bassel, 2015).

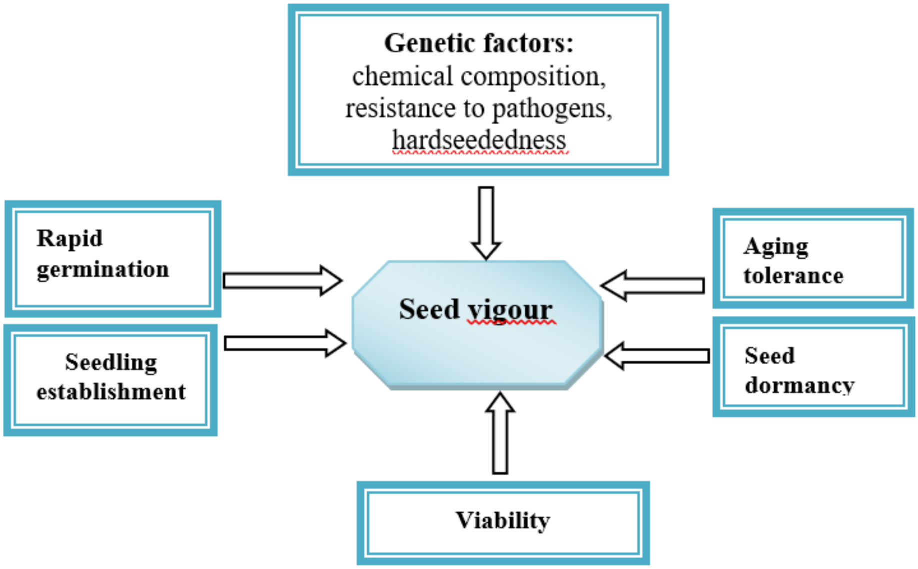

Figure 1. Factors affecting seed vigour

Seedling vigour is expressed by the length of radicle, stem and weight. Low vigour and pronounced seed dormancy are closely related to seed germination (Tomaz *et al.*, 2015), as well as to its size: larger seeds are inclined to produce more vigorous seedlings (Ambika *et al.*, 2014). Seed quality depends not only on purity, health, viability and vigour (Van de Venter, 2000), but also on many other factors, such as seed production locations, genetics among varieties, seed crop management, etc. (Velijević *et al.*, 2016). The seed production carried out under conditions of water stress, insufficient amounts of nutrients, extreme temperatures, often leads to the production of seeds with poor vigour, the so-called low vigorous seeds. Moreover, mechanical damages caused during harvest and processing, as well as improper storage, also adversely affect seed vigour (Jovičić, 2014). Even though numerous definitions of seed vigour accentuate field performance, seed vigour also has significant effects on seed storage. Low/poor seed vigour will result in low storage potential (Van de Venter, 2000).

a) Seed deterioration

Malik and Jyoti (2013) defined seed deterioration as "deteriorative alterations occurring with time that increase the seed's exposure to external challenges and decrease the ability of the seed to survive". High-vigorous seeds are seeds with good traits (Hampton, 1999). On the other hand, low-vigorous seed lots produces seedlings with smaller leaf area index, lower dry matter accumulation and the decreased growth rate of the crop. The main reason for the vigour loss is ascribed to seed deterioration during its development, harvest, drying and storage that begins when the seed is physiologically mature and is prolonged during storage (Basu and Groot, 2003).

Seeds with low vigour are mostly a consequence of seed deterioration processes. Seed deterioration results in the loss of seed quality in the course of time. The following factors are the essential reasons for seed deterioration: temperature, relative humidity, seed moisture content, and pest infestation (Akash *et al.*, 2022). It can be stated that the seed deterioration process is attributed to a series of interrelated "events", among which genetic damage, alternations in respiratory activities, changes in enzymes, proteins, hormones, the accumulation of toxic metabolites are included (Hampton, 1999). Moreover, lipid peroxidation, membrane disruption, DNA damage, impairment of RNA, and protein synthesis are also included (Akash *et al.*, 2022), as well as cytological, physiological, biochemical and physical modifications in seeds (Malik and Jyoti, 2013) (Figure 2). According to Akash *et al.* (2022), there are some other changes occurring in the process of seed deterioration, such as: breaking up of functional structures, biochemical changes that lead to ATP lower levels, the decrease in sugar content, the lack of ability of ribosomes to separate, degradation and inactivation of enzymes (amylase, dehydrogenase, oxidases, phospholipase), development and induction of hydrolytic enzyme activities, meristem exhaustion, intensification of leachates in seeds, the increase of the free fatty acid content, and reduced respiration.

There are the following three types seed deterioration (Farhadi *et al.*, 2012; Akash *et al.*, 2022):

1. *Field weathering.* Deterioration of seeds exposed to unfavourable air and weather conditions, i.e. to high air relative humidity and excessive temperatures in the period after seed maturation and prior to harvest is

considered to be caused by field weathering. According to Khatun *et al.* (2009) physiological changes can occur if seeds, even physiologically mature seeds, remain on maternal plants under adverse atmospheric conditions. This may result in the formation of hard seeds or off-colour seeds in legumes. Seed quality and viability are affected not only by weather conditions, but also by storage conditions (Jyoti and Malik, 2013).

2. *Harvest and post-harvest deterioration.* Inappropriate methods and procedures applied during harvest and handling of seeds can greatly alter seed quality. Mechanical damages are the most common and significant reasons for seed deterioration after harvest and during storage. Due to poor practices, microflora easily accesses seeds and seeds become susceptible to fungal infections, while storage potential is quite reduced (Akash *et al.*, 2022). If seeds are harvested under the most favourable conditions of seed maturation, and if post-harvest handling of seeds was appropriate, then moisture and temperature are the most significant factors influencing succeeding deterioration. If moisture is lost to the atmosphere, the deterioration rate rises considerably and reaches a maximum when the eRH is approximately 85-90% (Probert *et al.*, 2007).

3. According to Probert and Hay (2000) results of the seed deterioration rate was lowest when the eRH ranged from about 10% to 15%. Therefore, international standards prescribe that seeds for long term storage should be dried at the equilibrium relative humidity of 10–15% (FAO/IPGRI, 1994). Moreover, seed deterioration after harvest affects numerous biological, physiological and biochemical changes (Wang *et al.*, 2012).

4. *Storage deterioration*. Seed capability of being stored for certain time without loss of its quality is a seed trait largely genetically regulated. Storability of seeds is affected by seed quality during storage. It also depends on pre-storage conditions including environmental factors during pre- and post-harvest stages, as well as the seed moisture content and relative humidity and the temperature of storage buildings, the storage period and biotic agents (Kapoor *et al.*, 2011; Khan *et al.*, 2017). The degree of seed deterioration at the moment when seed lots are brought into storage affects storage potential. If the storage ambient is under any stress, such as alternations in temperature and relative humidity, high vigour seed lots in comparison to low vigour lots will endure stresses longer and their quality will deteriorate more slowly. Even if the low temperature of the storage room and the low seed moisture content are maintained, seed traits after storage depend on seed lot vigour (ISTA, 2009). High ambient temperatures and the high seed moisture content intensify deterioration of seeds (McDonald, 1999). These effects on life-span of seeds vary over species, and structural and biochemical composition of seeds (Shaban, 2013).

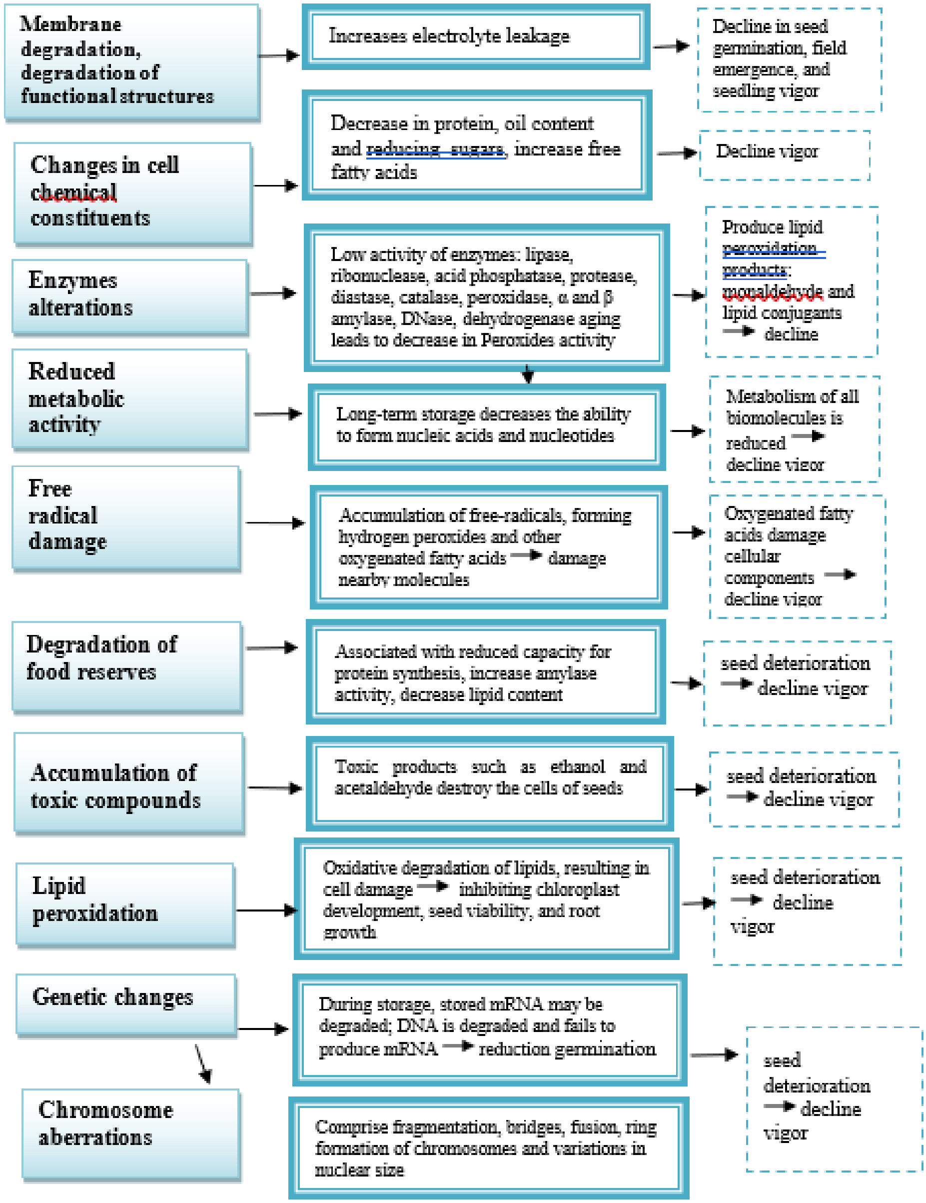

Figure 2. Major physiological and biochemical events of deterioration in seed

Marcos-Filho *et al.* (2015) give a schematic presentation of the relationships between seed germination and vigour in association to the progress of deterioration (Figure 3).

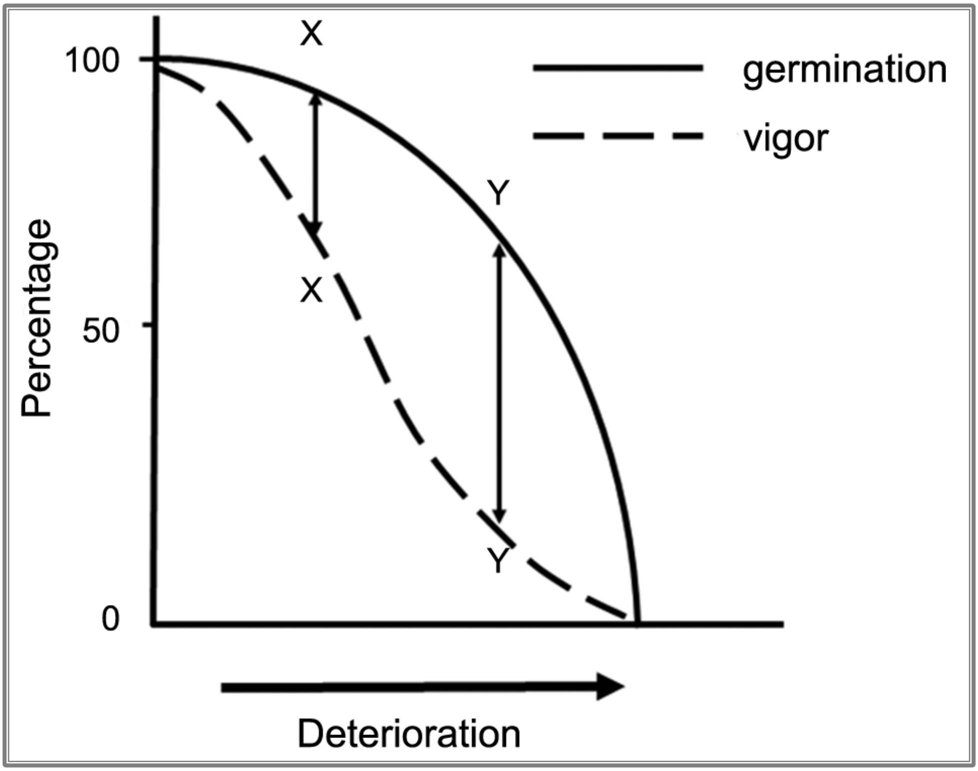

Figure 3. − Relationships between seed germination and vigour in association to the progress of deterioration d (?). The X and Y points on the germination and vigour curves correspond to different seed lots and illustrate the increasing difference between germinability and vigour as deterioration progresses. A high quality seed lot (X) that is less deteriorated will show relatively small difference in results from germination and vigour tests. However, a lower physiological quality seed lot (Y) with higher deterioration level will have higher germination performed under optimum conditions, but extremely low vigour.

Environmental factors affecting seed vigour

The longer seed life-span is the faster vigour is lost and seeds become progressively more susceptible to stress occurring between imbibition and radicle emergence (Bewley *et al.*, 2013).

Drought stress in the process of the seed development may cause serious reduction in seed vigour. Hydrolases (enzymes such α-amylase, β-amylase and α-glucosidase) have a crucial role during seed germination as they break down starch into sugar with water. Drought stress restrains the activity of these enzymes and adversely affects the metabolism of carbohydrates. In many plants, the accumulation of the osmolytes under conditions of drought stress is positively associated with tolerance to water stress. It is considered that these compounds (proline, glycine betaine-GB and soluble sugar) have an adaptive role in interfering with osmotic adjustment and protecting structures within cells in plants under stress (Biju *et al.*, 2017).

Seed vigour is susceptible to the increased level of the carbon dioxide concentration and varies over species (Lamichaney and Maity, 2021). Little work has been done to study impacts of elevated carbon dioxide (e[CO_2]) on seed vigour - the first reports were presented in 2017 (Hampton et al., 2013; Lamichaney and Maity, 2021). Given that e[CO_2] affects physiological performances of plants and the seed development, it is likely that such modifications will change seed quality. With this hypothesis Lamichaney *et al.* (2019) set up the experiment with rice and determined that atmospheric [CO_2] above 610 ppm decreased seed vigour of rice. These authors showed that seeds harvested from elevated [CO_2] up to 610 ppm did not have lower seed vigour. On the other hand, seed harvested at 720 ppm had decreased germination by approximately 10%. An increase in abnormal seedlings and dead seeds were characteristic of seeds physiologically deteriorated and it was attributed to the loss of seed vigour. Furthermore, e[CO_2] at 720 ppm decreased the content of seed nitrogen, substrate availability, and its consequent change in location.

According to Wang *et al.* (2012) stress induced by high temperatures and humidity (HTH) during the development and maturation of soya bean seeds in the field often led to seed deterioration. Seed deterioration occurring in the pre-harvest period has a stronger role than deterioration occurring in post-harvest period of the soya bean seeds. Wei et al. (2020) set the trial with two soya been cultivars Ningzhen No. 1 and Xiangdou No. 3. The seeds of the former cultivar are susceptible to pre-harvest deterioration under the HTH conditions, while the seeds of latter cultivar are resistant to pre-harvest deterioration under the HTH conditions. The authors compared effects of these conditions on the formation of seed vigour during physiological maturity. According to these authors, the more tolerant cultivars to HTH conditions were, the higher seed vigour was. Stress had an effect on seed vigour via adverse effects on signal and metabolic pathways, the ultrastructure of cells as well as physiology and biochemistry in soya bean leaves, pods, cotyledons, and embryos.

The higher moisture is the lower seed vigour is. Many cultivated plants were bred for fast imbibition after sowing to accelerate seed germination. Therefore, these plants are exceptionally susceptible to water stress, because oxygen accessibility is reduced. The activity of enzymes is also limited, which adversely affects carbohydrate metabolism, reduces water potential and soluble calcium and potassium, and changes the hormones of seeds (Abido *et al.*, 2020; Khaeim *et al.*, 2022). The objective of the study carried out by Khaeim *et al.* (2022) was to test maize seed germination and seedling development under effects of different abiotic stresses. The higher water level was the greater seed germination was. Nevertheless, when the optimum water level was achieved, seed germination decreased due to waterloging. Furthermore, water excess limited availability of oxygen for seeds. The temperature was another parameter that considerably impacted seed germination. Khaeim *et al.* (2022) applied temperatures in the range from 5 to 40 ^{o}C and determined that germination was to some extent higher at 20 ^{o}C than at 25 ^{o}C due to changes in enzymes that occurred at the higher temperature. Maize seeds begin to germinate at the average temperatures of about 20-30 ^{o}C. When temperatures exceeded this range, the status of cell energy and the activities of certain enzymes altered, because protein synthesis was thoroughly reduced, and the ATP content was raised extensively.

When seeds do not absorb water there is a lack of metabolic reaction, which furthermore obstructs activities of respiration and enzymes, synthesis of proteins, digestion of reserves, translocation and assimilation, embryo growth, breaking of the seed coat, radicle growth and, subsequently, the seedling formation (Carvalho and Nakagawa, 2012; Cardoso, 2012; Stefanello *et al.*, 2017).

Stefanello *et al.* (2017) set up two experiments to determine impacts of light, temperature and water stress on seed germination and vigour of linseed. The authors determined that the highest seed germination and vigour occurred at a constant temperature of 20 °C, regardless of light treatments. The lower osmotic potential of the medium was the more significant reduction in linseed seed germination and vigour was. Osmotic potentials corresponding to ≤ –0.30 MPa were detrimental to germination and no normal seedling developed starting at –0.50 MPa. Besides, lack of water in the medium can entirely hinders germination.

Seed filling is a critical developmental stage that essentially affects seed vigour, synthesis and accumulation of different components in seeds that develop. Various temperatures, especially temperatures that are elevated during the day, affect grain filling that subsequently results in poor or good grain quality (Abayawickrama *et al.*, 2017; Liu *et al.*, 2017). According to Wang *et al.* (2020), who observed effects of different temperatures during seed filling of hybrid rice seeds, properties of seed filling can affect seed vigour as a result of their effects on starch accumulation and structure. Moreover, these authors determined that contents of starch, amylose and, amylopectin, then relative intensity and the diameter of starch granules differed over various seed filling properties obtained under effects of different temperatures. Liu *et al.* (2017) revealed that the average starch granule size at the increased environmental temperature during the grain filling stage was considerably greater than those at lower temperatures.

Dormancy and seed vigour

Seed dormancy or seed inactivity represents the impossibility of maximum seed germination in a certain period of time immediately after harvest. This phenomenon usually occurs in wild species and not often in cultivated plants (Yogeesha *et al.*, 2006). Seed dormancy is a complex trait encompassing dormancy components that are divided into physiological (dormancy of embryo, endosperm and seed coat), morphological, morphophysiological, physical and combinational (physical + physiological) dormancy (Finch-Savage and Leubner-Matzger, 2006).

Multiple authors report a key link between the content of ABA (abscisic acid) and seed dormancy. In the study carried out by Reed *et al.* (2022), effects of ABA on seed dormancy were well described. Throughout the development of seeds, biosynthesis of abscisic acid (ABA) in seeds is affected by the genotype and the environment, stimulating various depths of primary dormancy. Light, temperature, after-maturing, or chilling mitigate dormancy in these seeds Lower amounts of ABA can be accumulated in seeds in dependence on various genotypes or environmental conditions during the period of seed filling, which can result in non-dormant seeds and there is no need to break primary dormancy. Non-dormant seeds, under conditions when their needs for water and temperature for germination are fulfilled, change a lower gibberellin (GA) to ABA ratio to a higher one, thus advancing germination. Secondary dormancy can be induced in seeds in which primary dormancy is eliminated or in non-dormant seeds with relative dormancy under extreme conditions of temperature, lack of oxygen, light or ageing stress, in the prolonged period of time. Alleviation of secondary dormancy and relative dormancy is possible with time under suitable conditions of light, temperature, after-maturing, and/or chilling (Reed *et al.*, 2022).

Seed dormancy of grasses is an undesirable trait when meadows and pastures are established and often negatively affects the establishment of high-quality pastures. On the other hand, seed dormancy under natural environmental conditions can be a positive trait, if germination of seeds is delayed and seedlings are developed under favourable agroecological conditions (Stanisavljević *et al.*, 2010). For instance, not fully matured embryo, while the cause of red clover seed dormancy is in most cases the seed coat which is hard and impermeable to water and gasses. A high percentage of hard seeds causes reduced germination and the initial growth of seedlings, which affects the competitiveness of other component (grass/legume) in the mixuture (Kimura and Islam, 2012).

CONCLUSIONS

This study encompasses the analyses of key aspects related to seed vigour, which is a vital factor in the process of plant germination and growth under different environmental conditions. Issues related to factors that affect vigour stability and decline over time were also observed. Genetic, physiological and environmental factors that play a key role in seed vigor maintenance or disruption were analyzed. A special emphasis was placed on factors of the environment, such as temperature, moisture/humidity and light, which could significantly affect seed vigour and its ability to germinate and grow. Furthermore, the important state of seed dormancy was highlighted, as it is a state in which the seed cannot germinate even under optimal conditions. This can be a result of complex interactions between genetic and environmental factors. In particular, the temperature plays an important role in activating the seed dormancy process, while moisture and light affect the rate and efficiency of germination. Moreover, genetic variations that directly affect seed vigour were identified. These variations provide new insights into the adaptive mechanisms of plants to different environmental conditions. It was determined that elevated CO_2 concentration (720 ppm) adversely affected seed vigour, which resulted in the increased number of abnormal seedlings and the decrease in the nitrogen content in seeds, and its availability and translocation. These findings point out the importance of seed quality monitoring in order to identify areas susceptible to problems in the process of food production and high-quality seed production. The study provides a comprehensive overview of essential aspects related to seed vigour and points out the importance of an integrative approach to the determination of seed vigour of specified plant species. These findings provide a foundation for further studies and the development of strategies to improve production of food and seeds and agricultural products as a whole.

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[1]Natalia KOVALZHY[1], Serhii RIEZNIK[1], Andrii BUTENKO[2*], Dmytro HAVVA[1], Vasyl DEGTYARJOV[1], Anna HOTVIANSKA[3], Oksana BONDARENKO[3], Nataliia NOZDRINA[3]

ACTIVITY OF CELLULOSE–DEGRADING MICROORGANISMS IN TYPICAL CHERNOZEM UNDER DIFFERENT FERTILIZATION SYSTEMS OF STRAWBERRIES (*FRAGARIA*)

SUMMARY

To determine the effect of fertilization systems on indicators of cellulose–degrading activity of microorganisms of a typical deep heavy loamy chernozem under the bed technology of growing garden strawberries using drip irrigation. Methods. Comparative profile, microbiological, mathematical and statistical. The cellulose–destroying activity of soils was carried out by the method of deep sowing of soil suspension on a dense agarized medium (Hutchinson–Clayton agar). The glucose formed after the decomposition of carboxymethyl cellulose (CMC) was determined by titration with sodium hyposulfite. Also, the activity of cellulose–destroying microorganisms was additionally evaluated by the rate of decomposition of cotton fabric (size 10*10 cm) during six months. The degree of decomposition was calculated based on the difference in weight. Results. Statistical analysis of the results of our research confirmed the significant influence on indicators of the number of cellulose–degrading microorganisms and cellulase activity of such factors as sampling depth and fertilization system, in particular, sampling depth has a more significant effect than fertilization system and vice versa, the fertilization system has a more significant effect on decomposition fabrics The highest activity of cellulase is characteristic of chernozem of the fallow area, and among the strawberry variants, the highest indicators were recorded under the condition of using organic fertilizers. An increase in cellulase activity at a depth of 20–30 cm in arable land as a result of

[1]Natalia Kovalzhy, Serhii Rieznik, Dmytro Havva, Vasyl Degtyarjov, State Biotechnological University, Alchevsky St., 44, UA61000, Kharkiv, UKRAINE
[2]Andrii Butenko*, (Corresponding author: andb201727@ukr.net), Sumy National Agrarian University, 160 H. Kondratieva St., UA40021, Sumy, UKRAINE;
[3]Anna Hotvianska, Oksana Bondarenko, Nataliia Nozdrina, Dnipro State Agrarian and Economic University, 25 Sergey Efremov St., UA49600, Dnipro, UKRAINE.
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plowing plant residues was noted. The highest cellulase activity was recorded in chernozem from the fallow area (6.4–11.2 μg of glucose per 1 g of soil in 7 days) and somewhat lower in strawberries, and comparing fertilization systems, we are talking about the positive effect of organic fertilizers (5.2–6.7 μg of glucose per 1 g of soil for 7 days). Conclusion. The largest number of cellulose–destroying microorganisms was recorded in summer, but enzymatic activity is greater in spring and autumn. Perennial strawberry plantations have higher biological activity compared to arable land, and a comparison of different fertilization systems shows the advantage of organic fertilization. Cultivated soils are characterized by a decrease in the activity of soil enzymes compared to fallow. In the strawberry area, under the organo–mineral system of fertilization, the decomposition of cotton fabric is more intense, even compared to fallow.

Keywords: typical chernozem, fertilization systems, soil microorganisms, enzymatic activity, cellulase.

INTRODUCTION

At the present stage of soil genesis, the most influential factor there is human agricultural activity (Tikhonenko, 2011; Bulyhin *et al.* 2018). Agrogenic soil formation is fundamentally different from natural, especially the rate of transformation of organic and mineral parts of the soil (Demydenko, 2021; Rieznik *et al.* 2023). Activity cellulose–degrading microorganisms in the soil is one of the most important indicators of its overall biological activity (Soares *et al.* 2012; Rieznik *et al.* 2021). The decomposition of cellulose is brought about by complex communities of interacting microorganisms (Mahanta *et al.* 2014; Dimova *et al.* 2023). Products of cellulose hydrolysis are available as carbon and energy sources for other microbes that inhabit environments in soil (Fomenko *et al.* 2021; Long *et al.* 2024; Karbivska *et al.* 2020). Human agricultural activity often leads to the suppression of soil biosphere functions and has a negative impact on the ecosystem (Kanivets, 2015; Volkohon *et al.* 2019; Kolisnyk *et al.* 2024). Therefore, the study of soil biological activity under different farming practices is especially relevant.

MATERIAL AND METHODS

Typical deep, medium–humus, heavy loam chernozems on the loess (molik, mollisol) on the territory of the State Biotechnological University, Kharkiv, Ukraine (Forest–Steppe zone of Ukraine), were chosen as the objects of research. The options for the study were selected plots under a natural phytocenosis (fallow land since 1946), a plot under a traditional system of cultivation (corn, the predecessor of which was sunflower with yield 2.7 t ha^{-1}, arable land for more than 100 years) and options for growing garden strawberries with drip irrigation under different fertilization systems. In the variant of growing corn, 40 t ha^{-1} of cattle manure was applied as the main fertilizer in the fall, followed by plowing to a depth of 22–24 cm. In March, harrowing was carried out, and in May the Leka hybrid corn (a simple interline corn hybrid of

Ukrainians selection) was sown with a sowing rate of 70,000 seeds ha$^{–1}$. Pre–sowing cultivation and sowing were carried out to a depth of 4–6 cm. During sowing, 160 kg ha$^{–1}$ of nitroammophoska ($N_{16}P_{16}K_{16}$) were applied. Top dressing was carried out in the phase of 5–7 leaves with ammonium nitrate (N – 34.4%) in a dose of 80 kg ha$^{–1}$. Fallow and arable land in our studies served as a kind of control in the study of microbiological indicators of the soil. An experiment with strawberries of the Roxana variety was planted in the fall of 2018 on an area of 0.3 ha, the predecessor of which was pure steam, and the average yield is 15–17 t ha$^{–1}$. The plot is divided into 4 options (each option has 4 rows): 1. Without fertilizer. 2. Mineral fertilizer system ($N_{64}P_{64}K_{64}$). 3. Organo–mineral fertilization system ($N_{64}P_{64}K_{64}$ + 50 t ha$^{–1}$ of humus). 4. Organic fertilization system (50 t ha$^{–1}$ of humus). Nitroamophoska ($N_{16}P_{16}K_{16}$) was used as a fertilizer at a dose of 400 kg ha$^{–1}$ and cattle manure – 50 t ha$^{–1}$. Strawberries were planted using a tape method of 25*130 cm with row shift, the distance between plants 25 cm. The formed ridges were covered with a black plastic wrap after laying the drip tape. Watering was carried out as needed to ensure constant soil moisture within 75% of the full soil moisture content.

The growing technology involves mulching the rows with straw and treatment with herbicides based on clopyralid 300 g l$^{–1}$ + fluazifop–p–butyl 150 g l$^{–1}$ at the end of May. Plant feeding is carried out by fertigation with a solution of urea–ammonium nitrate (UAN–32) at the rate of 120 kg ha$^{–1}$ at the end of March. Protection of plants from pests and diseases was carried out in several stages with the following tank mixtures: at the beginning of May, an acaricide based on two active substances, hexityazox 204 g l$^{–1}$ + abamectin 36 g l$^{–1}$, is applied; after two weeks, the insecticide pyridaben, 150 g l$^{–1}$ + pirimifos–methyl, 200 g l$^{–1}$ + acetamiprid, 50 g l$^{–1}$ was applied with the addition of liquid complex fertilizers with trace elements in chelated form; two weeks later, the fungicide cyprodinil 375 g l$^{–1}$ + fludioxonil 250 g l$^{–1}$ was added, also with the addition of liquid complex fertilizers with trace elements in chelated form. All drugs are introduced in accordance with the manufacturer's recommendations.

The selection of soil samples was carried out three times, layer by layer, every 10 cm to a depth of 40 cm, and in strawberry areas – and from the ridge. The research was conducted in 2021, and individual soil samples were taken by season. To count the number of cellulose–destroying microorganisms, the method of deep seeding of a soil–water suspension on a dense agar medium (Hutchinson–Clayton agar) containing carboxymethylcellulose (CMC) was used. Cellulase activity was determined based on the ability of the enzyme to decompose biopolymers (CMC) to glucose, the amount of which is determined iodometrically by back titration with sodium hyposulfite. The activity of cellulose–destroying microorganisms was additionally evaluated by the rate of decomposition of cotton fabric. Pieces of cotton fabric measuring 10*10 cm were buried in the ground for six months, and the degree of decomposition was calculated based on the difference in weight. All specified methods of studying the biological activity of the soil are described by Volkohon *et al.* (2010) and

Shchukovs′kyy *et al.* (2002). Analyzes were performed in triplicate. Analyzes were performed in triplicate.

Mathematical analysis of the data was performed with Microsoft Excel 2010 and Statgraphics 18.1 trial. Multifactor ANOVA and Correlation analysis were used.

RESULTS AND DISCUSSION

Our previous studies indicate a significant increase in biological activity and acceleration of the processes of mineralization of organic substances under the organo–mineral fertilization system (Kovalzhy, 2021; Karbivska *et al.* 2022). It was also confirmed that the improvement of physical and chemical indicators under the organic and organo–mineral fertilization system (Dehtiar'ov *et al.* 2021). In this article, we decided to highlight in more detail the annual dynamics of the activity of cellulose–destroying microorganisms (Table 1).

First of all, it should be noted that the number of cellulose–decomposing microorganisms is greatest in the upper part of the soil profile at a depth of up to 10 centimeters. The exception is the option of arable land, where the soil is turned over annually during plowing. Therefore, the number of bacteria and their activity will increase in the spring and autumn at the depth of wrapping plant residues and fertilizers (10–20 cm). In other versions, the number of cellulose–destroying microorganisms is regulated by hydrothermal indicators and the amount of plant residues that enter the soil. Therefore, in variants with strawberry, an unnatural situation is also created with the formation of a ridge mulched with a non–transparent plastic wrap. Under the plastic wrap, optimal conditions are created for the rapid decomposition of cellulose, and the supply of fresh plant residues is limited. Therefore, the largest number of cellulose–destroying microorganisms in the soil was recorded in the fallow variant (45.3 CFU*106 per 1 g dry soil), and in the experiment with strawberry – under the condition of applying organic fertilizers (6.0 CFU*106 per 1 g dry soil). This is consistent with the results of the study obtained by a team of Indian scientists Sangma *et al.* (2018).

Enzymatic activity of chernozem gradually decreases with depth and has similar dynamics with the number of cellulose–destroying microorganisms. In strawberry variants, a significant decrease into enzymatic activity into the ridge was recorded due to rapid mineralization of plant residues and organic fertilizers (Table 2). We also note the increase in cellulase activity at a depth of 20–30 cm in arable land as a result of plowing plant residues. The seasonal dynamics of enzymatic activity is slightly different from the dynamics of the number of microorganisms, and the correlation analysis showed a weak relationship between these indicators r=23. An increase in enzymatic activity is observed in spring and autumn, which indicates the additional influence of other factors such as: humidity, the presence of a substrate and the content of humus in the soil. In general, it was found that the highest activity of cellulase is characteristic of chernozem of the fallow area, and among strawberry variants, the highest indicators were recorded under the condition of using organic fertilizers. At that time, it is worth noting that the enzymatic activity of the typical chernozem we studied is significantly lower than in the chernozems of the forest–steppe zone of Ukraine, but the seasonal dynamics and general trends are similar (Rieznik *et al.* 2021; Radchenko *et al.* 2022; Radchenko *et al.* 2024). In particular, the highest activity of cellulase was recorded in chernozem in the fallow area (6.4–11.2 µg of glucose per 1 g of soil for 7 days) and somewhat lower in strawberries, and comparing fertilization systems we are talking about the positive effect of organic fertilizers (5.2–6.7 µg of glucose per 1 g of soil for 7 days).

Table 1. Dynamics of the number of cellulose–destroying microorganisms

Variants	Depth, cm	Hutchinson–Clayton agar, CFU*10^6 per 1 g dry soil		
		spring	summer	autumn
Fallow	0–10	3.98	45.32	1.32
	10–20	3.07	31.61	0.67
	20–30	0.61	4.55	0.28
	30–40	0.38	3.13	0.12
Corn for grain	0–10	3.14	9.37	0.63
	10–20	4.34	3.55	1.56
	20–30	0.73	1.10	0.43
	30–40	0.50	0.58	0.28
Strawberries (without fertilizers)	ridge	3.12	2.17	0.82
	0–10	4.27	8.82	1.10
	10–20	3.06	4.02	0.65
	20–30	0.47	1.92	0.23
	30–40	0.32	0.21	0.14
Strawberries (organic fertilization system)	ridge	3.12	5.97	0.70
	0–10	4.91	28.31	2.66
	10–20	3.10	6.77	1.48
	20–30	0.56	0.96	0.28
	30–40	0.33	0.81	0.20
Strawberries (organo–mineral fertilization system)	ridge	3.80	6.01	2.55
	0–10	4.83	18.09	1.57
	10–20	2.76	8.28	0.77
	20–30	0.48	0.89	0.20
	30–40	0.31	0.74	0.15
Strawberries (mineral fertilization system)	ridge	3.49	4.40	1.00
	0–10	3.87	15.33	1.35
	10–20	2.41	6.66	0.74
	20–30	0.48	2.07	0.19
	30–40	0.28	1.38	0.15
Fertilizer system	S.E.		1.05	
	$LSD_{0.5}$		2.73	
Soil layer	S.E.		0.83	
	$LSD_{0.5}$		2,32	

We observed a similar trend in the decomposition of cotton fabric, but the lack of correlation with other indicators indicates that the fabric and plant remains are decomposed by enzyme systems of a complex of microorganisms (Dimova *et al.* 2021). And according to data Datsko & Zakharchenko (2023) the intensity of tissue decomposition is significantly influenced by hydrothermal parameters, tillage, fertilization system and other parameters. In particular, according to Zinaida Dehtiarovas (2022) data, favorable conditions for increasing cellulolytic activity at a depth of 20–30 cm are often found in fields. Similar data on the decomposition of cotton fabric were obtained in our research also.

Table 2. Dynamics of the activity of cellulose–destroying microorganisms

Variants	Depth, cm	Cellulase, µg of glucose per 1 g of soil for 7 days			The degree of decomposition of cotton fabric, %
		spring	summer	autumn	
Fallow	0–10	11.2	6.4	9.9	28.9
	10–20	8.9	7.7	6.4	23.8
	20–30	6.7	5.4	5.2	23.6
	30–40	7.0	1.5	3.6	18.1
Corn for grain	0–10	3.6	4.9	7.7	16.9
	10–20	4.6	4.6	5.4	10.5
	20–30	5.2	2.4	4.0	13.2
	30–40	3.1	1.7	3.1	9.5
Strawberries (without fertilizers)	ridge	2.0	2.8	3.3	23.2
	0–10	8.6	3.6	5.2	34.2
	10–20	4.6	3.3	3.0	29.3
	20–30	2.7	2.7	2.6	26.2
	30–40	2.4	2.4	2.1	19.5
Strawberries (organic fertilization system)	ridge	2.7	4.2	2.6	19.0
	0–10	6.7	5.2	6.7	25.9
	10–20	4.8	4.6	5.8	24.5
	20–30	4.2	3.3	3.0	25.2
	30–40	2.0	2.0	2.6	19.8
Strawberries (organo–mineral fertilization system)	ridge	3.0	3.0	2.7	32.6
	0–10	6.4	4.8	6.8	35.7
	10–20	5.4	2.5	5.6	33.8
	20–30	4.2	2.1	3.7	30.1
	30–40	4.0	2.0	1.1	25.9
Strawberries (mineral fertilization system)	ridge	2.0	1.6	2.7	24.1
	0–10	6.7	3.6	5.8	28.6
	10–20	5.8	3.0	4.0	27.7
	20–30	3.3	2.4	3.3	26.9
	30–40	2.7	1.0	2.4	17.7
Fertilizer system	S.E.		0.22		0.84
	$LSD_{0.5}$		0.53		2.21
Soil layer	S.E.		0.18		0.67
	$LSD_{0.5}$		0.49		1.88

The factor analysis confirmed the significant influence on indicators of soil biological activity of such factors as sampling depth and fertilization system (Table 3). But the statistical analysis of the results of our research indicates a more significant influence of the sampling depth than the fertilization system on the indicators of the number of cellulose–degrading microorganisms and cellulase activity. At the same time, let's note that on the contrary, the fertilizer system has a more significant effect on the decomposition of the fabric.

Table 3. A two–way ANOVA for the effects of fertilizer system and soil layers on the number of cellulose–destroying microorganisms and their activity

Influence factor		Cellulose–destroying microorganisms	Cellulase	The degree of decomposition of cotton fabric
Fertilizer system	F–Ratio	4.60	28.74	65.25
	P–Value	0,0005	0,0000	0,0000
Soil layer	F–Ratio	16.21	69.84	30.11
	P–Value	0,0000	0,0000	0,0000

CONCLUSIONS

Mathematical analysis data indicates significant changes in biological processes under the influence of human agricultural activity and allows to distinguish "agrochernozem" from natural analogues.

Agrogenic soils are characterized by a decrease in the number of cellulose–destroying microorganisms. Cultivated soils are characterized by decrease in activity of soil enzymes in comparison with a fallow. Perennial strawberry plantations have higher biological activity compared to arable land, and a comparison of different fertilization systems shows the advantage of organic fertilization. The highest number of cellulose–destroying microorganisms was recorded in summer, but the enzymatic activity is greater in spring and autumn.

On the strawberry site, under the organo–mineral fertilization system, cotton fabric decomposition is more intense, even compared to fallow.

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Blažo LALEVIĆ[1*], Teofil GAVRIĆ[2], Amina STAMBOLIĆ[2], Amer SUNULAHPAŠIĆ[3], Vedrana KOMLEN[4], Lejla ČENGIĆ[2], Saud HAMIDOVIĆ[2]

ANTIFUNGAL POTENTIAL OF PEPPERMINT, BASIL AND SAGE ESSENTIAL OILS

SUMMARY

Synthetic fungicides are the most effective protection against plant pathogens, but, their uncontrolled and long-term use can lead to many harmful effects: environmental degradation, human health problems and pathogen resistance. The biological compounds contained in essential oils have no harmful effects on humans or the environment and can therefore be an alternative to synthetic fungicides. Essential oils are products of plant metabolism and often show antifungal, antiviral, antibacterial and insecticidal effects. The aim of this study was to investigate the antimicrobial potential of essential oils from peppermint, basil and sage essential oils obtained by hydrodistillation, on *Fusarium* sp. and *Aspergillus* sp. The experiment was carried out on potato dextrose agar. After inoculation of the agar with fungal mycelia, paper discs impregnated with 10 µl of oils were placed on the agar surface. In the control, the impregnation was carried out with distilled water. The inhibition zones were measured after 3, 6 and 9 days. The results showed that peppermint oil had the highest antimicrobial potential compared with other oils. Sage essential oil showed the lowest antifungal suppression. A negligible zone of inhibition was observed in the control. A statistically significant influence betwwen the oils and the incubation period was found in this study. Our results confirm the potential use of peppermint essential oil for the suppression of *Fusarium* sp. and *Aspergillus* sp. growth.

Keywords: antifungal potential, *Aspergillus*, *Fusarium*, inhibition zone, plant essential oils.

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[1] Blažo Lalević[*] (corresponding author email: blazol@agrif.bg.ac.rs), University of Belgrade, Faculty of Agriculture, Nemanjina 6, Belgrade-Zemun, SERBIA
[2] Teofil Gavrić, Amina Stambolić, Lejla Čengić, Saud Hamidović, University of Sarajevo, Faculty of agriculture and food science, Zmaja od Bosne 8, Sarajevo, BOSNIA AND HERZEGOVINA;
[3] Amer Sunulahpašić, Ministry of agriculture, water management and forestry, Travnik, BOSNIA AND HERZEGOVINA
[4] Vedrana Komlen, The University "Džemal Bijedić", Agromediterranean Faculty, University Campus, Mostar, BOSNIA AND HERZEGOVINA
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INTRODUCTION

Agricultural plant production is often associated with phytopathogen infestation (Köhl *et al*., 2019) and yield losses (Ampt *et al*., 2019). Chatterjee *et al*. (2016) found that the occurrence of plant disease leads to an annual loss of 10-15 percent of major crops worldwide. Peng *et al*. (2021) point out that more than two-thirds of plant diseases are associated with the occurrence of pathogenic fungi. Therefore, plant diseases need to be controlled using different techniques (Tsegaye *et al*., 2018), which requires redesigning agroecosystems to improve plant resistance to pathogens (McDonald and Stukenbrock, 2016). Pesticides show high efficiency in suppressing the occurence of *Fusarium* (Arie, 2019) and *Aspergillus* (Geetha *et al*., 2016); species of these fungal genera are frequently detected on various plant materials (Kalman *et al*., 2020; Perrone *et al*., 2007). However, Aktar *et al*. (2009) demonstrated that the use of pesticides is associated with higher plant productivity, avoidance of yield losses and control of disease vectors. On the other hand, the extensive use of pesticides led to significant degradation of the agroecosystem and human health (Salameh *et al*., 2006; Zheng *et al*., 2016). Therefore, several alternative methods to replace pesticides have been proposed (Nile *et al*., 2019).

The pesticidal properties of many plant species and their products are well-known and may represent formulations with potential applications against pathogens (Stevenson *et al*., 2017). Nxumalo *et al*. (2021) described the possibility of medicinal plant extracts as an alternative technology for synthetic chemicals. Several *in vitro* studies have been conducted to determine the potential of various plant extracts in suppressing growth of fungal genera such as *Candida*, *Aspergillus*, *Penicillium*, etc. (Azaz *et al*., 2004; Chuang *et al*., 2007; Hammer *et al*., 1999; Omran and Esmailzadeh, 2009).

The objective of this paper is to evaluate the antifungal potential of the essential oils of peppermint, basil and sage against *Fusarium* sp. and *Aspergillus* sp. in *in vitro* experiment.

MATERIAL AND METHODS

Fusarium sp. and *Aspergillus* sp. used in this research belong to the collection of microorganisms Department of Microbiology at the Faculty of agriculture and food science in Sarajevo (Bosnia and Herzegovina). These fungal isolates were previously cultivated and stored at 4 °C on the nutrient medium potato dextrose agar (PDA).

The shoots of peppermint, basil and sage were used for the extraction of plant material. Extracts were obtained through hydrodistillation (30 g of plant material and 400 ml of distilled water) using Neo Clevenger-type apparatus. The antifungal properties of plant extracts were determined using a disc diffusion assay. Potato dextrose agar (Himedia, India), previously sterilized at 120 °C for 20 min, was inoculated with pure cultures of *Fusarium* sp. and *Aspergillus* sp. Fungal mycelia were placed at four places on the agar surface, about 5 mm from the edge of the Petri dishes. In the center of the agar plate, one sterile filter paper disc (about

5 mm in diameter) containing 10 µl of the plant extract was placed. In control, distilled water was used for the impregnation of the disc. All experiments were performed in triplicate. Incubation of Petri dishes was performed at 22 °C for 9 days. All experiments were performed in 4 replications. The zone of inhibition (expressed in centimeters) was measured on the 3, 6 and 9 day of incubation.

For the determination of the statistical significance of obtained results, SPSS software (version 22, SPSS Inc., Chicago, IL) was used. P<0.05 was chosen as a parameter of statistical significance between the treatments.

RESULTS AND DISCUSSION

Our results showed that all tested essential oils had an inhibitory effect on the growth of *Fusarium* sp. (Table 1). The degree of inhibition depended on the essential oil and the incubation period.

All tested oils showed significantly higher values of the inhibition zone compared to the control (Table 1). After three, six and nine days of incubation, peppermint essential oil showed the statistically highest effect. The diameter of the inhibition zone with peppermint essential oil was 1.58, 0.68 and 0.23 cm, respectively. Sage essential oil showed a larger diameter of the inhibition zone after three days, while after six and nine days, the effect of basil essential oil was more pronounced compared with sage. In all treatments, the diameter of the inhibition zone statistic decreased significantly with increasing of incubation time for peppermint and sage essential oils. In the control, no zone of inhibition was observed after 6 and 9 days.

Table 1. Diameter of inhibition zone (cm) of *Fusarium* sp.

D	Essential oils											
	peppermint			basil			sage			control		
	Time of incubation (days)											
	3	6	9	3	6	9	3	6	9	3	6	9
1	1.6	0.7	0.2	0.8	0.4	0.2	0.8	0.3	0.0	0.3	0.0	0.0
2	1.5	0.6	0.2	0.6	0.2	0.0	0.8	0.3	0.0	0.3	0.0	0.0
3	1.3	0.5	0.2	0.5	0.2	0.0	0.8	0.3	0.1	0.2	0.0	0.0
4	1.9	0.9	0.3	0.8	0.3	0.1	0.7	0.2	0.0	0.2	0.0	0.0
A	1.58^{a}	0.68^{a}	0.23^{c}	0.68^{a}	0.28^{c}	0.75^{c}	0.78^{a}	0.28^{b}	0.03^{c}	0.25^{a}	0.0^{b}	0.0^{b}
T	0.83^{a}			0.41^{b}			0.36^{c}			0.08^{d}		

Legend: D – discs; A – average; T – total average

Legend: D – discs; A – average; T – total average

The essential oils of peppermint, basil and sage had an inhibitory effect on the growth of *Aspergillus* sp. (Table 2). Significantly lower values for the diameter of the inhibition zone diameter were observed for all essential oils during incubation. As in the experiment with *Fusarium* mycelia, peppermint essential oil showed the highest zone of inhibition compared with other treatments (0.63, 0.35 and 0.15 cm after 3, 6, and 9 days of incubation, respectively). Basil essential oil showed a stronger effect than sage essential oil, especially after three and six days. At the end of the incubation period, the same values were found for the diameter of inhibition zone. In the control, a negligible zone of inhibition was observed after three days; further incubation revealed no zone of inhibition.

Table 2. Diameter of inhibition zone (cm) of *Aspergillus* sp.

D	Essential oils									Control		
	peppermint			basil			sage					
	Time of incubation (days)											
	3	6	9	3	6	9	3	6	9	3	6	9
1	0.7	0.4	0.2	0.6	0.3	0.1	0.4	0.2	0.1	0.1	0.0	0.0
2	0.7	0.4	0.2	0.5	0.2	0.0	0.3	0.1	0.0	0.0	0.0	0.0
3	0.5	0.3	0.1	0.4	0.2	0.0	0.4	0.2	0.0	0.0	0.0	0.0
4	0.6	0.3	0.1	0.5	0.2	0.0	0.2	0.1	0.0	0.0	0.0	0.0
A	0.63[a]	0.35[b]	0.15[c]	0.5[a]	0.23[b]	0.03[c]	0.33[a]	0.15[b]	0.03[c]	0.03[a]	0.0[b]	0.0[b]
T	0.38[a]			0.25[b]			0.17[c]			0.01[d]		

Legend: D – discs; A – average; T – total average

Essential oils from medicinal and aromatic plants have antimicrobial properties and can be used to combat plant pathogens. Essential oils from the *Lamiaceae* family are known as antifungal agents (Santra and Banarjee, 2020). Plants from the *Lamiaceae* family are distributed worldwide and represent a cost-effective source for the extraction of essential oils that can be used in agriculture (Feng *et al*., 2011; Mamgain *et al*., 2013). Essential oils from *Lamiaceae* are often used to control fungal pathogens in crop production, such as *Fusarium*, or fungi responsible for food spoilage, such as *Aspergillus* (Couladis *et al*., 2004; Soliman and Badeaa, 2002).

Peppermint essential oil was recommended as the best inhibitor of *Fusarium* growth compared with other natural products (Kumar *et al*., 2016). Helal *et al*. (2006) found that the application of peppermint essential oil was effective against 11 fungi, including *Aspergillus* species. Our results are consistent with the report of Guynot *et al*. (2003) who found that peppermint essential oil inhibited the growth of *Aspergillus flavus* and *A. niger*.

Kocić-Tanaskov *et al*. (2011) reported the inhibition of *Fusarium* species growth using different concentrations of basil essential oil. The highest inhibitory effect of basil oil against *Fusarium* growth was described by Hashem *et al*. (2010). Our results are in agreement with those of Pandey and Dubey (1994), who described moderate to poor antifungal activity of basil essential oil.

Our results showed that the lowest inhibition zone of fungal growth was achieved with sage essential oil. Ferdes *et al*. (2017) described the low effect of sage essential oil on the growth of *A. niger* and *Fusarium oxysporum*. Sage essential oil showed no significant suppression of the growth of *Aspergillus flavus* (Foltinova *et al*., 2017). However, the fungotoxic effect of sage essential oil was observed at higher concentrations of this product (Daferera *et al*., 2003).

CONCLUSION

This study confirms that the presented essential oils can be used to control the growth of *Fusarium* and *Aspergillus* mycelia. The highest inhibition zone was observed with the use of peppermint and the lowest with the use of sage essential oil. Further studies will focus on the inhibition of other fungal species and a detailed content analysis of these natural plant products.

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Amina ŠERBO[1], Jasna AVDIĆ[1], Fatima PUSTAHIJA[2], Mirela SMAJIĆ MURTIĆ[1], Senad MURTIĆ[1*]

PHYSIOLOGICAL RESPONSE OF BASIL PLANTS (*OCIMUM BASILICUM* L.) TO DROUGHT

SUMMARY

Basil (*Ocimum basilicum* L.) is one of the most widely cultivated medicinal and aromatic species and the understanding of its physiological behavior under drought is of great importance for both basil producers and plant scientists. The aim of this study was to evaluate the physiological response of basil plants to different drought levels. This study found that drought significantly increased the activities of superoxide dismutase, guaiacol peroxidase, pyrogallol peroxidase and catalase, indicating that these enzymes play a prominent role in detoxification of reactive oxygen species and thus in plant drought tolerance. Exposure of basil plants to moderate and severe drought resulted in higher accumulation of proline and total phenolics and flavonoids, suggesting that basil plants under prolonged drought conditions tend to improve their drought tolerance by inducing the production of these compounds in leaves. Interestingly, mild drought did not negatively affect the photosynthetic pigment content as well as proline and total phenolic and flavonoid content in basil leaves as compared with control plants. In light of this fact, as well as the fact that the basil plants have the ability to produce protective macromolecules and antioxidants in high amounts in severe drought conditions, it can be concluded that basil plants tolerate drought conditions well.

Keywords: antioxidants, defense mechanisms, leaves, osmoregulation, water shortage

INTRODUCTION

Plants, as sessile organisms, are unable to escape unfavorable conditions in their environment; however, they have the ability to adapt to environmental change by regulating their own growth and development (Knudsen *et al.*, 2018).

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[1]Amina Šerbo, Jasna Avdić, Mirela Smajić Murtić, Senad Murtić (corresponding author: murticsenad@hotmail.com), University of Sarajevo, Faculty of Agriculture and Food Science, Zmaja od Bosne 8, Sarajevo, BOSNIA AND HERZEGOVINA

[2] Fatima Pustahija, University of Sarajevo, Faculty of Forestry, Zagrebačka 20, Sarajevo, BOSNIA AND HERZEGOVINA

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Over the course of the evolution plants have developed a very sophisticated mechanisms to sense and respond to environmental variations including drought caused either by water shortage or by the presence of excess salt in the soil (Lamers *et al.*, 2020). Regardless of the cause of drought, the fact is drought causes various biochemical and physiological changes in plants, which adversely affect crop yield and consequently agricultural production even in areas that are relatively well supplied with water resources. This problem has sharply increased over the past decades as a result of global warming (Gao *et al.*, 2018; Seleiman *et al.*, 2021).

Drought triggers a wide array of responses in plants ranging from molecular, cellular to whole-plant levels. The responses of plants to drought at the whole-plant level include, among others, a reduction in leaf area, stomatal closure, leaf folding and leaf abscission, all in order to reduce water loss (Wongla *et al.*, 2023). Under drought conditions, plants also transport more nutrients to the roots, improving their expansion and thus the ability to capture water from deeper soil layers. At the cellular level, plants tend to increase the production of osmolytes such as sugar alcohols and proline amino acid with a view to maintain homeostasis under drought conditions (Mahmood *et al.*, 2019). Plants also activate both enzymatic and non-enzymatic antioxidative defense system to scavenge reactive oxygen species (ROS) whose production in the plant cells is elevated under drought (Fetsiukh *et al.*, 2022). Enzymatic antioxidative defense system consists of numerous antioxidant enzymes such as superoxide dismutase, guaiacol peroxidase, pyrogallol peroxidase and catalase, which play a key role in ROS degradation (Rajput *et al.*, 2021). Non-enzymatic antioxidative defense system is comprised of different compounds with antioxidant properties such as ascorbic acid, carotenoids, glutathione, and phenolic compounds. Some of these compounds act directly in the ROS detoxification, while some act indirectly as cofactors for antioxidant enzymes (Chaudhary *et al.*, 2023).

The responses of plants to drought vary greatly among plant species which depend mainly on plant genotype, drought severity and duration as well as the interaction between plants and environment in which they grow (Farjam *et al.*, 2014). The plant response to drought is also highly influenced by the developmental stage of the plant (Bandurska, 2022). In this light, an understanding the physiological response of plants to water deficit is of a crucial importance for predicting plant behavior under drought. It is also a key determinant for improving crop productivity in a changing climate (Fahad *et al.*, 2017).

Basil (*Ocimum basilicum* L.) is one of the most widely cultivated medicinal and aromatic species in the world, with its origins traced back to India. It has been used as a spice throughout the world for centuries, and recently, there has been an increasing focus on its medicinal properties (Romano *et al.*, 2022).

Like a number of other plant species, basil is often exposed to adverse environmental conditions, including primarily the soil water deficiency. Therefore, the aim of this study was to evaluate the physiological response of basil plants to different levels of drought stress. We hypothesized that drought-affected basil plants would exhibit increases in physiological performance measures such as proline content, total phenolic and flavonoid content, total antioxidant capacity and antioxidant enzymatic activities in leaves. We also hypothesized that drought-affected basil plants would exhibit declines in photosynthetic pigment content in leaves as compared to non-stressed basil plants.

MATERIAL AND METHODS

Experimental design

The experiment was conducted from the end-August 2023 to mid-September 2023 in a greenhouse at the experimental station of the Faculty of Agriculture and Food Science in Sarajevo (43°49′34.41″ N and 18°19′18.47″ E). Over the course of the experiment, air temperature in greenhouse was maintained at 25 ± 4 °C during the day and 18 ± 4 °C during the night. White shade cloth was used to reduce light and heat intensity during hot days.

Basil plants used in the experiment were produced in the nursery near the greenhouse and there was no observed significant difference between basil plants in terms of vigor, size and appearance. Before setting up an experiment, the basil plants were in pre-flowering stage (Figure 1).

Figure 1. Basil plants used in the experiment

For the drought treatment experiment, basil plants were grown in pots (8 cm diameter × 12 cm height, one plant per plot) previously filled with substrate Florabella (Klasmann-Deilmann, Germany).

Four drought stress levels were applied in the experiment and each level included twelve individuals: (1) non-stress (70-80% of the maximum substrate water-holding capacity was designated as the control (non-stress) treatment), (2) mild drought stress (three days without water), (3) moderate drought stress (six days without water), and (4) severe drought stress (nine days without water). After drought treatments, basil plants were regularly watered until the end of experiment (18th day of experiment).

The leaves of basil plants were collected twice over the course of the experiment: on the 9th day of experiment and on the 18th day of experiment (after a 9-day water recovery period). During the 9th day of the experiment, fresh leaves of basil plants were sampled to study the following physiological traits: proline content, photosynthetic pigment content including chlorophyll *a*, chlorophyll *b* and total carotenoids, and the activities of following antioxidant enzymes; superoxide dismutase, guaiacol peroxidase, pyrogallol peroxidase and catalase. For this purpose, three fully developed and healthy leaves from each basil plant were collected and immediately frozen in liquid nitrogen and kept at -80 °C until analysis.

After sampling, basil plants in all drought treatments were re-watered to 70-80% substrate water-holding capacity for the next nine days to assess the impact of drought treatments on the phenolic and flavonoid biosynthesis in plants. At the end of the drought/re-watering experiments (18th day of experiment), five fully developed leaves from each basil plant leaves were cut, dried in oven at 40 °C for 48 h, ground into powder with an electric blender, and then placed in paper bags until analysis.

Chemicals

Potassium dihydrogen phosphate (KH_2PO_4), dipotassium phosphate (K_2HPO_4), bovine serum albumin, Bradford reagent, dithiothreitol (DTT), polyvinylpyrrolidone-40 (PVP-40), guaiacol, pyrogallol, hydrogen peroxide, cytochrome C oxidase, ethylenediaminetetraacetic acid (EDTA), xanthine and xanthine oxidase and were obtained from Sigma-Aldrich (St. Louis, MO, USA). The other chemicals used throughout the analysis, i.e. ninhydrin reagent, proline amino acid, glacial acetic acid, acetone, ethanol, Folin-Ciocalteu reagent, sodium carbonate, gallic acid, aluminum chloride, sodium hydroxide, catechin, iron (III) chloride, 2,4,6-tripyridyl-s-triazine and iron (II) sulfate heptahydrate were obtained from Merck (Darmstadt, Germany).

Protein extraction and determination

0.5 g fresh leaves of basil plants were frozen in liquid nitrogen and then ground into a fine powder using a pestle and mortar. In order to separate the soluble proteins from the homogenized powder, 1.5 mL of a 50 mM potassium phosphate buffer (pH 7.0) containing 1 mM EDTA, 1 mM DTT and 0.05% PVP-40 was added. The homogenate was centrifuged at 10,000 × g for 10 min at 4 °C to obtain a clear supernatant. The resulting supernatant fraction was then transferred to a new tube for protein concentration and antioxidant enzyme activity measurements. Protein concentration was measured using the Bradford method with bovine serum albumin serving as the reference standard (Bradford, 1976).

Superoxide dismutase activity assay

Superoxide dismutase (SOD) activity was determined spectrophotometrically using the method of McCord and Fridovich (1969). This method was based on the SOD ability to inhibit reduction of cytochrome C by superoxide anions generated from xanthine-xanthine oxidase system. The assay reaction mixture (850 - 1.000 µl) consisted of 50 mM phosphate buffer (pH 7.8), 0.1 mM EDTA, 50 µM xanthine, 10 µM cytochrome C, and xanthine oxidase in amounts sufficient to cause an increase in absorbance of 0.025 min^{-1} at 550 nm. The protein extract (10 - 50 µl) were added to the reaction mixture and the rate of reduction of cytochrome c was followed spectrophotometrically at 550 nm. The SOD activity was expressed in units of SOD activity (U) per mg of protein. One unit of SOD activity was defined as the amount of SOD needed to inhibit 50% of the reduction rate of cytochrome C.

Catalase activity assay

Catalase (CAT) activity was determined spectrophotometrically using the method of Aebi (1984). The reaction medium was prepared by mixing 950 µl of the reaction mixture (10 mM H_2O_2 in 50 mM potassium phosphate buffer pH 7.0) and 50 µl of protein extract. CAT activity was assayed by observing the rate of decrease in absorbance at 240 nm at an interval of 10 sec up to 120 sec, as a consequence of H_2O_2 decomposition. The results were expressed as micromoles decomposed H_2O_2 per min per mg of protein.

Guaiacol peroxidase activity assay

Guaiacol peroxidase (GPOD) activity was determined spectrophotometrically using the method of Chance and Maehly (1954). 900 µl of the reaction mixture (50 mM potassium phosphate buffer pH 7.0 supplemented with 18 mM guaiacol and 5 mM H_2O_2) and 100 µl of protein extract were mixed to obtain reaction medium. GPOD activity was assayed by observing the rate of increase in absorbance at 470 nm at an interval of 15 sec up to 180 sec, as a result of guaiacol oxidation. The results were expressed as micromoles tetraguaiacol (product of guaiacol oxidation) per min per mg of protein.

Pyrogallol peroxidase activity assay

Pyrogallol peroxidase (PPOD) activity was determined spectrophotometrically using the method of Maehly and Chance (1954). The reaction medium was prepared by mixing 950 µl of the reaction mixture (50 mM potassium phosphate buffer pH 7.0 amended with 20 mM pyrogallol and 1 mM H_2O_2) and 50 µl of protein extract. PPOD activity was assayed by observing the rate of increase in absorbance at 430 nm, at an interval of 15 sec up to 180 sec, as a result of pyrogallol oxidation. The results were expressed as micromoles purpurogallin (product of pyrogallol oxidation) per min per mg of protein.

Proline estimation

Proline content was determined spectrophotometrically using the method of Bates et al. (1973). 0.5 g fresh leaves of basil plants leaves were homogenized in 10 mL of 3% aqueous sulfosalicylic acid, and then filtered through a glass microfiber filter to a plastic test tube. 2 mL of filtrate was mixed with 2 mL of freshly prepared ninhydrin reagent (2.5 g of ninhydrin in 40 mL 6 M orthophosphoric acid and 60 mL of glacial acetic acid) and 2 mL of glacial acetic acid in a test tube and then the mixture was incubated for 1 h at 100 °C. After incubation, 4 mL of toluene was added, the mixture was gently mixed with a vortex for 30 sec and then left at room temperature for 15 min. The upper reddish layer of mixture was transferred to cuvette and absorbance was read at 520 nm using toluene as blank. A proline standard curve ranging from 0 to 5 µg mL^{-1} was used to determine the proline levels of each sample, and the obtained values were than recalculated to fresh mass of leaves (µg g^{-1} FM).

Estimation of photosynthetic pigment content

The content of chlorophyll *a*, chlorophyll *b* and total carotenoids was determined spectrophotometrically using the method of Lichtenthaler and Weliburn (1983). 0.2 g of fresh basil leaves was extracted with 10 mL of pure acetone using a pestle and mortar. After that, the extract was filtered through coarse filter paper into a 25 mL volumetric flask and diluted to the mark with extract solution (pure acetone). The filtrates were assayed spectrophotometrically at 662, 645 and 470 nm and the concentrations of chlorophyll *a*, chlorophyll *b* and total carotenoids (mg mL^{-1}) were determined using the following equations:

c (chlorophyll *a*) = 9.784 x A662 - 0.990 x A644

c (chlorophyll b) = 21.426 x A644 - 4.650 x A662

c (total carotenoids) = 4.695 x A440 - 0.268 x (c chlorophyll *a* + c chlorophyll *b*)

The obtained values were than recalculated to fresh mass of leaves (mg g^{-1} FM).

Extraction of phenolic compounds

1 g of ground and dried basil leaves was placed in an Erlenmeyer flask (100 mL) and macerated with 40 mL of 30% ethanol solution for 6 h with frequent shaking and then left to stand at room temperature for 18 h. Thereafter, the extract was filtered through a coarse filter paper into a 25 mL volumetric flask and then diluted to the mark with extract solution. Each extract was tested for total phenolic and flavonoid content and total antioxidant activity.

Total phenolic content estimation

The total phenolic content was determined spectrophotometrically using the Folin-Ciocalteu method (Ough and Amerine, 1988). 0.1 mL of extract, 6 mL of distilled water, and 0.5 mL of Folin-Ciocalteu's reagent (previously diluted 1:2 with distilled water) were addedto a 10 mL flask and then mixed thoroughly for 5 min. After that, 1.5 mL of saturated solution of Na_2CO_3 was added and then the flask was filled to the mark with 30% ethanol solution and left at room temperature for 1 h. Thereafter, the resulting mixtures were assayed spectrophotometrically at 765 nm. The gallic acid standard curve ranging from 0 to 500 mg L^{-1} was used to determine the total phenolic content of each sample, and then the obtained values were recalculated to fresh mass of leaves (mg eq. GA 100 g^{-1} FM).

Total flavonoid content estimation

The total flavonoid content was determined spectrophotometrically using the Aluminium chloride colorimetric assay (Zhishen *et al.*, 1999). 1 mL of extract, 4 mL of distilled water, 0.3 mL 5% $NaNO_2$ and 0.3 mL 10% $AlCl_3$ were placed into a 10 mL flask and mixed thoroughly for 5 min. Then 2 mL of 1 mol L^{-1} NaOH was added and the flask was filled to the mark with distilled water. After 10 min, the resulting mixtures were assayed spectrophotometrically at 510 nm. The catechin standard curve ranging from 0 to 100 mg L^{-1} was used to determine the total flavonoid content of each sample, and then the obtained values were recalculated to fresh mass of leaves (mg eq. C 100 g^{-1} FM).

Total antioxidant capacity estimation

The total antioxidant capacity was determined spectrophotometrically using the ferric reducing antioxidant power (FRAP) assay (Benzie and Strain, 1996). A 10 mL volumetric flask was filled with 200 µL of distilled water, 100 µL of extract, and 2000 µL of FRAP reagent (0.3 mol L^{-1} acetate buffer (pH = 3.6), 10 mmol L^{-1} TPTZ (2,4,6-tripyridyl-s-triazine) and 20 mmol L^{-1} $FeCl_3$ x 6 H_2O in a ratio 10:1:1) and then left at room temperature for 15 min. The resulting mixtures were assayed spectrophotometrically at 595 nm. The $FeSO_4$ x $7H_2O$ standard curve ranging from 0 to 2000 µmol L^{-1} was used to determine the total antioxidant capacity of each sample and then the obtained values were recalculated to fresh mass of leaves (µmol Fe^{2+} 100 g^{-1} FM).

Statistical analysis

All assays were performed in triplicates and the results were expressed as means ± standard deviation. The significant differences between treatments were determined using the one-way analysis of variance (ANOVA) and least significant difference (LSD) test at 5% level of probability.

RESULTS

The study found that proline content significantly increased in basil leaves under both moderate drought and severe drought (Table 1). In this regard, the proline content in basil leaves under moderate (T3) and severe drought (T4) was 0.62 and 3.25 times higher, respectively, than the control group (T1).

Table 1. Effects of drought on proline and leaf photosynthetic pigment contents in basil plants

Drought treatment	Proline (µg g^{-1} FM[2])	Chlorophyll *a* (mg g^{-1} FM)	Chlorophyll *b* (mg g^{-1} FM)	Carotenoids (mg g^{-1} FM)
T1 (control)	30.03 ± 2.01[c]	1.02 ± 0.25[a]	0.72 ± 0.06[ab]	0.50 ± 0.05[b]
T2 (mild)	30.61 ± 1.12[c]	1.00 ± 0.15[a]	0.85 ± 0.34[a]	0.65 ± 0.11[a]
T3 (moderate)	48.11 ± 10.1[b]	0.73 ± 0.09[b]	0.67 ± 0.06[b]	0.52 ± 0.05[b]
T4 (severe)	97.67 ± 14.91[a]	0.56 ± 0.14[c]	0.35 ± 0.13[c]	0.35 ± 0.06[c]
$LSD_{0.05}$[1]	6.91	0.13	0.14	0.05

[1]Averages denoted by the same letter in the same column indicate no significant difference (P < 0.05)

[2]fresh mass

This study also showed that moderate and severe drought negatively affects the chlorophyll *a* content in basil leaves. As compared with chlorophyll *a*, the content of chlorophyll *b* in basil leaves was first raised in response to mild drought stress and then began to decrease with increasing drought duration. Total carotenoid contents in basil leaves have followed a similar pattern as chlorophyll *b* in response to drought (Table 1).

Antioxidant enzyme changes in response to drought stress in basil leaves were determined by observing the activities of following antioxidative enzymes; SOD, PPOD, GPOD and CAT. SOD activity in leaves of basil plants exposed to drought was significantly higher than those under non-stress conditions (control group). This study also found that SOD activity under mild and moderate drought stress was significantly higher than those under non-stress and severe drought conditions (Table 2).

Table 2. Antioxidant enzyme changes in response to drought stress in basil leaves

Drought treatment	SOD[2] activity (U min^{-1} mg^{-1} protein)	PPOD activity (µmol min^{-1} mg^{-1} protein)	GPOD activity (µmol min^{-1} mg^{-1} protein)	CAT activity (µmol min^{-1} mg^{-1} protein)
T1 (control)	4.08 ± 1.76^c	3.03 ± 1.98^c	0.15 ± 0.04^c	0.100 ± 0.025^b
T2 (mild)	11.42 ± 5.22^a	16.28 ± 3.81^a	0.26 ± 0.17^b	0.188 ± 0.030^a
T3 (moderate)	10.11 ± 4.32^a	15.61 ± 5.56^a	0.35 ± 0.14^b	0.178 ± 0.019^a
T4 (severe)	7.73 ± 4.22^b	11.52 ± 9.66^b	0.50 ± 0.11^a	0.176 ± 0.022^a
$LSD_{0.05}$[1]	2.53	3.55	0.10	0.018

[1]Averages denoted by the same letter in the same column indicate no significant difference (P < 0.05)

[2]SOD (superoxide dismutase), PPOD (pyrogallol peroxidase), GPOD (guaiacol peroxidase), CAT (catalase)

PPOD activity showed similar patterns of change to those observed for SOD activity. PPOD activity in leaves of basil plants first increased during mild drought, but then decreased, especially under severe drought conditions.

Drought treatment also caused a significant increase in GPOD activity compared with well-watered basil plants. In this study, GPOD activity in leaves of stressed basil plants gradually increased, reaching the highest value under severe drought stress.

Drought also significantly increased CAT activity in leaves of basil plants as compared to well-watered plants, regardless of drought duration. However, the differences in CAT activity among the drought treatments were not significant.

Table 3. Antioxidant properties of basil plants under drought conditions

Drought treatment	Total phenolics (mg 100 g^{-1} FM[2])	Total flavonoids (mg 100 g^{-1} FM)	Total antioxidant capacity (µmol Fe^{2+} 100 g^{-1} FM)
T1 (control)	$32.88 \pm 1.19^{c*}$	2.43 ± 0.69^b	50.47 ± 1.94^c
T2 (mild)	33.07 ± 0.77^{bc}	2.51 ± 0.12^b	52.41 ± 3.22^{bc}
T3 (moderate)	37.91 ± 0.98^a	2.98 ± 0.06^a	58.04 ± 3.16^a
T4 (severe)	35.00 ± 0.21^b	2.78 ± 0.10^a	54.79 ± 3.16^b
$LSD_{0.05}$[1]	2.21	0.26	2.84

[1]Averages denoted by the same letter in the same column indicate no significant difference (P < 0.05)

[2]fresh mass

In the current study, total phenolic content significantly increased in basil leaves under moderate and severe drought as compared with non-stressed basil plants. On the other hand, mild drought stress did not affect the total phenolic content of basil leaves compared to control conditions (Table 3).

The total flavonoid content in basil leaves showed a similar pattern of change as that observed for total phenolic content. The study results also showed that the total antioxidant capacity (FRAP value) was higher in drought-stressed plants as compared to control plants. Under moderate stress, the increase was more noticeable, and then under severe and mild drought stress.

DISCUSSION

Effects of drought on proline and leaf photosynthetic pigment contents in basil plants

Drought strongly affects all aspects of plant physiology which leads, among other things, to osmotic imbalance in plant cells. Most scientists agree that plants under drought stress conditions tend to maintain the osmotic balance and turgor pressure in plant cells by increasing the accumulation of proline as one of the major organic osmolytes (Ashraf and Foolad, 2007; Chahine *et al.*, 2021; Spormann *et al.,* 2023). Apart from acting as an excellent osmolyte, proline enhances cell membrane stability and reduces the production of reactive oxygen species. It also acts as a signaling/regulatory molecule able to trigger specific gene expression essential for plant responses to stress conditions (Kavi Kishor *et al.,* 2022).

In this study, the proline content in basil leaves significantly increased under both moderate drought and severe drought (Table 1). Numerous studies have also reported a similar increase in proline content in different plants subjected to drought (Fu *et al.,* 2018; Hosseinifard *et al.,* 2022). Interestingly, in this study, mild drought stress did not significantly affect proline accumulation in basil leaves. Considering the fact that proline is a reliable biomarker of stress (Hayat *et al.,* 2012), these results indicate that basil plants tolerate short-term drought conditions well. These results are in line with those of Kalamartzis et al. (2020) and Driesen et al. (2023).

In the current study, moderate and severe drought drastically decreased the content of all the studied photosynthetic pigments including chlorophyll *a*, chlorophyll *b* and total carotenoids in basil plants as compared to control treatment. These results are basically consistent with the previous studies (Dias *et al.,* 2018; Hu *et al.,* 2023), suggesting that exposure of plants to long-term drought conditions causes a considerable damage to photosynthetic pigments and thus to plant photosynthetic capacity. Most scientists believe that drought induces loss of thylakoid cell membrane structural integrity within chloroplast, resulting in degradation of photosynthetic pigments (Chaves *et al.,* 2009; Li *et al.,* 2020). In addition, drought negatively affects the plant's ability to take up nitrogen, iron and other nutrients necessary for the synthesis of photosynthetic pigments as well as for maintaining the structure and function of chloroplasts (Umair Hassan *et al.*, 2020), and this is probably an additional reason for the decrease of the photosynthetic pigments content under long-term drought conditions.

In this study, mild drought stress did not negatively affect chlorophyll *a*, chlorophyll *b* and total carotenoid contents in basil leaves (Table 1). Moreover, total carotenoid contents in basil leaves under mild drought stress were significantly higher ($p < 0.05$) than those in the corresponding controls (non-stressed plants). Numerous studies have reported that carotenoids play a key role in protecting photosynthetic apparatus from photodamage caused by drought-induced oxidative stress (Latowski *et al.*, 2011; Ashikhmin *et al.*, 2023), and therefore increasing or maintaining the content of total carotenoids in the leaves under drought conditions is of great importance for photosynthetic efficiency. In addition, given that the carotenoids act both as light-harvesting pigments and as stress signals in plants, the maintenance of total carotenoid contents in leaves of plants exposed to drought is considered to be an important indicator for assessing plant drought tolerance (Mibei *et al.*, 2016). From this point of view, basil can be regarded as a drought-tolerant plant. However, it is important to note that prolonged drought conditions have a serious negative impact on the photosynthetic pigment synthesis, which ultimately leads to a decrease in their content in the leaves (Wang *et al.*, 2018). This hypothesis is supported by the results of the current study.

Enzymatic antioxidant responses to drought in basil plants

Under drought stress conditions, many physiological processes in plants, including photosynthesis, respiration as well as nutrient uptake and translocation, are negatively affected (Phansak *et al.*, 2021). These physiological disorders unavoidably led to overproduction of ROS that can cause damage to all classes of biomolecules (Oguz *et al.*, 2022).

However, over the course of evolution, plants have evolved complex defense systems against the accumulation and production of ROS, and among them, the enzymatic ROS-scavenging system is certainly one of the most important (Nadarajah, 2020). This defense system consists of numerous enzymes that have the ability to protect plant cells from the harmful effects of ROS. Some of them, such as SOD, CAT and peroxidases (PODs) are of great importance for maintaining the level of ROS in plant cells.

SOD acts as the first line of intracellular defense against superoxide radical anions which are produced in the large amounts in the early stage of stress as a result of the one-electron reduction of molecular oxygen. This enzyme catalyzes the dismutation of superoxide radical (O_2^-) to molecular oxygen (O_2) and less toxic hydrogen peroxide (H_2O_2) which is subsequently decomposed to H_2O by CAT or PODs. However, their mechanisms for converting H_2O_2 into H_2O are not the same; PODs convert H_2O_2 into H_2O using a wide variety of substrates as an electron donor, while CAT does not require an electron donor for the conversion of H_2O_2 into H_2O (Gusti *et al.*, 2021; Ren *et al.*, 2016).

In this study, SOD activity in leaves of basil plants under drought conditions was significantly higher than those under non-stress conditions (Table

2). The study results also showed that SOD activity under mild and moderate drought stress was significantly higher than that under non-stress and severe drought conditions, indicating that SOD activity first increased under mild and moderate drought, and then began to decrease under severe drought conditions. A similar pattern of changes in SOD activity was observed in leaves of *Medicago sativa* L. (Tina *et al.*, 2017) and *Ilex verticillata* (L.) A.Gray. subjected to drought (Xie *et al.*, 2023). These results strongly support the hypothesis that SOD play a crucial role in detoxification of ROS in plant cells in the early stages of drought. This finding is also consistent with a number of other studies that have reported high SOD activity at the initial stage of plant exposure to drought (Lu *et al.*, 2010; Ighodaro and Akinloye, 2018; Duong *et al.*, 2023).

In this study, PPOD activity has followed a similar pattern as SOD in response to drought. PPOD activity first increased during mild drought, but then decreased, especially under severe drought conditions (Table 2). These findings provide further support for the hypothesis that prolonged drought conditions cause an imbalance in the plant's defense system which, among other things, leads to a decrease in SOD and PPOD activity (Kapoor *et al.*, 2020). These findings align with those reported by Liu *et al.* (2011) and Ulusu et al. (2022).

In the current study, drought treatment caused a significant increase in GPOD activity compared to control (non-stressed plants). Interestingly, GPOD activity in leaves of stressed basil plants gradually increased over the course of the experiment, reaching the highest value under severe drought stress, which was inconsistent with the pattern observed for SOD and PPOD activity. Namely, in comparison with GPOD activity, SOD and PPOD activity was the highest under mild drought stress.

In this study, drought also caused a significant increase in CAT activity compared to well-watered plants, regardless of drought duration. In the scientific literature, there are many reports on CAT activity in plants under drought conditions and they are extremely heterogeneous. CAT activity has been shown to increase, remain unchanged, or decrease under drought depending mainly on the experimental conditions and plant species (Jiang and Zhang, 2002; Luna *et al.*, 2004; Song *et al.*, 2022; Mishra *et al.*, 2023). In this regard it is important to note that CAT has a very low affinity for H_2O_2, which means that it is effective only at high concentrations of H_2O_2. This is perhaps one of the reasons for the low CAT activity in the initial phase of stress, observed in earlier studies. On the other hand, CAT has a very fast turnover rate. One molecule of this enzyme can convert more than 2 million molecules of H_2O_2 to H_2O per second, indicating that CAT is one of the most important parts of the enzymatic antioxidant defense system in plants (Racchi, 2013). The results of this study support this hypothesis.

Non-enzymatic antioxidant responses to drought in basil plants

Under drought conditions, plants also activate a non-enzymatic antioxidant defense system to achieve redox homeostasis in plant cells. The non-enzymatic antioxidant defense system of the plant comprises a variety of non-enzymatic antioxidant molecules including ascorbic acid, carotenoids, glutathione and phenolic compounds. They not only protect the plant cells against ROS, but also play a prominent role in plant growth and development by modifying numerous signaling pathways related to cell division, cell elongation, abscission and cell death (Kasote *et al.*, 2015). Among them, phenolic compounds appear to be the most important since they exhibit direct and indirect antioxidant properties. In this regard, phenolic compounds can directly neutralize ROS through electron donation; however, they also exhibit indirect antioxidant activity by inducing the production of endogenous antioxidant enzymes (Kumar and Goel, 2019).

In the current study, exposure of basil plants to moderate and severe drought conditions caused a significant increase in total phenolic content as compared with control plants (Table 3). Increased synthesis of the phenolic compounds in plants exposed to prolonged drought has been observed in numerous studies (Park *et al.*, 2023; Šamec *et al.*, 2021), indicating that the accumulation of phenolic compounds in plant cells is one of the important defense strategies adopted by plants to cope with deleterious effects of drought (Ahlawat *et al.*, 2023).In this study, mild drought stress did not affect the total phenolic content of basil leaves compared to control conditions. Given the fact that phenolic accumulation in plants serves as a reliable biomarker of stress (Jańczak-Pieniążek *et al.*, 2022), the absence of significant changes in total phenolic content between mild drought and control treatment indicates that basil plants tolerate short-term drought conditions well. This hypothesis is also supported by the results of the proline and photosynthetic pigment content analysis.

The results of this study demonstrated that severe drought caused a significant decrease in the total phenolic content of basil leaves as compared with moderate drought; however, the total phenolic content in basil plants under severe drought conditions was still significantly higher than in well-watered plants. Król et al. (2014) reported that exposure of plants to long-term drought stress can cause disturbances in phenolic biosynthetic pathways, which consequently leads to a decrease in their content in leaves. Several other studies also provide evidence that long-term progressive drought negatively affects the synthesis of phenolic compounds in plants (Gutierrez-Gonzalez *et al.*, 2010; Chennupati *et al.*, 2012).In this study, the total flavonoid content showed a similar pattern of change as that observed for total phenolic content. The obtained results were expected since flavonoids are one of the most abundant and widespread phenolic compounds in plants (Mutha *et al.*, 2021). The study results also showed that the total antioxidant capacity (FRAP value) in the leaves of basil plants increased significantly in all drought stress treatments as compared with control plants. The increase was more noticeable under moderate stress, followed by severe and mild drought stress. The same pattern was observed in the relationship between drought stress levels and total phenolic and flavonoid contents, indicating that phenolic compounds are one of the main contributors to the antioxidant capacity of plants. These findings align with those of earlier research (Lyu *et al.*, 2023; Zeng *et al.*, 2023).

CONCLUSIONS

The study revealed that exposure of basil plants to drought significantly increased the activities of SOD, GPOD, PPOD and CAT in plant leaves, indicating that these enzymes play an important role in ROS detoxification and thus in plant drought tolerance. Exposure of basil plants to moderate and severe drought also resulted in higher accumulation of proline and total phenolics and flavonoids, indicating that basil plants under prolonged drought stress conditions tend to improve their drought tolerance by inducing the production of these compounds in leaves. The study results also revealed that moderate and severe drought had a negative effect on the content of photosynthetic pigments, i.e. chlorophyll *a*, chlorophyll *b* and total carotenoids. In the current study, mild drought did not negatively affect the content of photosynthetic pigments as well as proline and total phenolic and flavonoid content in basil leaves. These findings lead to the conclusion that basil plants tolerate drought conditions well. This conclusion is further supported by the fact that the basil plants have the ability to produce protective macromolecules and antioxidants in high amounts even in severe drought conditions.

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Natalija PAVLOVIĆ[1], Željko DOLIJANOVIĆ[2], Milena SIMIĆ[1], Željko KAITOVIĆ[1], Vesna DRAGIČEVIĆ[1], Milan BRANKOV[1]

EDAMAME - VEGETABLE CROP OF THE FUTURE: PRODUCTION CHALLENGES AND CHEMICAL PROFILE

SUMMARY

Edamame is a vegetable, immature soybean (*Glycine max* [L.] Merr.) and an excellent plant–based protein source which is low in calories, high in essential amino acids, dietary fibre, phospholipids, vitamins, minerals, and many other bioactive compounds. Secondary metabolites in edamame include various phenolic compounds, isoflavones, and saponins. Besides providing fresh, green and highly nutritious beans as the main product, edamame biomass can be utilized at the same time for animal feeding or as green manure. Germination, seedling emergence and stand establishment are very important initial phases in development that have a great impact on the whole vegetative cycle. Weed management is one of the key elements for reaching full genetic and yield potential. Irrespective that edamame could enrich the soil with N through the N–fixation process, optimal nutrition which includes application of mineral and organic fertilizers, biofertilizers as well as foliar fertilizers are required for successful production. Ensuring timely water supply to edamame is another crucial factor that greatly contributes. Edamame, as a vegetable crop, is highly sensitive to environmental fluctuations, thus optimization and the development of adequate cropping technology are essential for successful production. As nitrogen fixing legume rich in bioactive compounds, with various benefits for human health and suitability for crop rotation, edamame hugely contributes to sustainable agriculture.

Keywords: germination and emergence; fertilization; weed management; water supply; chemical composition; sustainable agriculture

[1] Natalija Pavlović, Milena Simić, Željko Kaitović, Vesna Dragičević, Milan Brankov (*corresponding author: mbrankov@mrizp.rs) , Maize Research Institute "Zemun Polje, Belgrade, SERBIA

[2] Željko Dolijanović University of Belgrade, Faculty of Agriculture, Belgrade, SERBIA

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INTRODUCTION

Edamame soybean is a vegetable and a highly nutritious crop, so its presence in the human diet is constantly increasing. Edamame popularity rapidly grows from China and Japan, where it is a common part of the diet. Edamame production is primarily concentrated in China, Japan, and Taiwan, while the main consumers of edamame are China, Japan, Korea, the USA, Taiwan, Thailand, and Europe (Nair *et al*. 2023). China is the biggest edamame producer (Dong *et al.* 2014). The vegetable soybean (or edamame) account approximately 2% of world soybean production (Dar *et al.* 2023). Production technology for this crop is challenging because of several critical points during the vegetation period that can negatively affect edamame development. The important points include the reduced germination rate of the seed, high water demands, sensitivity to pests and weak competition against weeds, through nutrition, up to unequal beans maturation and a short harvesting window governing appropriate storage for maintaining the quality of beans. All of these potentially limiting factors must be mitigated to ensure the successful production. Table 1 presents an overview of the various studies which aimed to deal with some of the challenges mentioned.

Different numbers in superscript next to the surnames of authors represent specific areas covered by the scientific paper. The scientific paper does not have to cover all the specific areas listed under the same letter.
[1] sowing/planting; [2] plant nutrition; [3] weed management; [4] irrigation; [5] harvesting and processing, [6]proteins, carbohydrates, lipids; [7]fibers and macro/micronutrients; [8]isoflavones and pigments; [9]vitamins, amino acids, [10] antinutrients; [11] chlorophyll and enzymes

[*]This study refers to abiotic stresses (photoperiod, temperature and water) soybean is a light–sensitive plant that has a specific physiological response to the duration of daylight in a 24–hour cycle. It is a short–day plant, therefore it requires shorter daylight period that have to fall below a critical threshold to induce flowering. Sunlight controls duration of pod filling and ageing of leaves (Staniak *et al.* 2023). Day length and light intensity contributes significantly to crop development, growth and yield (Jankauskiene *et al.* 2021). Temperature is the key factor dictating when flowers will appear after floral induction (Heatherly and Elmore 2004). Even with optimal temperature conditions, if the day length is not short enough it will postpone the start of reproductive development (Setiyono *et al.* 2007). This is of particular importance when edamame yield potential is considered. Contrary, sowing edamame (commonly determinant in plant growth) when duration of daylight is too short, will induce flowering during the early vegetative stages, growth will be stopped and developed plants will be stunned, overall resulting in low yield. High temperatures in early spring combined with sufficient sun radiation will induce earlier flowering, thus prolonging duration of reproductive stages and in all positively affect yield (Cooper 2003), in indeterminate soybeans. The longer the vegetation period, the more nodes and pods per plant are formed, and total fresh pod weight is increased (Zhang and Kyei-Boahen 2007).

Table 1. Edamame soybean research around the world

Technology production	Chemical composition	Technology production + Chemical composition	Reviews
Duppong, (2005);[4]	Sikka *et al.* (1978);[6, 7]	Simmone *et al.* (2000);[5; 8]	Ravishankar *et al.* (2016);[1, 5-9]
Zhang and Kyei-Boahen (2007);[1]	Yanagisawa *et al.* (1997); [9]	Rao *et al.* (2002);[1, 6, 9]	Zeipina *et al.* (2017);[1, 2, 6, 9]
Pornprom *et al.* (2010);[3]	Kumar *et al.* (2009);[8, 9]	Carpenter, (2007);[1, 6, 8]	Zhang *et al.* (2017);[1-6, 8]
Sharma and Kshattry (2013);[1]	Li *et al.* *(2012);*[6]	Saldivar *et al.* (2010);[5, 6, 10]	Miles *et al.* (2018);[1-5]
Li *et al.* (2013);[1]	Carson *et al.* (2012);[8]	Carson, (2010);[1, 6, 8]	Djanta *et al.* (2020);[1-3, 5-9]
Williams and Nelson (2014);[3]	Song *et al.* (2013);[6, 9]	Carson *et al.* (2011);[1, 6]	Nair *et al.* (2023);[1-9]
Kering and Zhang (2015);[1]	Xu *et al.* (2016);[6, 9, 10]	Santana *et al.* (2012);[5, 6, 8, 9, 10]	Staniak *et al.* (2023);*
Abugho *et al.* (2015);[3]	Liu *et al.* (2019);[6-9]	Li *et al.* (2014);[1, 9]	
Ogles *et al.* (2016);[1]	Carrão-Panizzi, (2019);[6, 8]	Maruthi and Paramesh (2016);[2, 6]	
Crawford, (2017);[1, 3]	Jiang *et al.* (2020);[6]	Nolen *et al.* (2016);[1; 6]	
Williams *et al.* (2017);[3]	Hertamawati and Rahmasari (2021); [6, 9]	De Oliveria *et al.* (2019);[2, 6, 7]	
Williams and Bradley (2017);[3]	Yu *et al.* (2021);[6, 9]	Shiu *et al.* (2020);[4, 5, 9]	
Crawford and Willams (2018);[1]	Guo *et al.* (2022);[6, 9]	Jankauskiene *et al.* (2021);[1, 6, 7]	
Crawford and Willams (2019);[1]	Agyenim-Boateng *et al.* (2023);[6-9]	Moloi and Van der Merwe (2021);[4, 6, 9, 10]	
Lord *et al.* (2019); [1,5]		Liu *et al.* (2022);[2, 6, 7]	
Williams *et al.* (2019);[3]		Hlahla *et al.* (2022);[2, 3, 6, 7, 8]	
Dhaliwal and Williams (2020);[1, 5]		Moloi *et al.* (2022);[2, 4, 10]	
Carneiro *et al.* (2020);[5]		Brooks *et al.* (2023);[2, 6, 7]	
Chen *et al.* (2021);[4]			
Fogelberg and Mårtensson (2021);[1, 2]			
Lestari *et al.* (2021);[2]			
Hasanah *et al.* (2021);[1, 2]			
Zeipina *et al.* (2022);[1]			
Li *et al.* (2022);[3]			
Saputra and Anggraini (2022);[2]			
Simiderle *et al.* (2022);[2]			
Netty, (2023);[2]			

Since environmental factors are crucial for edamame production, many researchers evaluated growth of edamame in different agroecological conditions. Jankauskiene et al. (2021) evaluated the potential of growing edamame varieties in Lithuania as a country with a lack of heat and light). The temperature varied approximately from 14°C in May to 18°C in August and precipitation was around 240 mm during the growing season. Five out of six varieties were suitable for Lithuanian climatic conditions. Vegetation period lasted 105 days to the end of R7 stage. They stated that temperature and precipitation were positively correlated with plant height, while better branching of some varieties didn't enhance yield. The yield was correlated with weather conditions and variety. The variety 'Chiba Green' was the most suitable for cultivation under Lithuanian climatic conditions, with the lowest plant height, but the highest yield, the weight of 100 pods, photosynthetic intensity and chlorophyll index. Furthermore, Nolen *et al.* (2016) proved the possibility to grow edamame in Virginia, USA, in early spring, by sowing it in high tunnels, providing optimal temperature conditions for plant development. Ogles *et al*. (2016) investigated characteristics of different edamame cultivars from III to VI maturity group (MG) grown in Central Alabama, USA, during 2014–2015 growing season. Eleven cultivars were investigated. Yield in 2014 was higher than in 2015, which was owing to higher air temperatures (about 33°C) during the R1 stage. In 2014 pod yield varied from 10.2 to 16 t/ha, while next year it varied from 2.6 to 13.5 t/ha. Number of pods per plant was related to maturity group – earlier MG, lower number of pods per plant.

Differences between edamame and conventional soybean

Unlike conventional soybeans, whose mature beans are commonly used for livestock feed and for processing in various industries, edamame is regularly used as a vegetable. Edamame is a determinant soybean and the main difference between edamame and common soybean is the harvesting time (Zeipina *et al.* 2017). Common soybean is harvested at the R8 stage of development (full maturity), while edamame is harvested earlier, in the R6 stage (full seed) (Figure 1). It is very important to perform harvesting during the period when the pods are green and avoid discoloration to yellow (Zeipina, 2017). At the full seed stage beans take 80–90 % of pod width (Konovsky *et al.* 2020). Any delay in the harvest, pods are turning into yellow and quality is declining. Edamame reaches the full seed stage 75–100 days after sowing (Zeipina *et al.* 2017), depending on a maturity group. There is a difference in the storage conditions needed for edamame and conventional soybeans. Edamame, being harvested with higher moisture content, requires specific handling and storage conditions to maintain its quality. Common soybeans, with a low moisture content (10–13 %) can be stored for a long time without quality degradation, while edamame, with a moisture content of 80 %, has a shorter shelf life (Crawford, 2017). Cooling immediately after harvesting is significant for preserving beans freshness and they are mainly stored in freezers. According to Saldivar *et al*. (2010) when stored in bags with air at 4 °C, the appearance of the edamame remained relatively unchanged, while storing at 25 °C in open air conditions led to significant changes, such as yellowing, browning, shrinking, and wilting. The study also showed that storing edamame pods in a nitrogen atmosphere at 25 °C caused a rapid decrease in the levels of all soluble sugars. Overall, the research concluded that maintaining a low temperature during storage is more crucial than atmospheric conditions in preserving the nutritional and sensory qualities of fresh edamame pods. Maintaining post–harvest seed quality becomes challenging because of high respiration rates (Nair *et al.* 2023). Therefore, storage practices such as blanching, freezing, and storing at low temperatures need to be implemented shortly after harvest. Blanching and storing at low temperatures preserve the high sugar concentration. While the yellow color of the pod is a sign of converting sugar to starch, increasing the maltose content by boiling is a way to maintain and improve the sweetness of edamame seeds (Nair *et al.* 2023).

Figure 1. Edamame soybeans (left hand side: VE to VC; middle: R2; right hand side: R6). *VE – growth stage when the coleoptile breaks through the soil surface. VC – cotyledons have been pulled through the soil surface. R2 – the plant is beginning full bloom. R6 – the "green bean" stage, total pod weight peaks. Org. photos.

The differences between the nutrient profile of edamame and common soybeans are expected considering genotype differences as well as the fact that the moment of harvest occurs at different stages. According to Carson *et al*. (2011) the lipid content is lower in edamame, the protein content is similar to common soybean and varied 12.9-18.4 g/100g and 36.1-39.5 g/100 g, respectively. Compared with conventional soybeans, edamame is richer in vitamins and other valuable nutrients. Edamame contains more vitamins A, C, K and, B than common soybean (Takahashi and Ohyama 2011). Edamame has higher content of Fe while common soybean has higher level of crude fat (Sikka *et al.* 1978). Edamame soybean harvested at the R6, contained higher concentration of asparagine, alanine, and glutamic acid compared to common soybean harvested in the same development stage (Yanagisawa *et al*. 1997).

Edamame has a lower content of trypsin inhibitor, as well as cysteine, and methionine in proteins (Nair *et al.* 2023). According to Tsou and Hong (1991) edamame, compared with grain soybean, has higher level of starch (83.20 vs. 0.66 mg/g), total sugar content (110.2 vs. 102.4 mg/g) sucrose (95.14 vs. 62.05 mg/g), glucose (13.4 vs. 11.18 mg/g) and fructose (8.95 vs. 0.73 mg/g), and lower level of raffinose (0.16 vs. 14.85 mg/g), stachyose (0.95 vs. 25.38 mg/g) and crude fiber (44.9 vs. 52.7 mg/g) measured on dry weight basis.

A production process of edamame is similar to that of conventional soybeans, with slight differences such as plant density and harvest time. Optimal plant density reduces competition among plants, allowing them greater access to water, nutrients, and sunlight accessible, which overall increase photosynthesis efficiency (Staniak *et al.* 2023). Providing enough space for development is important for edamame soybeans, which branch more than common soybean (Zeipina *et al.* 2022). Edamame seeds are larger, therefore the recommended sowing distance in the row is greater and the planting density is lower (Zeipina et al. 2017). These results in a lower plant density compared with common soybeans, which also provide more space for weeds. Sharma and Kshattry (2013) stated that branching is positively correlated with number of pods. Lower plant density affects greater branching and pod number, resulting in a higher yield, while at higher densities, fewer pods and branches are formed and thus, the yield is lower (Dhaliwal and Williams 2020). According to Hasanah et al. (2021) spacing of 20 x 20 cm significantly reduced the number of productive branches per plant on edamame on 5.8. At a spacing of 30 x 20 cm, it increased to 6.6, while at 40 x 20 spacing, there were 6.4 productive branches per plant. Zhang *et al.* (2013) concluded that common soybean had better emergence than edamame because it is less sensitive to deeper planting. On 3 cm depth, edamame and common soybean had equal emergence rate, while on deeper planting depth (4 and 5 cm), edamame had lower emergence rate.

Due to its short vegetation period, edamame is suitable for multiple cropping during the same year (double or triple cropping), depending on maturity group, climate and latitude. However, taking all the mentioned factors into account, introducing edamame varieties for the first time can be challenging and it may be difficult for farmers to achieve the expected yield and quality (Djanta *et al.* 2020).

Seed quality and vigor of edamame

High–quality seed is the primary prerequisite for successful agricultural production. Good germination and strong seedling establishment are crucial for successful seedling adaptation to environmental conditions, overcoming stress factors and efficiently utilizing available resources (Cardarelli *et al.* 2022). Seed treatments with microorganisms are beneficial for crops sown directly in the field, such as soybeans, whose development and yield potential strongly depend on symbiosis with microbes (Cardarelli *et al.* 2022).

Kering and Zhang (2015) indicated that for common soybean germination and emergence, soil moisture status has greater importance than seed size. They concluded that under insufficient moisture levels, neither small nor large seeds can finish the initial seed development phases. Crawford and Williams (2018) stated that seed size did not have an influence on edamame crop emergence. Emergence from small seeds was 7.6 % while from large seeds it was 76.8 %. Williams and Bradley (2017) stated that common soybean had better emergence than edamame. Also, seed size affected emergence because smaller seeds need less time to emerge. Williams et al. (2019) noticed 7.2 % higher emergence of standard soybean compared to edamame soybean (67.7 vs. 60.5 %). According to Crawford and Williams (2019), total emergence remained consistent across various seed sizes, but the small seeds had 10 % faster emergence compared with the large seeds, such as edamame have.

The temperature, moisture, and oxygen status of the soil affects seed development and can limit seedling development outside of the optimal range (Li *et al.* 2022). The fastest emergence of three tested edamame cultivars was at 22 °C, while at 10° C, plants weren't able to emerge (Fogelberg and Mårtensson (2021). Initial development stages of edamame (from germination to VE) was postponed up to 17 days after sowing under unfavourable soil conditions including cold and overly moist soil (Sharma and Kshattry 2013). Seed vigor is crucial for germination and emergence and depends on a greater extent on appropriate harvest and storage management (Li *et al.* 2022). Mechanical damage to edamame seeds leads to a decrease in germination, therefore, it is important to handle the seeds with the utmost care during planting to avoid seed injury (Miles *et al.* 2018). Edamame seedlings are also sensitive to damage influenced by penetration through the soil and pest attacks. To maintain high germination rates conditions during storage, play important role (Ravishankar *et al.* 2016). Appropriate fertilization system can increase low germination (Maruthi and Paramesh 2016)

The seed coat has a protective role against biotic and abiotic stress and it controls the speed of seed hydration, slows down imbibition, reduces the risk of injury to dry seeds, and, through thickness, it could induce dormancy and slower germination, which is of particular importance for edamame (Li *et al.* 2022). From this standpoint, some practices, such as cover crops, can reduce edamame emergence because of lower contact between the seed and the soil (Crawford *et al.* 2018*)*.

Edamame cropping

The main problems in edamame production, includes the high pressure of diseases and pests, leading to increasing use of pesticides, a deficiency of germplasm material (a bottleneck) and a high-water demand (Nair *et al.* 2023). To obtain the maximum genetic potential of edamame soybeans, it is necessary to implement every required practice that will result in the expected yield and quality.

Weed management

To control weeds in edamame, there are many practices like crop rotation, rotary hoeing, inter–row cultivation, herbicide use and extensive hand weeding

(Zhang *et al.* 2017). After sowing first six weeks is crucial weed control (Sharma and Kshsttry 2013). If pre-emergence herbicides effectively manage weeds, it is possible to avoid post-emergence herbicides (Pornprom *et al.* 2010). Pre-emergence herbicides help plants in competition, prevents weeds from becoming too large to control and give the possibility to reduce the use of chemicals, costs, and environmental harm (Pornprom *et al.* 2010).

Many published studies are related to weed control using herbicide (Pornprom *et al.* 2010; Abugho *et al.* 2015; Williams and Nelson 2014; Williams *et al*. 2017; Crawford and Williams 2018; Williams *et al*. 2019), and only a few of them are related to environmentally friendly practices (Crawford *et al.* 2018; Crawford and Williams 2018). In the future more attention needs to be paid to evaluation of integrated weed management practices in edamame production, which include combined implementation of cultural, mechanical, biological, chemical, and preventive strategies to control weeds.

Williams and Nelson (2014) investigated tolerance of edamame and commom soybean to bentazon, fomesafen, imazamox, linuron, and sulfentrazone, indicating linuron and fomasafen as the friendliest herbicides for edamame. Results obtained from Crawford and Williams (2018) study showed that edamame plants, developed from larger seeds, in the presence of *Abutilon theophrasti*, were taller, produced 7.5 % more biomass and had higher LAI, than those from smaller seeds, in the early stages of growth, two to eight weeks after emergence. Williams *et al*. (2019) evaluated tolerance of edamame on application of pre-emergence herbicides consisted of flumioxazin as well as its mixture with chlorimuron, metribuzin, acetochlor, and pyroxasulfone (not registered for weed control in edamame). Research included edamame cultivars as well as standard soybean cultivars. For edamame, injuries from flumioxazin-based treatments, 2 and 4 weeks after treatments were 2.9 % and 0.9 %, while for common soybean it 1.7 and 0.7 %, respectively. Crawford *et al.* (2018) investigated the impact of different cover crops (winter–killed oilseed (*Raphanus sativus* L.), two *Brassica napus* L. treatments early–killed and late–killed, two *Secale cereale* L. treatments early–killed and late–killed, and bare–soil control) on soil moisture level, edamame emergence, weed density, and weed biomass. The results indicated that cover crops preserved soil moisture by reducing evaporation. Among the tested cover crops, late–killed *Seccale cereale* preserved the highest amount of water, while edamame emergence had the lowest value. Early–killed *Secale cereale* reduced weed density by 20 % and suppressed early–season weed growth by 85 %, indicating it as the best solution for weed management.

Fertilization

A commonly used practice to enhance soybean growth is seed inoculation with biofertilizers, i.e., microorganisms which can increase the availability and supply of nutrients. It is not necessary to inoculate seed with biofertilizer every year and in some cases, microbiological fertilizers might express even a negative effect on yield (Fogelberg and Mårtensson 2021). Also, essential macro- and microelements can be provided during the vegetation period, through foliar fertilization or fertigation. By application of foliar fertilizer, it is possible to supply the plant with micronutrients (Brankov *et al.* 2020), especially when nutrient availability in the soil is limited (Fernández and Brown 2013). It is important to underline that foliar fertilization allows the addition of nutrients in small quantities (Patil and Chetan 2018), while the plant's response is faster compared with soil application (Fageria, 2009). It is important to mention that biofertilizers and foliar fertilizers cannot exclude or replace the use of mineral fertilizer, but serves as supplement to maintain plant nutrition on optimal level.

Maruthi and Paramesh (2016) investigated the effect of different fertilization systems on the edamame quality finding that the best emergence and seed quality parameters were achieved in the treatment that included the full NPK rate + the full rate of farmyard manure + *Bradyrhizobium* + PSB. Compared with the treatment that included only the full NPK rate, the combined treatment had 12.4 % higher field emergence and 2.0%t more protein. Thus, a higher nutrition rate promotes seed quality, increasing nutrients reserve in seed. In South Asia, edamame was involved in an intercropping system and it can reach yields of up to 10 t ha^{-1} (Ravishankar *et al.* 2016). The edamame residues can improve soil nutritional status, nitrogen by 129 kg ha^{-1}, potassium by 21 kg ha^{-1} and organic carbon by 0.09 percent (Ravishankar *et al.* 2016). Deficiency in essential nutrients such as Zn could result in poorer growth, yield potential, and bean quality. De Oliveira *et al.* (2019) examined different methods of Zn fertilization (application on the soil; foliar application at V4; V8 or R4 stage). Compared with other treatments, foliar application of Zn at R4 influenced the greatest accumulation of Zn in edamame beans which concentration reached 45.05 mg/kg. The highest mean protein content from all of three cultivars was under R4 (42.3%). Moloi and Khoza (2022) examined the effect of foliar selenium application on the photosynthetic capacity, antioxidative enzyme activities, and yield parameters of a drought–susceptible edamame cultivar. Foliar applied selenium at the V1 increased the level of photosynthesis at the vegetative stage, as well as antioxidative capacity at the flowering stage. Netty (2023) investigated how different rates of organic (compost: 10 or 20 t/ha) and inorganic fertilizers (Urea, SP-36 and KCl – in 100, 75, 50 or 25 % of the recommended rate) complement each other in purpose to enhance plant growth and yield. Compared with the control treatment (100 % inorganic fertilizers), 10 t/ha of compost + 50 or 75 % of inorganic fertilizer was the most effective in enhancing plant height, total number of leaves per plant and number of productive branches. Brooks (2023) evaluated the effect of different date (at-planting and split 50% applied at-planting and 50% at R1) and amount (22.4, 44.8, 67.2 and 89.6 kg N/ha) of nitrogen fertilizer, as well as S fertilizer (22,4 kg/ha), on yield, nutrient uptake, C, N, and S concentrations in plant tissue, chemical and physical quality of edamame. In two of three tested years, for every kg of Nitrogen added in soil yield increased 8.98 kg/ha and 29.9 kg/ha. Application of different fertilization treatments didn’t have influence on content of N, C, S C:N, C:S ratio in plant tissues. Uptake of N, C and S. N uptake was higher than applied amount of N fertilizer, while S uptake was lower than applied amount.

Crop arrangement and sowing date

The sowing date had a strong influence on yield. Zeipina *et al.* (2022) tested the possibility of growing edamame soybeans at higher latitudes by combining different cropping practices. They tested direct edamame sowing in the field and production of transplants in a greenhouse and then sowing in the field) and plant densities (13 plants m^{-2} and 20 plants m^{-2}). The growing season and sowing method had a significantly affected the yield. Among two tested cultivars, both reached the highest yield at higher plant density and production in the greenhouse. The results of these studies indicate that is possible to successfully produce edamame in tested region which has specific climate conditions. Li *et al*. (2014) conducted a study in Harbin, China to test the impact of different sowing date (3 May, 15 May, 27 May, and 8 June) on fresh pod yield and chemical composition of edamame beans. It was shown that with delayed sowing date, the fresh pod yield and the content of oil and sucrose was decreased. They documented that late sowing increased the protein accumulation, free amino acids and the content of glucose and fructose. The content of raffinose and stachyose, which are characterized as anti-nutrients was also increased with delaying sowing date.

Water requirements of edamame

Generally, soybean has high water demands during the vegetation period, as indicated by the transpiration coefficient that ranges from 600 to 700 L, making soybean an inefficient water consumer (Hrustić *et al.* 1988). Edamame as a determinant soybean is more sensitive to water deficit than standard soybean. Soybean is especially sensitive to water deficiency during germination, flowering, pod formation, and bean filling. However, between seed emergence and flowering, soybeans are less susceptible to water stress. During imbibition, seeds need to absorb water in 90–150 % of their dry mass to germinate (Hrustić *et al.* 1988). What is more, 65 % of the total water requirement for the entire vegetation period is consumed during the flowering, pod formation and bean–filling stages (Kranz and Elmore 2022). Hrustić *et al.* (1998) found that optimal photosynthesis could be achieved only if cellular turgor is maintained by an adequate water supply, while the water deficit and decrease in turgor pressure thereby reduce net assimilation, overall organic matter production and agricultural yield.

Research by Mertz-Henning *et al.* (2017) showed that the greatest decrease in soybean yield occurred when the water deficit stress happened during the reproductive stage. Moreover, it was observed that protein content was higher while oil content was lower in plants exposed to a water deficit during the reproductive stage. Drought also leads to a reduction in the number of nodes and internodes length (Hrustić *et al*. 1988). The level of soil moisture significantly impacts the flowering intensity, pod quantity, and plant height of edamame

(Zeipina *et al.* 2022). Drought stress during the flowering stage decreased the edamame yield, qualification rate (relation between total pods and fully filled green pods without shape abnormalities) and qualified pod dry weight, while the total number of pods per plant was not significantly affected (Chen *et al.* 2021). Furthermore, Moloi and Khoza (2022) concluded that drought stress reduces the photosynthetic capacity, antioxidative enzymes and yield of edamame. They examined the response of edamame soybean on applied selenium foliar fertilizer and drought stress (30 percent water holding capacity) occurred at V3. Owing to pronounced sensitivity to drought of edamame, Hlahla *et al.* (2022) tested the biochemical response and detected the compounds which may serve as indicators of drought tolerance in different edamame cultivars sensitive or tolerant to drought stress. Cultivars were exposed to drought stress at V3 by decreasing water holding capacity of soil from 100 to 30 %. Because of reduction of carotenoids and hemicellulose content, as well stomatal conductance in drought sensitive cultivars and high level of starch in drought tolerant cultivars they stated that those parameters could be indicative factors of drought stress in edamame. According to Moloi and van der Merve (2021), total soluble sugars during the flowering stage and proline during the pod–filling stage has a protective role in edamame during drought stress. Total soluble sugars content increased 1.75-fold, from approximately 1.2 to 2.1 mg glucose/g fresh weight. Proline content increased 2.25-fold (approximately from 0.09 to 0.22 mg /g fresh weight) under drought stress treatment (30% water holding capacity) compared to control (100% water holding capacity), in drought tolerant cultivar.

Harvest and bean quality

Due to the fact that malnutrition (hidden hunger) is a persisting problem worldwide, the quality of products containing essential minerals, vitamins, and other bioactive compounds is a requirement for successful agricultural production. Edamame soybeans are a source of protein, amino acids, carbohydrates, vitamins, minerals, antioxidants, fibers, and soflavones. Compared with snap peas and green peas, consuming edamame increases the intake of essential minerals such as iron, zinc, magnesium, phosphorus, calcium, potassium, sodium, copper and manganese are because of their higher concentration in edamame beans (Takahashi and Ohyama 2011). Flavour, texture and sensory traits define the quality of edamame. Sucrose enhances the sweetness, while compounds like saponin, isoflavonoids, and L–arginine contribute to the bitterness (Zeipina *et al*. 2017). Amino acids contribute to edamame giving it a sweet (Ala, Gly, Ser, Thr, Pro) rich (Asp, Glu) or bitter (Arg, His, Iss, Leu, Met, Phe, Val) flavor, while some are neutral (Tyr, Cis, Lis) (Guo *et al.* 2022). Volatile compounds are related to aroma and the main volatile compounds were aldehydes and alcohols (Guo *et al.* 2022). From this standpoint, it is of particular importance to harvest edamame while maintaining the highest bean quality. Moisture content outside the optimal range indicates that the beans are either fully ripened or have not yet reached maturity, making them unsuitable for classification as vegetable and market use (Agyenim-Boateng *et al.* 2023).

Dry conditions during seed maturation will have an enhanced effect on the quality of the beans (Nair et al. 2023).

Important nutrients in edamame beans

Edamame beans on a dry weight basis contain 40.2 % of proteins, 23.2 % of fatty acids (palmitic, stearic, oleic, linoleic and linolenic acid), with linoleic acid as the most abundant; 7.4 % of total soluble sugars (sucrose, fructose and glucose); 1401.1 µg/100 g of total reduced folates; 12130.7 µg/100 g of total carotenoids (β–carotene, α–carotene, β–cryptoxanthin, zeaxanthin and lutein the most presented); 548.9 µg/g of total tocopheroles content; 1633.5 µg/g of total isoflavones; 1889.1 mg/100 g and 7.38 mg/100 g of Mg, K, and Fe, respectively (Agyenim-Boateng *et al.* 2023). Xu *et al.* (2016) investigated chemical and physical traits of edamame beans, depending on development stage. Accordingly, the intensity of bean green color progressively decreased from the R5 to R6. After R6 it was rapidly transformed to a yellow color, owing to chlorophyll degradation. Protein and lipid content followed seed development and maturation (protein content was gradually increased from 34.8 at R6 to 37.6 g /100g dry weight at R8, while lipid had a period of stagnation around the R6 (17.2 g /100 g dry weight and increased to 18.2 g /100 g dry weight at R8). Further, Liu *et al.* (2019) evaluated different edamame soybean genotypes to compare nutritional compositions of fresh and matured seeds (R6 and R8). Protein concentration varied among genotypes and there was no clear illation, is protein content higher at full maturity stage or at full seed stage. It varied between 34.6–43.9 % at R6 and R8 38.4–42.5 %. Soluble sugar concentration was significantly higher at R8 than at the R6. Content of sucrose was also increased, from 55.6 to 70.5 %. Xu *et al.* (2016) reported that at the R6, edamame had the highest sucrose level and its content was declining through bean maturation. Total sugar content tended to decline from 43.9 at R6 stage to 39.9 g/100 g dry weight at R8. At R1 sucrose level was 6.51 g /100 g dry weight. At R6, sucrose level increased 41 %, while at R8 it increased only 7 % relative to the level in R1. Contrary to this, Santana *et al.* (2012) stated that sucrose level was 44 % higher in R8 compared to R6. Obtained from three cultivars, the mean values of raw fat, raw protein, and soluble sugar content were 18.92, 37.38 and 6.39 g /100 g dry weight, respectively. First two components promote texture, while sucrose contributes to sweetness (Guo *et al.* 2022). Results obtained from Guo *et al.* (2022) indicated that alanine, glutamine, and asparagine are the most represented among analyzed amino acids, with average content 0.92 mg/g, 3.20 mg/g and 1.22 mg/g, respectively. Li *et al.* (2012) confirmed that sucrose takes up 71.7 % of the total sugar content and found a negative correlation between protein and total soluble sugar and sucrose. They also concluded that a protein increase of 10 mg/g is followed by a sugar decrease of 4.3 mg g^{-1}, indicating that carbon was in that case involved mainly in protein synthesis regarding sucrose synthesis. According to Liu *et al.* (2019) most abundant mineral element was potassium (16.4-19,3 mg/g). The concentration of K, Na, Zn, and Mn was higher at R6, while Mg and Fe concentration was higher at R8.

Antinutrients in edamame beans

It is well known that soybean seeds are high in several anti–nutrients, such as phytic acid, trypsin inhibitors, oligosaccharides, phenols, saponins, etc. Phytic acid interacts with minerals, forming insoluble complexes with them (chelation) thus reducing the availability of divalent and trivalent metal ions of Zn, Fe, Ca, Mg, Mn, and Cu (Sharma, 2021) and decreasing their absorption in the digestive system. Despite this, it is worth mentioning the antioxidant effect of phytic acid (Kumar *et al.* 2010). Trypsin plays a key role in protein digestion, so trypsin inhibitors, as protease inhibitors, are considered as an antinutrient in legumes because protease inhibitors may inhibit pancreas growth and enlargement (Gemede and Ratta 2014). Oligosaccharides are also categorized as antinutrients due to their poor digestibility and ability to cause flatulence, a common digestive problem (Sharma, 2021). In edamame, beans are presented raffinose and stachyose. Above mentioned components cannot be entirely deemed as undesirable since, in addition to their antinutritive effects, their positive effects on human health are well-documented.

Xu *et al.* (2016) stated that the level of phytic acid increased significantly during seed development and maturation, whereas in edamame beans, the phytate concentration is lower than in the mature beans (R8). Additionally, total phenolic content had the lowest value at R6 compared to all other reproductive stages. The level of polyphenol thiamine was in stagnation between the R5 and R8, when it reached its maximum. The level of phytate reached the highest value at R8 (11.1 mg/g dry weight), while at R6 it was 43 % lower. Trypsin inhibitor activity was at the highest level at R6. The level of oligosaccharides, as an undesirable trait, started to increase from R1 through R6 and R8. Initial content of raffinose at R1 was 0.08 g /100 g dry weight. At R6 it increased 4.75-fold, while at R8 it increased 12.6-fold, to 1.09 g /100 g dry weight. Stachyose level tended to decrease from 0.36 g/100 g dry weight, at R1 stage, to 69.4 % at R6. However, at R6 it reached significantly higher concentration (5.13 g /100 g dry weight). Contrary, Santana et al. (2012) stated that raffinose did not differed significantly, while stachyose content was 62.9 % lower in R8 compared to R6. Results obtained from Saldivar et al. (2010) were in agreement with results from Xu et al. (2016) regarding to stachyose and raffinose content in mature seeds, but they did not found this oligosaccharides in immature edamame soybean harvested at R6. Results from Santana et al. (2012) showed that level of phytic acid is the highest at R8.

Bioactive compounds in edamame beans

Bioactive compounds presented in edamame beans are important from a nutritional point of view and also have potential health benefits. Isoflavones from soybeans such as: daidzein, genistein, and glycitein (Peñalvo *et al.* 2004), are biologically active compounds and have many health benefits for humans. They have estrogen–imiting functions and the first two act as antioxidants. It was proven that genistein indirectly inhibits the development of breast cancer cells and soybean isoflavones may help reduce the risk of heart disease (Munro *et al.*

2003). They could also help in the prevention, suppression, or inhibition of prostate cancer, increasing bone density and helping prevent osteoporosis (Munro *et al.* 2003). Carotenoids are considered as bioactive compounds due to their antioxidative activity and health benefits.

Carpenter (2007) reported that total isoflavones content varied from 48.92 to 132.78 µg/g, depending on edamame variety. Among them, the highest concentration had malonyl daidzin 24.8-67.7 µg/g and malonyl genistin 14.3-53.3 µg/g. Concentration of daidzein and genistein was below 1 µg/g, diadzin up to 5.7 µg/g, genistin up to 2.8 µg/g and glycitin up to 1.3 µg/g. Kumar *et al*. (2009) analyzed the content of tocopherols, isoflavones, total phenols, and antioxidant activity in different reproductive stages of common soybean, often used at R6. All four tocopherol isomers were presented in the beans, but at R5 and R6 δ-tocopherol had the highest concentration (39.6 and 44.8 µg/g), respectively. At later stages tocopherol level increased as well as total isoflavones content. The amount of daidzein, glycitein, genistein, and total isoflavones at R6 were 166.16 µg/g, 183.28µg/g, 375.57µg/g and 725.00µg/g, respectively. From R5 to full maturity these components increased 4.6-, 3.12-, 9.6-, and 5- fold, respectively. Free radical scavenging activity, total antioxidative activity, and total phenolic content was at the highest level in immature seeds, and decreased with maturing. Percent of reduction of DPPH radicals decreased from 58.5 % at R5 to 43.9 % at full maturity. According to Carrão-Panizzi et al. (2019) total isoflavones total β-glucosides, total malonyl glucosides, and total aglucones was lower at R6 of edamame compared to R8: 224.84 vs. 475.00 mg/100 g, 22.56 vs. 112.98 mg/100 g, 194.44 vs. 349.04 mg/100 g and 3.74 vs. 13.00 mg/100 g, respectively. In edamame beans, content of lutein and β-carotene, depending on variety, varied from 895 to 2119 µg /100 g dry weigh, and from 291 to 491 µg/100 g, respectively (Simonne *et al.* 2000).

Legumes, characterized by a low glycaemic index and high fiber content, play an important role in reducing in the risks of obesity, diabetes, cardiovascular diseases, cancer and high levels of cholesterol (Sharma, 2021). Two major storage proteins that belong to the globulin family known as glycinin (11S) and β–conglycinin (7S) are the primary sources of protein in soybeans and are considered high–quality proteins due to their balanced amino acid profiles, which provide all essential amino acids, limited only in methionine (Zarkadas *et al.* 2007). Other authors identified edamame as a complete protein source (Hertamawati and Rahmasari 2021, Lord *et al.* 2019). According to Song *et al.* (2013), the content of free amino acids in edamame varied between 4.6 and 10.2 mg g^{-1} dry matter including all 23 amino acids and all essential amino acids are present of 0.5 mg g^{-1} dry matter. Soybean protein is high in lysine, which is typically low in other common crops (Agyenim-Boateng *et al.* 2023). Liu *et al*(2019) reported that concentration of free amino acid was higher at R6 and varied between 74 and 129.2 mg/g. The most abundant amino acids were: arginine, alanine, serine, glutamic acid, and aspartic acid. The content of saturated fatty acid (palmitic acid + stearic acid) was generally higher at R6 while the concentration of unsaturated fatty acid (oleic acid + linoleic acid + linolenic acid) was higher at R8. The gama–aminobutyric acid (GABA) is also reported to be present in edamame beans and the average concentration was 0.437 mg g^{-1} dry matter. Flooding treatment before harvesting can enhance the level of GABA in edamame, without negative consequences on yield (Shiu et al. 2020). Among organic acids, citrate (2.8 mg g^{-1} dry matter) and malate (2.1 mg g^{-1} dry matter) have the highest concentrations and are related to sensory attributes (Song *et al.* 2013). Edamame contains lecithin, an essential component which supports brain development and can enhance memory and cognitive abilities.

Edamame – part of sustainable diet and sustainable agriculture

Edamame contributes to sustainable agriculture and a sustainable and healthy diet. The soybeans cultivation supports and improves soil fertility by facilitating nitrogen fixation (Agyenim-Boateng *et al.* 2023) through a symbiotic relationship with nitrogen–fixing bacteria, reducing the reliance on mineral nitrogen fertilizers at the same time. Due to its short vegetation, edamame could also be included in rotation as a double crop or even as a cover crop, increasing the status of organic matter and nitrogen. Even more, including edamame soybeans in crop rotation in both organic and conventional production might help break pest and disease cycles. Because of the short vegetation period and simultaneous flowering and grain filling, its biomass could also be successfully used as a feed (Zhang *et al.* 2017), i.e., for forage and silage production. From this standpoint, edamame growing increases crop diversity, fitting into various cropping systems. Due to its richness in protein, dietary fiber, vitamins, minerals and bioactive compounds, edamame has a high nutritional value. Edamame can be eaten fresh, in soups, salads, or as a snack. It can be processed by boiling or roasting (Duppong and Hatwerman-Valenti 2005). Thus, by including edamame in diets, nourishment with essential nutrients is supported (Agyenim-Boateng *et al.* 2023).

On the market, it can be sold fresh or frozen, in pods or as beans. Edamame popularity rapidly grows worldwide. The world population recognizes edamame as a healthy and tasty food. The health benefits distinguish edamame as a specialty food that is part of a healthy and sustainable diet. As a result of above, it is estimated that edamame consumption and market needs will rapidly grow over time, while cropping technology is still evolving. This gives opportunity to agro-science and agro–business to develop cropping practices and genotypes adapted to various agro–climates and expand edamame growth over arable areas.

CONCLUSION

Based on its unique nutritional profile, edamame soybean has a great potential to be included in healthy and sustainable nutrition, in regard to the standard soybean. Low in antinutrients and calories, but high in protein, vitamins, minerals, and other bioactive compounds which have heath promoting role, it could be concerned to be nutraceutical. Nevertheless, growing edamame as a vegetable crop could be challenging, particularly when weed control and irrigation are considered. Furthermore, as a legume crop, its cropping is beneficial due to the nitrogen fixation. So, from the nutritional and agro-ecological standpoints, edamame is multiple significant, whereas cropping system should be adjusted to the local agroecological conditions.

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***Alper Ahmet ÖZBEY*[1], *Nebi BİLİR**[2]**

SEEDLING MORPHOLOGY AND QUALITY IN CRIMEAN JUNIPER (*JUNIPERUS EXCELSA* BIEB.)

SUMMARY

Junipers (*Juniperus* sp.) have 1.56 million ha natural distribution which of total 23.3 million ha Turkish forest area. However, 1.1 million ha (71%) of junipers and 9.6 million ha (41%) of Turkish forest area is unproductive. Seedling quality and morphology is one of the important tools in conversion of unproductive forest to productive forest.

Seedling quality and morphology were investigated based on seedling height and root-collar diameter, and sturdiness index of 2+0 years containerized and bare-root seedlings of Crimean juniper (*Juniperus excelsa* Bieb.) grown at Forest Nursery from southern Türkiye. For the purpose, 150 seedlings were sampled randomly from each seedling type in a forest nursery to collect seedling height and root-collar diameter data. Sturdiness index was calculated by ratio between the height and diameter.

Collected data was performed for comparison of the seedling types for the characteristics by one-way analysis of variance, and to be determined relations among the characteristics by correlation analysis. The seedlings were distributed to quality classes of Turkish Standard Institute and Sturdiness index.

Studied bare-root seedlings (16.3 cm) showed higher height growth performances than containerized (14.5 cm) seedlings opposite to root-collar diameter (2.04 mm and 1.86 mm), while the seedlings showed large differences within seedling type for the characteristics. Averages of sturdiness index were 72.5 and 88.5 in containerized seedlings and bare-root seedlings, respectively.

Seedling types indicated significant ($p \leq 0.05$) differences for the characteristics. Positive and significant ($p \leq 0.05$) phenotypic relations were estimated among the characteristics in seedling types. Distribution of seedlings to quality classes varied for the characteristics, classes and seedling type.

Results of the study were discussed based on nursery practices and plantation forestry of the species.

Keywords: Afforestation, Diameter, Height, Nursery, Variation.

[1]A. Ahmet Özbey, South-Western Anatolia Forest Research Institute, Antalya, TÜRKİYE

[2]Nebi Bilir*, (corresponding author: nebibilir@isparta.edu.tr), Faculty of Forestry, Isparta University of Applied Sciences, Isparta, TÜRKİYE

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INTRODUCTION

Junipers (*Juniperus* sp.) are one of the largest taxa by 8 species at 1.6 million ha natural distribution which of 1.1 million ha (~71%) is unproductive in total 23.4 million ha Turkish forest area which of 9.7 million ha is unproductive (OGM, 2024). However, Crimean juniper (*Juniperus excelsa* Bieb.) has 82% natural distribution in Turkish junipers (OGM, 2014). Crimean juniper is also native in Albania, Greece, North Macedonia, Bulgaria, Cyprus, Syria, Lebanon, Azerbaijan, Crimea, Russia at 500 and 2700 m (Farjon, 2005, Figure 1). Crimean juniper has drought, heat and cold tolerant characteristics. It is getting importance based on climate change and adaptation ability to different ecological conditions. The species is used widely in afforestation, rehabilitation and other forestry and landscape practices because of these advantages at different ecological conditions (Eser, 2021a).

Figure 1. Natural distribution of Crimean juniper in the world

Seedling quality and morphology is one of the important tools in conversion of unproductive forest to productive forest, and also forestry and landscape practices. While seedling quality and morphology have important roles in these practices, there are limited studies carried out on seedling quality and morphology of Crimean juniper (i.e., Gülcü and Gültekin, 2005; Eser and Gülcü, 2019; Eser, 2021a). In these studies, morphology of seedling type at the same age and sturdiness index have not yet been compared. It is also known that many abiotic and biotic factors could impact on morphology and quality of seedlings such as seedling type or seed source (Yücedağ *et. al*., 2010; Eser, 2021b; Uslu and Bilir, 2020; Yücesan and Yıldırım, 2021; Öye and Bilir, 2024), and nursery conditions and practices (i.e., Dewald and Feret, 1987; Bilir, 2019 and 2021). It is getting importance of new studies carried out on different seed sources, nurseries and morphological attributes.

Quality and morphology of containerized and bare-root seedlings of Crimean juniper, produced in Beyler-Antalya Forest Nursery from southern Türkiye, were examined to estimate variation, correlation and distribution of seedlings to quality classes based on seedling height and root-collar diameter, and sturdiness index to contribute nursery practices and plantation forestry of the species in this study.

MATERIAL AND METHODS

In this study, 150 seedlings of the species were sampled randomly from each 2+0 years containerized (**CS**) and bare-root (**BRS**) seedlings (Figure 2) called also seedling type in the study. The seedlings were grown originated from natural forests selected phenotypical of the species (30°31' N latitude and 29°44' E longitude, and 1350 m) in Beyler- Antalya Forest Nursery (30°39' N latitude and 29°51' E longitude, and 1035 m) established at 85.4 decare at southern part of Turkey. Averages of annual temperature and total precipitation were 11.3 °C and 636 mm. Average of P^H was 8.02 at the nursery. Seedling height (**SH**) and root-collar diameter (**RCD**) of the sampled seedlings were measured at end of growing period of 2023.

The collected data was performed by following model of ANOVA for comparison of seedling types at SAS Package (SAS, 2004).

$$Y_{ij} = \mu + P_j + e_{ij} \quad (1)$$

Where Yij is the observation from the jth seedling of the ith type, μ is overall mean, Pi is the random effect of the ith type, and eij is random error.

Figure 2. A view from sampled seedlings

The seedlings were classified according to the Seedling Quality Classification of Turkish Standard Institute for the Seedling height (**SH**) and root-collar diameter (**RCD**) (Anonymous, 1988, Table 1). The seedlings were also classified for Sturdiness index (**SI**) (Aphalo and Rikala, 2003) as (Table 1):

$$\mathrm{SI} = \frac{SH(mm)}{RCD(mm)} \quad (2)$$

Table 1. Seedling quality classes of Turkish Standard Institute in the species

Quality classes	Seedling height (SH, cm)	Root collar diameter (RCD, mm)	SI
First class	8≤	2 ≤	SI≤50
Second class	8> SH ≥6	-	50<SI≤60
Cull	6>	-	SI>60

The characteristics were related by Pearson' phenotypic correlation analysis (SAS, 2004).

RESULTS AND DISCUSSION

Characteristics

Bare-root seedlings (16.3 cm) showed higher height growth performances than that of containerized (14.5 cm), while it was opposite in root-collar diameter (2.04 mm and 1.86 mm) (Table 2, Figure 3). Similar growth performances were also reported in Stinking juniper by Eser (2021a), while containerized seedlings of Taurus cedar and Brutian pine had higher growth performances than bare-root seedlings for seedling height and root-collar diameter (Çetinkaya and Bilir, 2019; Bilir, 2019; Eser, 2021b). Averages of seedling height and root-collar diameter were found 21.9 cm and 5.2 mm in 1+1-year containerized seedlings, and 24.7 cm and 3.7 mm in 2+0 year bare-root seedlings of Crimean juniper, respectively in another forest nursery and seed source (Eser, 2021a).

Table 2. Averages ($\bar{x}$) and coefficient of variation (CV%) for the characteristics in the containerized (**CS**) and bare-root (**BRS**) seedlings

	CS		**BRS**		**Total**	
	$\bar{x}$	**CV**	$\bar{x}$	**CV**	$\bar{x}$	**CV**
SH (cm)	14.5	19.6	16.3	18.2	15.4	19.7
RCD (mm)	2.04	19.6	1.86	17.6	1.95	19.2
SI	72.5	18.7	88.5	17.0	80.5	20.3

Many abiotic such as seedling type, nursery conditions, nursery practices, soil characteristics (i.e., Dewald and Feret, 1987; Dilaver *et al*., 2015; Yılmazer and Bilir, 2016; Yazıcı and Turan, 2016; Yazıcı, 2018; Eser and Gülcü, 2019), and biotic such species and seed source (i.e., Parker and Niejenhuis, 1994; Ivetić, and

Škorić, 2013) could impact on seedling morphology and quality in forest tree species. These results emphasized importance of these factors in nursery practices.

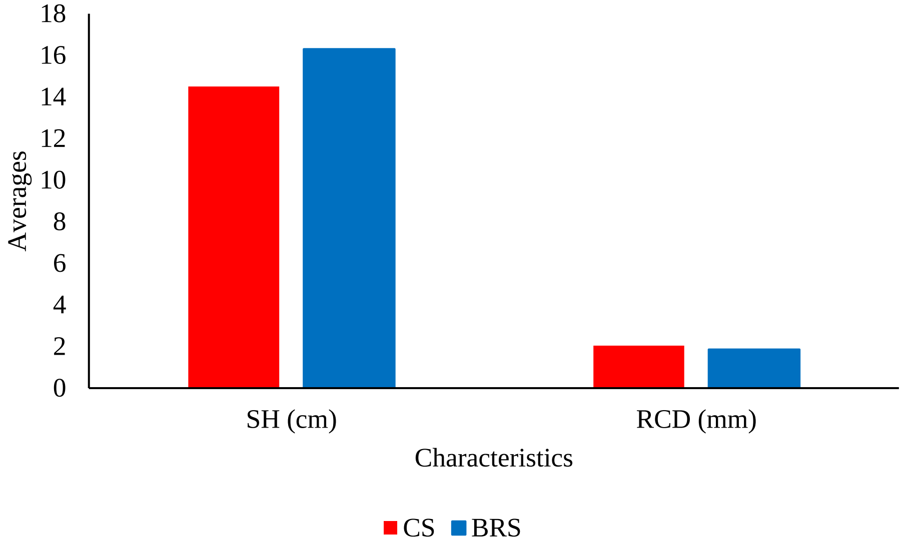

Figure 3. Averages of the characteristics for the seedling types

Averages of sturdiness index were 72.5 in containerized seedlings and 88.5 in bare-root seedlings. Besides, individual seedlings had large difference for the characteristics within seedling type (Table 2, Figure 4). For instance, seedling height ranged from 8.5 cm to 24.0 cm in containerized seedlings, while it was between 9.5 cm and 24.7 cm in bare-root seedlings. Sturdiness index had the lowest variation in seedling types (18.7% and 17.0%) opposite to the pooled types (20.3%) based on coefficient variation. Similar variations were also reported in different forest tree species (i.e., Dilaver *et al*., 2015; Çetinkaya and Bilir, 2019; Eser, 2021a, b). The variations indicated importance of individual selection in seed harvesting and tending in nursery practices for higher growth performance in the species.

Significant ($p \leq 0.05$) differences were found between seedling type for the characteristics according to results of analysis of variance. Significant ($p \leq 0.05$) differences between seedling types were also found in Crimean and Stinking junipers (Eser, 2021a), and in different forest tree species (i.e., Dilaver *et al*., 2015; Yılmazer and Bilir, 2016; Çetinkaya and Bilir, 2019; Öye and Bilir, 2024). The result emphasized importance of seedling type for afforestation practices and plantation forestry.

Significant ($p \leq 0.05$) and positive correlations were also reported between seedling height and root-collar diameter in Crimean and Stinking junipers by Eser (2021a, b), and in different forest tree species (i.e., Dilaver *et al*., 2015; Çetinkaya and Bilir, 2019; Uslu and Bilir, 2020). The correlations could be used in nursery practices of the species such as tending.

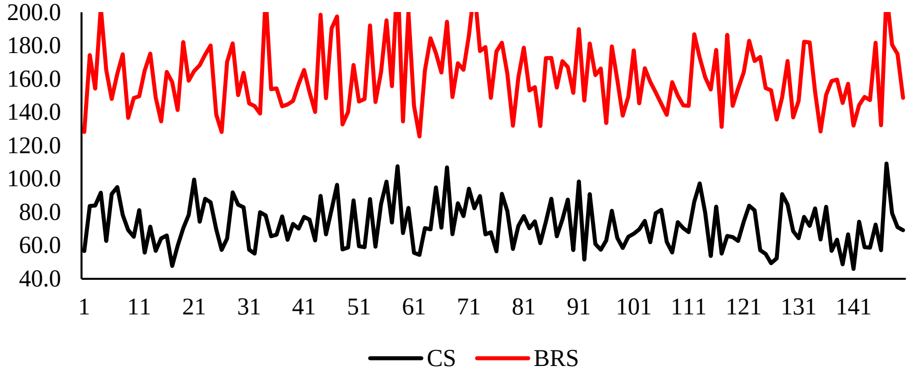

Figure 4. Individual sturdiness index for the seedling types

Table 3. Phenotypic correlations among the characteristics in the seedling type

	r	SH	RCD
CS	**RCD**	.549**	-
BRS		.567**	-
Total		.446**	-
CS	**SI**	.420**	-.505**
BRS		.450**	-.460**
Total		-.520**	.507**

**; Correlation is significant at the 0.01 level.

Seedling quality

Distribution of seedlings to quality classes changed for the characteristics, classes and seedling type (Table 4). The results were well accordance with results of early studies carried out in different forest tree species (i.e., Dilaver *et al.*, 2015; Yılmazer and Bilir, 2016; Çercioglu and Bilir, 2016; Bilir, 2019; Uslu and Bilir, 2020). All seedlings of seedling types were in first class (8 cm ≤ SH) for seedling height. Containerized seedlings (56% in first class) had better quality than bare-root seedlings (45.7% in first class) for root-collar diameter, and also for sturdiness index (Table 4, Figure 5).

Seedling height and root-collar diameter were different quality criterions, while they were combined in sturdiness index. Sturdiness index could also be accepted as balancing of above and under parts of seedlings. However, quality classes of sturdiness index have not been tested in the field, yet. The results of seedling quality showed importance of quality characteristics and classes.

Table 4. Distribution of seedlings to quality classes of Turkish Standard Institute (SH and RCD) and sturdiness index (SI) in seedling types

	Quality classes	SH	RCD	SI
CS	**First class**	100	56.0	2.7
	Second class	-	-	20.0
	Cull	-	44.0	77.3
BRS	**First class**	100	35.6	-
	Second class	-	-	2.0
	Cull	-	64.4	98.0
Total	**First class**	100	45.7	1.3
	Second class	-	-	11.0
	Cull	-	54.3	87.7

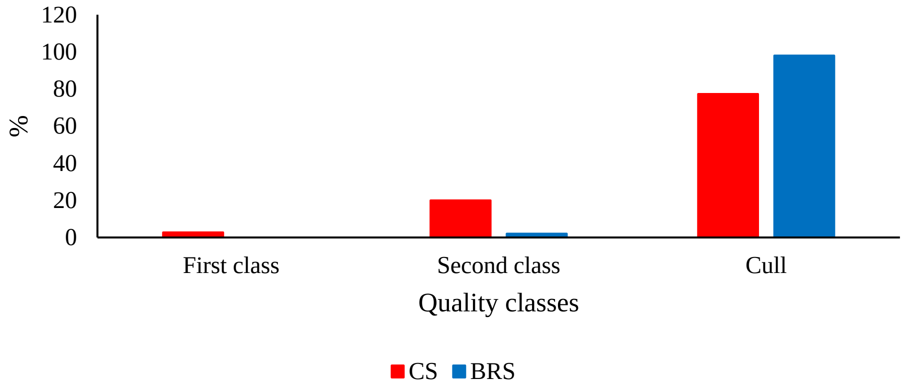

Figure 5. Distribution of seedlings to quality classes of sturdiness index for the seedling types

CONCLUSIONS

Averages height and sturdiness index of bare-root seedlings were higher than containerized seedlings, while containerized seedlings had higher root-collar diameter. Seedling types showed significant ($p \leq 0.05$) differences for the characteristics. The results indicated importance of seedling type and selection criterions in afforestation, plantation and other forestry practices of the species. All seedlings of the seedling types were in first class for seedling height, while containerized seedlings had better quality than bare-root seedlings for root-collar diameter, and also for sturdiness index. However, quality classes of sturdiness index have not yet tested in the field. The quality classes should be tested at field. There were positive and significant ($p \leq 0.05$) correlations among the characteristics of the seedling types. The relations could be used in nursery practices of the species.

The present study was carried out only one nursery and seed sources. Future studies should be carried out by new characteristics such as survival, fresh and dry weights in different forest nurseries on the species.

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Marija MARKOČ[1] and Jovo KRSTAJIĆ[2]

MULTICRITERIAL ANALYSIS OF AGROECOLOGICAL VINEYARDS IN MONTENEGRO: HOW MOTIVATION AFFECTS VITICULTURE?

SUMMARY

Viticulture in Montenegro relies on various traditional and extensive practices that promotes sustainability and resilience in winemaking industry. Even though agro-ecological management systems protect natural habitats and landscapes, there is an evident lack of connection between farmers and other stakeholders in Montenegrin pilot area. This is the first multi-criterial research within winemaking industry in Montenegro that have taken into consideration both natural and social context in order to propose innovative solutions that may enhance the agro-ecological transition in viticulture. Tailored research methodology enabled analysis of the Main Agro-ecological Structure (MAS) development of each farm, as well as the analysis regarding perception of favorable conditions for Territorial and Environmental Context to agro-ecological transition (TEC). The results of the research show that the pilot area is extremely favorable for agro-ecological transition due to the high degree of vulnerable environmental protection and presence of various ecosystem services. However, this study identified low degree of motivation among farmers due to complex administrative bureaucracy procedures connected to subsidy schemes, high production costs, complex terrain configuration followed by low infrastructure development, inadequate collaboration with HORECA sector leading to stock risks, social conflicts leading to negative competitiveness, lack of interest among youth to join the sector and insufficient networking with public authorities in joint development of territorial strategies and action plans. Introduction of innovative agro-ecological practices followed by raising the awareness among farmers about benefits of the studied management systems on their production performances will increase their internal motivation and thus create sustainable ambience in domain of viticulture that will act appealing for young farmers to initiate or improve their agri-business.

Keywords: agroecology, viticulture, motivation

[1] Marija Markoč (corresponding author: marija.markoc@t-com.me), House of Olives Ltd., Ul. 9. januara 211A, Stari Bar, 85000 Bar, MONTENEGRO

[2] Jovo Krstajić, Department for Social Welfare and Non-Governmental Organizations, Municipality of Bar, Bulevar revolucije 1, 85000 Bar, MONTENEGRO

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INTRODUCTION

Agroecology involves various approaches to solve actual challenges of agricultural production. However, agroecology initially dealt primarily with crop production and protection aspects, in recent decades, new dimensions such as environmental, social, economic and ethical are becoming relevant (Wezel *et al.*, 2009). The definition of agroecology introduced all the participants in the food system and this included the importance of re-establishing the close relationships between the people who grow the food and the people who eat it, while reducing the negative impacts of the intermediary system between the two. Agroecology became a way of building relationship-based market systems that are equitable, just, and accessible for all (Gliessman, 2007). According to Gliessman (2018), agroecology is the integration of research, education, action and change that brings sustainability to all parts of the food system: ecological, economic, and social. To improve its sustainability, viticulture should increase the provision of ecosystem services to decrease its use of inputs and the resulting environmental impact while maintaining high socioeconomic performance (Gary *et al.*, 2017). Romero *et al.* (2022) emphasize that the establishment of sustainable production patterns based on socio-economic and environmental criteria is a key strategy in progression toward viable and competitive wine production. Furthermore, this research demonstrates the necessity of establishing systems that make cultivation sustainable by promoting the quality of the wine grape and by implementing working methods with favorable social, economic, and environmental consequences for rural populations and the environment. Ranzani et al. (2023) shared that organic wine producers' attention to environmentally conscious production should be complemented by financial incentives or regulatory support, by involving policy-makers and sharing technical assistance to promote landscape enhancement and encourage sustainable land use practices in viticulture areas. Furthermore, consumer awareness on organic wines produced in sustainable agro-ecosystems and carbon footprint information could help create demand for such products. The MAS can be useful tool in the planning processes of the farms because it allows the quantification of the internal and external corridors, including natural vegetation; as well for defining the context of landscape management because it shows a series of cultural relations (economic, social, symbolic and technological) that are normally overlooked by the partial analysis of landscape ecology (León-Sicard *et al.*, 2018).

Viticultural tradition in Montenegro dates back before the Roman period, as stated by Maraš *et al.* (2015). Wine production in Montenegro is based on the autochthonous grape varieties Vranac and Kratošija that make Montenegro recognizable in the wine world (Sošić *et al.*, 2023). Viticulture and winemaking of Montenegro is still mainly based on autochthonous grapevine varieties ('Vranac', 'Kratošija', 'Krstač' and 'Žižak'). The available literature data indicate a long tradition and an important breeding history of grapevine evaluation in Montenegro, especially concerning 'Vranac' and 'Kratošija'. Among the producers of grapes and wine dominant position in Montenegro has the company “13. Jul – Plantaže” (94.5% of the total production, i.e. quantity of 145.000 hl of all types of wine) (Pajović-Šćepanović *et al.,* 2016). However, intensive development of viticulture and winemaking sector is taking place in the last decade in Montenegro, with increasing surface under vineyards, as well as the number of wineries and the number of wine types. The study of Pajović-Šćepanović *et al.* (2016) stated that technological conditions of production in the company “13. Jul – Plantaže” fully meet internationally accepted standards, while the other, small producers (family businesses) technology of wine production ranges from the use of modern equipment to the traditional methods of production. Smallholder agroecosystems that use agroecological practices and are surrounded by a moderately heterogeneous matrix have better attributes to initiate the agroecological transition process at the community level (Salazar-Rojas *et al.*, 2023). For the production practice in the agro-ecological conditions of the Podgorica subregion, the optimum loading is 16 buds per vine because it enables obtaining high yields as well as good quality of grapes for the Cardinal variety (Popović *et al.,* 2023.). Sošić *et al.* (2023) concluded that polyphenolic content of the analyzed wines, including other chemical characteristics, indicates that these Montenegrin autochthonous varieties can produce wines of exceptional properties and very good quality.

The last three decades have seen a significant increase in number of farm families diversifying their on- and off-farm production. The factors for diversification include environmental pressures, climate change, a decline in terms of trade in agriculture, low-income elasticity in commodities markets, and over-reliance on raw products (McGehee *et al.,* 2007). Kuznetsova *et al.* (2018) emphasized that in order for the agro-industrial complex to become a real driver for the development of other sectors of the economy, it is necessary to intensify investments in the fixed capital of the industry and improve the development of rural social infrastructure, to solve the problem of chronic poverty, with a priority focus on the growth of professionalism, productivity and wages, and a reduction in staff turnover. Despite the favourable natural conditions, especially for organic viticulture, a comparison of land use in the last two decades shows the abandonment and overgrowth of vineyards in the higher, so-called vineyard sites (Topole *et al.,* 2023).

In modern conditions, an integrated approach and systematic improvement of the social and economic situation is required, especially in rural areas, without which it is impossible to develop the country's agriculture and improve the quality of life. Besser and Mann (2015) concluded that farmers’ work satisfaction is positively affected by farm income proxies and monetary return, as well as by other quality factors besides economic returns, thus indicating importance of both internal and external motivation factor in the overall farmers’ satisfaction.

MATERIAL AND METHODS

Participants and Procedure

This study was conducted with the ten owners of agro-ecological vineyards in the area of Crmnica, Municipality of Bar, Montenegro. The average farm area was 1.25 ha, the smallest farm had 0.31 ha, while the largest one had 1.9 ha. Each of the onsite interview lasted from 1.5 to 2 hours. The average age of the participants was 44.67 years. The experience in viticulture varied, with 19.4% having the experience less than 5 years, 16.7% less than 10 years, 20.4% less than 15 years, and 43.5% more than 15 years of experience in viticulture. In terms of education, 11.1% completed high school, 56.5% hold an undergraduate degree, 23.1% hold master's degree, and 9.3% hold a PhD. Nine participants were Montenegrin (one is Russian) and all of them are Orthodox. The survey was conducted in two rounds, in May 2021 and May 2022, in order to compare the results and to evaluate the degree of agro-ecological transition and awareness raised in the Pilot area.

Instruments

In order to implement Multi-criteria analysis of the agro-ecological vineyards in Montenegrin pilot area (Crmnica), participants were requested to fill out surveys during the onsite visit, consisting of the analysis of the Main Agroecological Structure (MAS) development of each farms, as well as the analysis regarding perception of favorable conditions for Territorial and Environmental Context to agro-ecological transition (TEC).

This section shows the useful data for analysis of MAS development, with the final ranking that set up the starting point for the agro-ecological transition for each company, and traces a basic scenario for the entire Pilot area (Crmnica). The MAS can be seen as the internal configuration or spatial arrangement of the farm and the connectivity between its different sectors, patches and corridors of vegetation or productive systems and the exchange with external environment. Taking into consideration the internal configuration of the farm, in particular, the observation focuses on the degree of openness and on the exchange relationships (between the different living species and between the different cultural contaminations) that the same farm maintains with environment. The more the farm presents an articulated arrangement of its spaces, able to alternate different crops, preserve the presence of trees and hedges, keep functional small ditches and water bodies, the more will be able to offer vital connection systems, both internally and externally, with the surrounding environment. Furthermore, thanks to the qualitative questions included in the interview, it is possible to trace a first indicative identikit of the typical Crmnica farm. Considering the fragmented nature of the vine plots, even within the same company properties, the indicators CMELS, EEC, EIC, DEC and DIC, had been considered referring to the largest portions of the farm (Table 1), if exceeded 66% (2/3) of the overall land area. Otherwise, it was necessary to calculate the average value of an analysis conducted separately for each individual farm’s portions until at least 50% (half) of the total land area has been investigated.

The last four questions of the survey regarding Territorial and Environmental Context to the agro-ecological transition (TEC) allowed us to deepen perception regarding certain issues that external factors may cause to the agricultural holding, and vice-versa, regarding impacts that agricultural holding has on external environment. In particular, the two issues that we investigated were: the perception of the territorial context and its agro-ecological vocation (first two indicators: Economy and Production (EP) and Networks, Relations and Social recognition (NRS)); the perception of the degree of environmental impact of agricultural activity and its management (last two indicators: Compounds Toxic for the Environment and Human Health (CTEHH) and Farm Waste Management (FWM)). Both questions were useful for assessing whether the wider territorial context is favorable or not to the agro-ecological transition. The perception scales have been based on the satisfaction level, where 0 represents "no satisfaction at all" and 4 represents "highest satisfaction possible".

Table 1: The 10 MAS indicators

Indicator	Acronym	Description
Connection with the main ecological landscape structure	CMELS	Assesses the distance of the farm in relation to the nearby fragments of natural vegetation, mainly forest covers and bodies of water.
Extension of external connectors	EEC	Evaluates the percentage of the linear extension of live fences located in the perimeter of the farms.
Extension of internal connectors	EIC	Evaluates the percentage of the linear extension of the rows of vegetation but internally.
Diversification of external connectors	DEC	Evaluates the diversity of live fences or hedges located in the perimeter of the major agro-ecosystem.
Diversification of internal connectors	DIC	Evaluates the diversification of internal living fences.
Use and Soil Conservation	USC	Evaluates the distribution percentage of different covers within the farm and the conservation of the soil (evidences of erosion).
Management of Weeds	MW	Evaluates the management practices and systems of weeds control.
Other management Practices	OP	Evaluates the types of production systems (ecological, conventional or in transition) of each farm.
Perception – Awareness	PA	Evaluates the degree of conceptual clarity and awareness of producers regarding agro-biodiversity.
Level of Capacity of Action	CA	Evaluates the capacities and possibilities of farmers to establish, maintain or improve their MAS

The final ranking of the MAS development level of the interview farmers is obtained by combining it with the interpretation scale presented in the methodological document and reported below, as Table 2:

Table 2: Scale of MAS interpretation

MAS development	Value
High developed	80 – 100
Moderately developed	60 – 79
Slightly developed	40 – 59
Weakly developed, with cultural potential	20 – 39
Weakly developed, without cultural potential	10 – 19
No agro-ecological structure	1 - 9

The final calculation of the MAS was obtained by adding the resulting value of each of the aforementioned indicators, according to the following formulation:

$$MAS = CMELS + EEC + EIC + DEC + DIC + USC + WM + OP + PC + CA$$

The final ranking of the degree of vocation of the Territorial and Environmental Context of the Pilot Area to the agro-ecological transition is shown below (Table 3) combining it with the scale of interpretation present in the methodological document:

Table 3: Scale of TEC interpretation

MAS development	Value
Extremely favorable context for agro-ecological transition	49 – 62
Favorable context for agro-ecological transition	33 – 48
Unfavorable context for agro-ecological transition	17 – 32
Very difficult context for agro-ecological transition	1 - 16

The final calculation of the TEC is obtained by adding the resulting value of each of the indicators, according to the following formulation:

$$TEC = EP + NRS + CTEHH + FWM$$

RESULTS AND DISCUSSION

The Main Agro-ecological Structure (MAS) development

Connection with the main ecological landscape structure (CMELS)

Almost all of the selected farms are surrounded by natural elements. The different types of natural elements are mainly contiguous or even overlapping. This characteristic is the peculiarity of the landscape, where the terraces planted with vines alternate with large wooded areas, which often delimit the different properties. All of the farms have a high possibility for biotic interrelationships or even a high degree of biological connectivity with different natural elements (forests, shrubs, water sources – lake, rivers, streams etc.) located nearby the farms, while the vicinity between the natural elements ranges from less than 150 meters up to 450 meters.

Extension of external connectors (EEC)

The external connectors within the farms create discontinuity between the cultivated surface and the external environment, thus constituting the perimeter made up of "living" elements, among which the woods and hedges are the most present ones. Among "non-living" elements, the most present are asphalted roads, the mule tracks and dry stone walls, a distinctive feature of grapevine growing area of Crmnica (Figure 1).

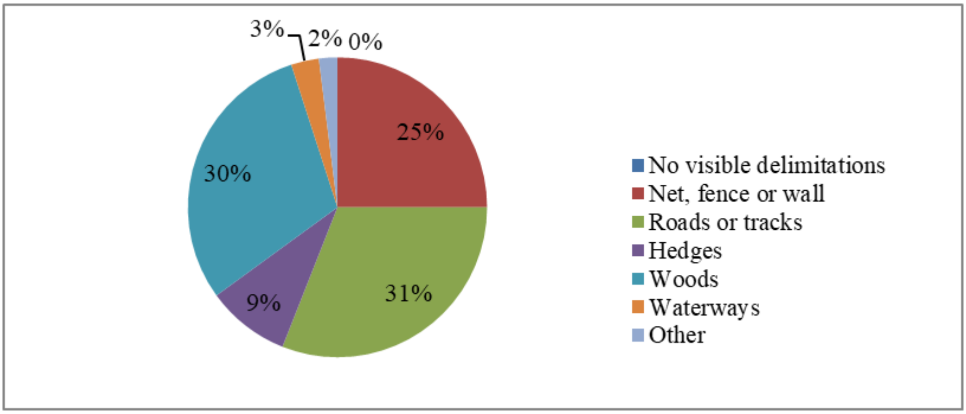

Figure 1: Percentage composition of external perimeter

Extension of internal connectors (EIC)

Internal dividers of the farms are the elements separating different parcels that belong to the same property. However, "living elements" are rare, except of a bit more present hedges (Figure 2). With the exception of three farms, the forest and waterways could not be identified. The "non-living" elements are widely present, such as asphalted roads, old stony mule tracks, as well as net and fences (dry stone walls).

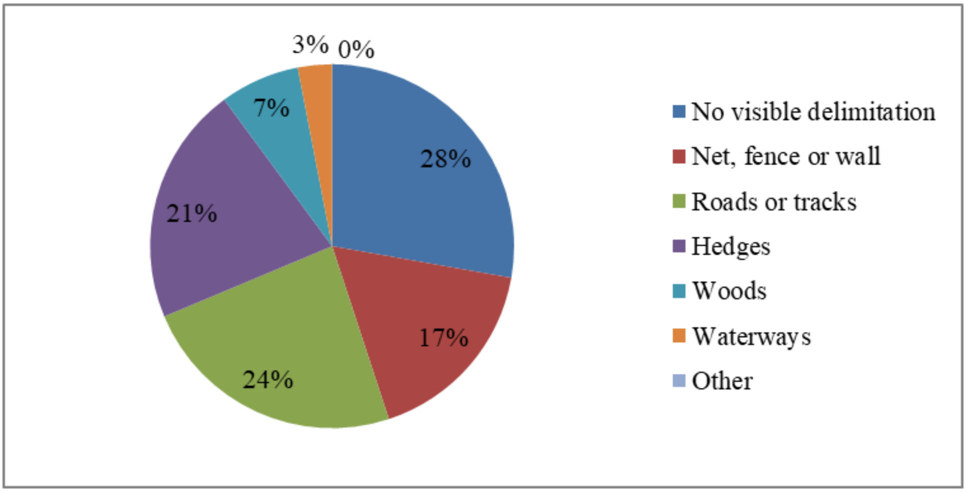

Figure 2: Percentage composition of internal dividers

Diversification of external connectors (DEC)

The degree of diversification of the “living” elements on the farm’s perimeter is very high within all the farms surveyed; and therefore, there are greater connection functions. These data confirm the high capacity of biotic interrelations between external and internal environment. The living perimeter present in all the areas analyzed is characterized by the presence of over 4 different tree species, or several layers of vegetation, of at least two rows of vegetation (8 farms) or by the presence of 2 to 3 different tree species, with at least two layers of vegetation, of at least two rows of vegetation (2 farms). There were no single species-layer-row cases; neither was a case without any living elements present, thus ensuring great interconnection function.

Diversification of internal connectors (DIC)

Widespread presence of “living” dividers in 5 out of 10 cases, 4 cases of quite widespread and sporadically widespread presence of biotic interrelationships between the crops and internal dividers and only 1 case of marginal presence of such relations, show great chances for agro-ecological activity. There is only 1 farm with presence of over 4 different tree species – several layers of vegetation – at least two rows of vegetation; 7 farms with the presence of 2 or 3 different tree species – at least two layers of vegetation – 1 or 2 rows of vegetation and 2 farms with mainly single species internal connectors, distributed in a single row. It should be emphasized that sporadic incidences were not taken into consideration, but only multi-layered structure, with multiple rows and species.

Use and Soil Conservation (USC)

The small sized and scattered plots, ingrained traditional extensive agricultural practices in Crmnica pilot area allowed diverse cultivation systems. Even though the grapevine is the leading plant species in this area, other Mediterranean fruit species such as pomegranate, figs, kiwi, peaches, apples, sporadical citrus trees and olive fruits, but also vegetables are cultivated as well. In certain cases, these examples cannot be characterized as poly-culture, due to sporadic and individual cases of other plants. All of the 10 farms have been characterized by the presence of at least two crops and there is no example of monoculture farm. Even though majority of the farms are located on the slopes and heavily accessible terrains, erosion is absent or limited and not very significant (8 farms). Traditional practices of building dry stone walls and terraces as anti-erosion measures limited the negative impact of erosion and enabled successful grapevine growing with efficient fertilization and irrigation of the crops.

Management of Weeds (MW)

Weed management, influenced by particular orographic conditions and the context of high slopes, takes place in most cases with the use of hand-operated machinery and tools (brush cutters), and without the support of agricultural vehicles such as tractors. At 6 out of 10 farms, there is neither presence of random strips nor areas of weeds, due to exclusively mechanical management.

Two out of 10 farms use chemicals combined with mechanic management. There is no exclusively chemical management of weed in any of the selected farms.

Other management practices (OP)

None of the companies involved in the multi-criteria survey is awarded with an organic certification. Only one farm is categorized as "conventional" farm even though there are no intensive agri-technical measures implemented in here, but the presence of chemical weed management and regular pests and disease protection with non-selective pesticides is practiced. The majority of the winemakers interviewed adopt integrated agricultural practices (90%) that emerged from ingrained traditional extensive practices regarding soil management, pruning techniques, rainfed agriculture, limited use of pesticides and similar.

Perception – Awareness (PA)

Even though the farmers show high degree of sensitivity towards the sustainable agriculture and environmental responsibility, it was noticed that farmers have a low motivation for implementing agro-ecological practices in order to shift their production to more sustainable approach. Furthermore, this pilot area became one of the leading tourism destinations within Bar municipality, and therefore it is necessary to raise the awareness and farmers' knowledge about biodiversity protection (flora and fauna, as well as water and soil resources). Finally, it is necessary to show the farmers that integrated agriculture and agro-ecological principles are not an additional financial nor labor burden for their performance. In order to fulfil these goals, participation of all the stakeholders is mandatory, such as integration of advisory services, local authorities and relevant ministries. Their contribution have to be given through various subsidy schemes as well as to overcome administrative bureaucratic procedures through the formation of working groups that would regularly visit the farms and work onsite with farmers who have low information skills or language barriers in order to identify their needs, propose the solutions and lead them through the process which will result with raised awareness among farmers and higher percentage of available funds withdrawal. This strategy would enhance both the internal and external motivation among experienced farmers, but also among young and interested beginners that initiate agricultural business.

Level of Capacity of Action (CA)

All participants believe that additional workload is necessary for agro-ecological production and therefore it has discouraging and demotivating impact. Furthermore, 80% of the farmers emphasize lack of economic and financial resources for their transition, 60% of them finds it unavailable to accentuate poly-cultivation. Encouraging data is that even 80% of the farmers is motivated to shift to organic farming. Furthermore, farmers are aware of the fact that customers prefer home-made products, made of domestic varieties by using traditional practices. Therefore, there is a high potential for agro-ecological transition; however, individual efforts will not gain a willing progress, but a multi-side contribution is strongly recommended (Figure 3).

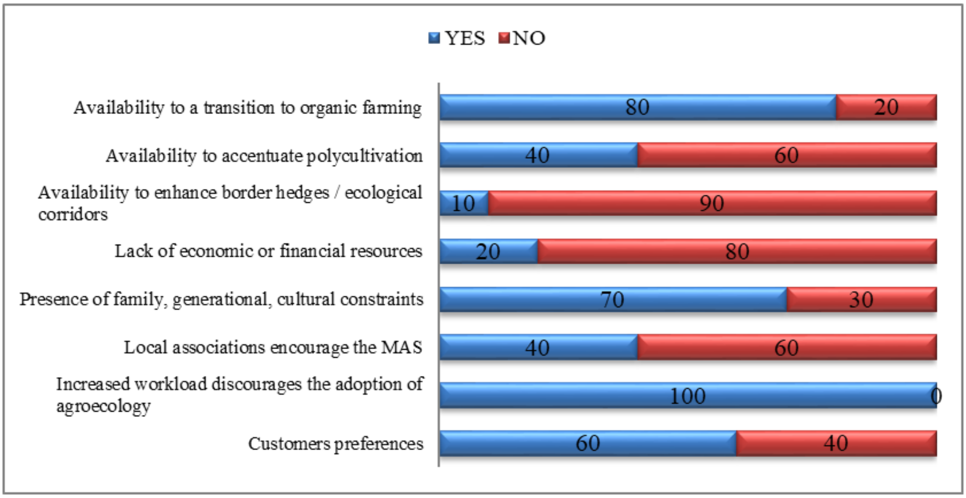

Figure 3: Capacity for Action

Regarding MAS analysis, all of the selected farms are devoted to the agro-ecological transition and adoption of ecological principles. Therefore, 2 out of 10 farms are classified as "high developed", while 6 holdings are "moderately developed" in an interval of just 8 points. Only one farm was at the lower limit of the "slightly developed" category, while one farm is "moderately developed" (upper limit of "slightly developed" category).

Territorial and environmental context (TEC)

Economy and Production (EP)

Farmers are mostly satisfied with "grape quality" and "wine quality". It is widely known that farmers from Crmnica region are very confident in the quality of their final products and therefore, the prices are often very high. Therefore, income from wine production in this area is slightly higher than the income coming from other agricultural activities. Even though most of the farmers are satisfied with their yield, it can be significantly improved by adopting certain innovative practices and diversifying their production that would have a positive environmental impact.

Network, Relations and Social recognition (NRS)

The perception of the social context also represents a useful indicator to understand if territory of Crmnica can be suitable for an agro-ecological transition (Figure 4). There is an evident intolerance and negative competition between local grapevine growers and wine producers. Furthermore, the role of the grapevine grower as the protagonist in rural development is not sufficiently recognized by the competitive authorities. Farmers increased their marketing skills by introducing accommodation facilities within their holdings. However, there is a problem with grey economy and "selling the products at the doorstep". Therefore, it is necessary to create a stronger network between small producers and HORECA sector. Regarding the small sized farms and high prices of mechanization and processing equipment, higher sustainability and productivity could be reached through farmers clustering and common resources sharing.

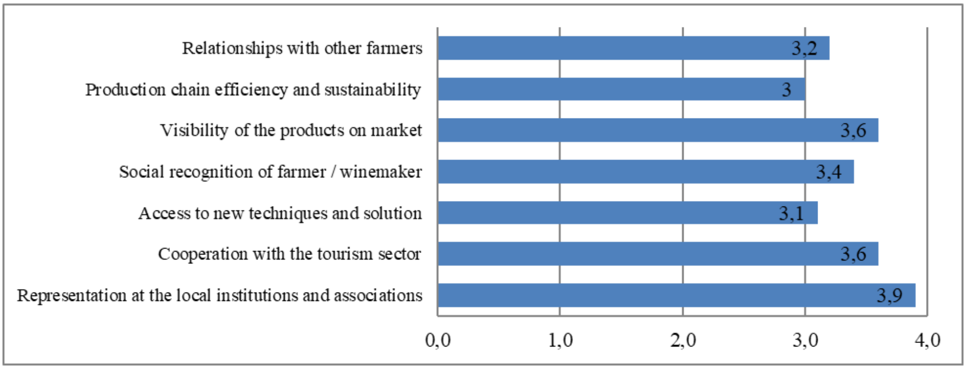

Figure 4: Network, relations and social recognition perception

Compounds Toxic for the Environment & Human Health (CTEHH)

There are no problems related to toxicity (high concentrations of nitrates or phosphates in both groundwater and surface water and soil) for both the environment and humans.

Farm Waste Management (FWM)

Waste management practices are related with producing organic manure through the composting of pruning residues.

Nine out of ten farms have been categorized as "extremely favorable" for agro-ecological transition regarding the perception of the territorial context.

CONCLUSIONS

Multicriteria analysis of agroecological vineyards in Montenegrin pilot area of Crmnica revealed great potential for agroecological transition of the whole area. However, this comprehensive analysis emphasized further analysis social factors impact, since low motivation of experienced winemakers have been identified. Therefore, this conclusion leads us to reject the hypothesis that agroecology transition is dependent from natural factors only. It's worth noting that this study has some limitations, such as a small sample size. Therefore, further research with a larger and more diverse sample, including other Montenegrin viticulture zones, may be needed to gain a deeper understanding of the potential links between agroecological transition and overall satisfaction of both young and more experienced farmers.

ACKNOWLEDGEMENTS

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***Polina LEMENKOVA*[1]**

MAPPING WOODLANDS IN ANGOLA, TROPICAL AFRICA: CALCULATION OF VEGETATION INDICES FROM REMOTE SENSING DATA

SUMMARY

This paper presents the application of the scripting algorithm GRASS GIS for calculation and visualization of vegetation indices using satellite data. The data include satellite images Landsat-8 OLI/TIRS covering tropical rainforests of central Angola. The images were acquired in July 2013 and July 2023. The methodology is based on using module 'i.vi' of GRASS GIS which automatically calculated 10 vegetation indices: DVI, NDVI, ARVI, EVI, GEMI, MSAVI2, NDWI, PVI, GARI and IPVI. The algorithms of data processing and calculation of vegetation indices are presented in the scripts. The results include the extracted information on distribution of bright green vegetation compared with other land cover types: tropical forests and coastal areas distinguished from artificial surfaces and urban areas, soils and coastal shores. The results indicated landscape dynamics in Angola with decline in tropical forests since 2013 until 2023. The machine-based workflow increases computational efficiency through fast processing of satellite data. The use of scripts demonstrated that programming method of automated information extraction from satellite images is effective for environmental monitoring of tropical African landscapes in rainforests.

Keywords cartography, programming, environment, ecology

INTRODUCTION

This paper studied the vegetation health in the tropical landscape of southern Africa, Angola, using calculation of vegetation indices. Knowledge of the size, distribution, and evolution of vegetation patches is an essential element of environmental studies on land cover change. Areas affected by deforestation or converted into settlements indicate environmental degradation in tropical regions. Environmental monitoring can be effectively implemented to detect such

[1] Polina Lemenkova (corresponding author: polina.lemenkova2@unibo.it), Alma Mater Studiorum – University of Bologna, Department of Biological, Geological and Environmental Sciences, Bologna, ITALY

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problems using satellite images (Sunny et al., 2022; Lemenkova, 2022a; Koon et al., 2023).

However, processing remote sensing (RS) data using Geographic Information System (GIS) involves manual work. In contrast, computer vision provides automated approach through algorithms (Dumay and Mainguet, 2009).

RS data has long been used for these purposes in various regions of Africa (Bardinet, 1981; Jacques et al., 1993; Lemenkova, 2023 a, b; Dawelbait et al., 2017). However, the application of RS data in Angola has mainly been used to map general land cover types using traditional GIS (Schneibel et al., 2016; Lourenco et al., 2022; Lehmann et al., 2023; Awadallah et al., 2015). In coastal areas, tropical forests are scattered in wetland ecosystems and are difficult to map using traditional approaches. In contrast, the machine approach can automatically detect areas covered by healthy vegetation discriminating landscape patches on satellite images by the difference in spectral reflectance of bright green leaves. This paper presents the use of such technological approach presented through programming.

STUDY AREA

We used the modules and libraries of the GRASS GIS software (Neteler et al., 2012) to map vegetation indices in central Angola, Figure 1.

Over the last 10 years, land cover types and vegetation in Angola have changed rapidly due to environmental and climate impacts and anthropogenic activities. This trend is visible by comparing satellite images on different data covering the same area. While the definitions of landscape patches and vegetation types are mainly determined using field surveys based on their size and level of complexity, showing the heterogeneity of the area, in the mountainous regions of Angola, the identification of landscape patches is difficult due to the inaccessible region for topographic studies. Therefore, it is worth investigating the dynamics of vegetation growth and plant status with freely available satellite images such as Landsat scenes (Ruppen et al., 2023). Landsat data are widely used for vegetation mapping, calculating vegetation indices, and ecological monitoring.

The landscapes of Angola are characterized by a mosaic of vegetation including coastal plains, tropical rainforests in the central regions, swamps and wetlands dominant in the mountainous regions and to grassy and semi-deciduous forests in the central regions of the country. The details of their distribution are shown in land cover map based on spatial data from the Food and Agriculture Organization (FAO), Figure 2.

The difference between these contrasting vegetation types can be detected using RS data. Specifically, the effective tool for land cover monitoring is the calculation of vegetation indices. Its effectiveness is explained by the difference in spectral reflectance in the Red/NIR channels of satellite images that well indicates the distribution of vegetation contrasting with other land cover types.

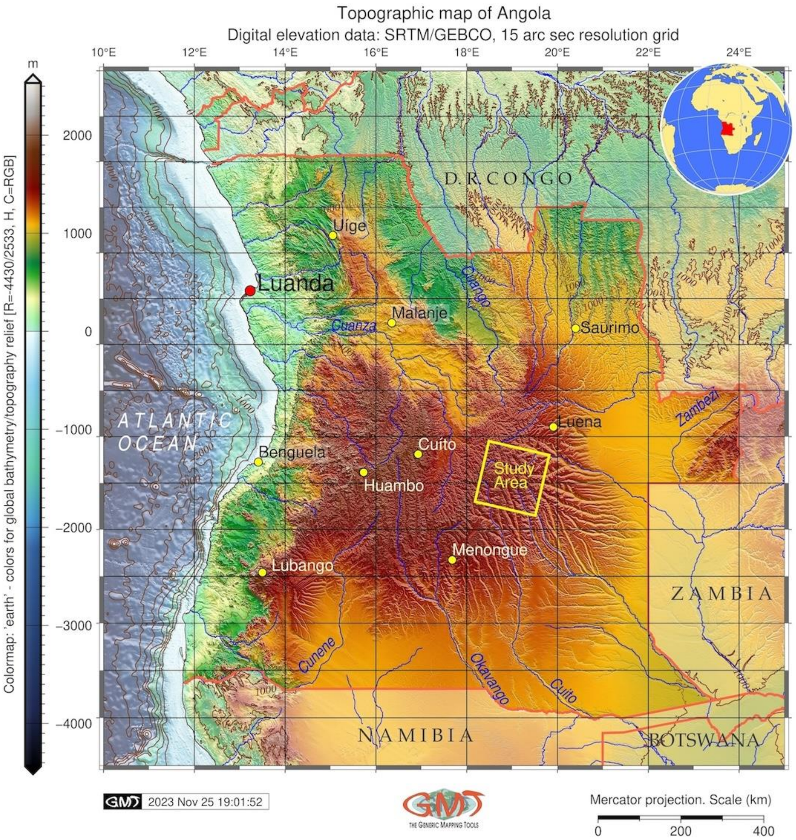

Figure 1. Topographic map of Angola, Africa

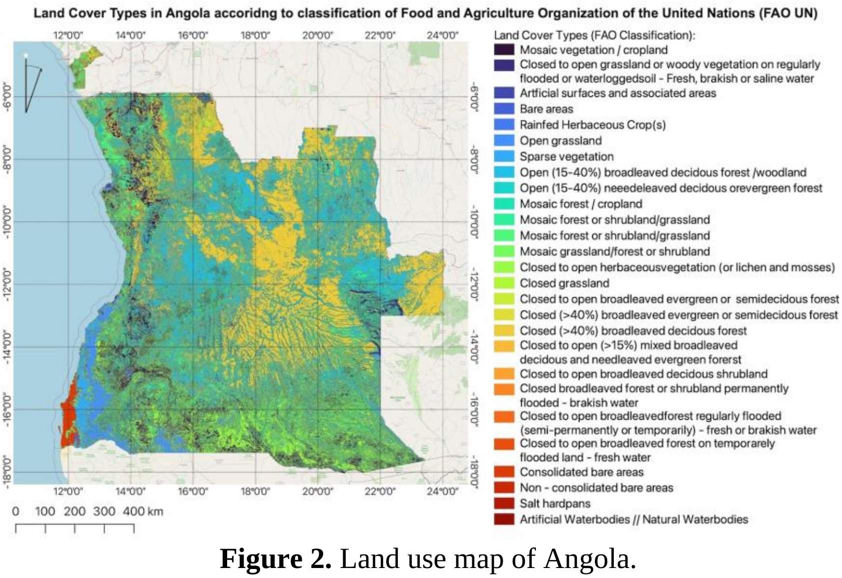

Figure 2. Land use map of Angola.

OBJECTIVES AND GOALS

The objective of this paper is twofold. First, we present an improved GRASS GIS scripting method for computing and visualizing 10 vegetation indices calculated using the 'i.vi' algorithm over study area in a mountainous region of central Angola. Second, we compare the performance of Landsat-8 OLI/TIRS imagery in vegetation mapping on 2013 and 2023. GRASS GIS programming algorithms have are novel method for computing vegetation indices which are usually calculated using traditional GIS approaches. This technique is chosen to improve the implementation vegetation monitoring. Unlike existing methods, this algorithm works automatically in data extraction for different vegetation indices that have been calculated for comparative analysis.

After providing an overview of the GRASS GIS algorithms and offering commentary on the code snippets and modules, we demonstrate how to use this application to map ten distinct vegetation indices using two satellite photos taken between 2013 and 2023. The results of the experiment are then presented, together with a consideration of their implications. In the end, we offer suggestions for potential future research in related fields and draw conclusions.

MATERIAL AND METHOD

Multispectral satellite images Landsat with 30 m resolution were enhanced by adding topographic layers (cities, roads, hydrographic network and country boundaries). The topographic map was produced using Generic Mapping Tools (GMT), a scripting toolset for processing and mapping spatial information using programming codes (Lemenkova, 2022 a; b). The condition for an accurate calculation of vegetation indices is that the images are cloud-free and acquired during the season of high vegetation cover and low humidity, which is October to May for tropical Africa. For these reasons, we used the selected images with cloudiness less than 10% and taken in July 2013 and 2023 during a dry period with low precipitation and humidity, Figure 3.

Launched in 1982, Landsat provides a valuable open source information to monitor vegetation and land cover types. For Angola, satellite images are used for environmental monitoring, such as landscape dynamics, urbanization (Temudo et al., 2019), urban restructuring, deforestation, degradation (Palacios et al., 2015, Lemenkova, 2024a), and increased agricultural activities (Mendelsohn, 2019, Lemenkova, 2024b). We used the latest sensor of Landsat products: OLI/TIRS. Compared to Landsat ETM+ where the usable swath range is limited to the central part of the image, Landsat OLI/TIRS is updated and improved in technical quality and characteristics (Lemenkova, 2023c). The 185 km swath in multispectral cameras enables frequent global coverage. Such a wide swath of is useful where clouds are a major obstacle to image acquisition in clear weather, as in tropical Africa.

Three software were used: 1) GRASS GIS for image processing (GRASS Development Team, 2022); 2) Generic Mapping Tools (GMT) for topographic mapping (Wessel et al., 2019); 3) QuantumGIS (QGIS.org, 2023) for GIS mapping. The codes were based on the existing works (Lemenkova, 2024c). Technically, this allowed to evaluate scripting approach in processing RS data. For environmental analysis, GRASS GIS supported monitoring vegetation cover and distribution of tropical forests.

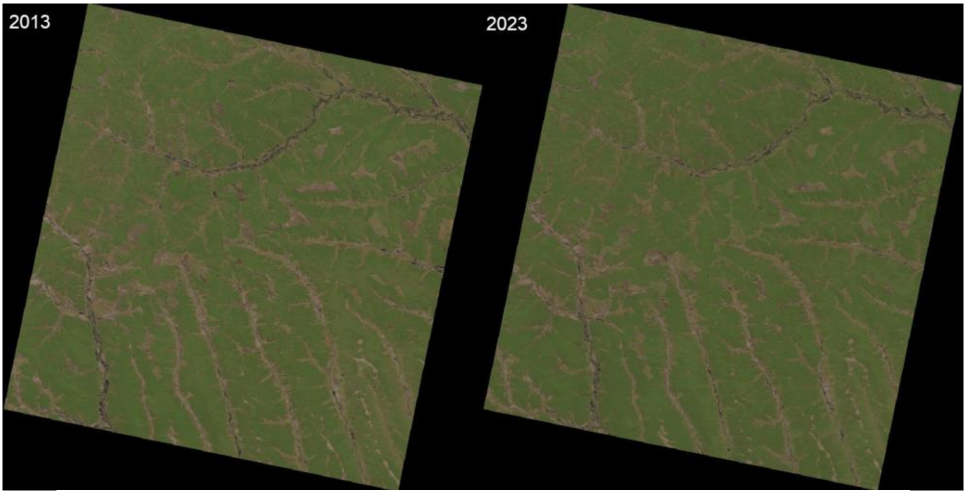

Figure 3: RS data: Landsat 8 OLI/TIRS scenes covering central Angola.

As a computer tool to help ecology and landscape studies, the calculation of vegetation indices is frequently employed in environmental monitoring. The principal methodology relies on the properties of plants whose chlorophyll content causes notable variations in the red and near-infrared regions of multispectral pictures. The presence of vivid green vegetation can thus be highlighted on satellite photos by utilizing the Red and NIR bands in a variety of combinations and formulas. As a result, it is possible to differentiate lush, vibrant vegetation from the surrounding, disparate land cover types, which include urban areas, bare ground, rivers, streams, and meadows adjacent to river valleys.

Numerous vegetation indices have been established in the past to extract data from grayscale photos. Because of its widespread use, the NDVI is the most well-known and well-liked of them. To make the contrast between the built-up regions and the vegetation better, we first employed NDVI and then computed other indices for comparison. Several indices with modified characteristics, such as enhanced atmospheric resistance (ARVI) or ground brightness correction (MSAVI), have been produced as NDVI modifications.

In this study, we tested 10 different vegetation indices: 1. Difference Vegetation Index (DVI); 2. Normalized Difference Vegetation Index (NDVI); 3. Atmospherically Resistant Vegetation Index (ARVI); 4. Enhanced Vegetation Index (EVI); 5. Nonlinear Vegetation Index for Global Environmental Monitoring (GEMI); 6. Modified Soil Adjusted Vegetation Index (MSAVI2) minimizes the effect of bare soil on the Modified Soil Adjusted Vegetation Index

(MSAVI2); 7. Normalized Difference Water Index (NDWI); 8. Perpendicular Vegetation Index (PVI) which is similar to difference vegetation index; 9. Green Atmospherically Resistant Vegetation Index (GARI); 10. Infrared Percentage Vegetation Index (IPVI).

Computing vegetation indices is an important part of ecological monitoring using RS data. The methods applied to the calculation of vegetation indices are based on multispectral transformations of pixels that form the raster structure. This approach converts the radiances recorded by the satellite sensor into quantities. The multispectral satellite images allow to numerically evaluate the state of chlorophyll content in leaves. In this way, the vegetation index reflects the growth stage of plants using these indicators. To calculate vegetation indices, the 'i.vi' algorithm was used by GRASS GIS. This module enables to separate vegetation cover from other land cover types, since it is based on automatic machine-based discrimination of spectral reflectance values of vegetation and rainforests on the satellite images.

Algorithm implementation of the GRASS GIS programming for image processing is as follows. First, the images were imported into the GRASS GIS project using the Geospatial Data Abstraction Library (GDAL): "r.in.gdal /Users/polinalemenkova/grassdata/Angola_2023/LC08_<...>_T1_SR_B1.TIF out=L8_2023_01" This is repeated for the 11 necessary bands of Landsat OLI/TIRS Then, the contents of the files were checked using the listing command: g.list rast. After that, the files were preprocessed by copying the Landsat bands to match the input structure of the "i.landsat.toar" module that will be used later to calibrate the digital number (DN) of the Landsat imagery. This is done using the following command: g.copy raster=L8_2023_01,lsat8_2023.1

Afterwards, the DN pixel values were converted to spectral reflectance values using DOS1 from digital number (DN) to reflectance. This is done using module i.landsat.toar which calculates reflectance and temperature of the "top of the atmosphere" for Landsat images. This step is necessary as it converts the DN to reflectance values before creating an RGB composite. Otherwise, the colours of the natural RGB composite do not look convincing but rather blurred. This conversion was done using metadata file with i.landsat.toar by the following command: "i.landsat.toar input=lsat8_2023. output=lsat8_2023_toar. sensor=oli8 method=dos1 date=2023-07-12 sun_elevation=44.08803962 product_date=2023-07-18 gain=HHHLHLHHL".

After the preprocessing, the next step involves the calculation of 10 vegetation indices. All indices were calculated using the GRASS GIS module "i.vi" and then visualized on the maps using a combination of map processing modules. First, the NDVI calculation was performed using the code: "g.region raster=lsat8_2023_toar.4 -p i.vi red=lsat8_2023_toar.4 nir=lsat8_2023_toar.5 viname=ndvi output=lsat8_2023.ndvi --overwrite r.colors lsat8_2023.ndvi color=ndvi" (here, the example of NDVI).

The next step included cartographic visualization and data representation using several GRASS GIS modules. First, the screen was launched using the 'd.mon' module: d.mon wx0. Then, the region was created to include the extent of the study area by 'g.region' as follows: "g.region raster=lsat8_2023_toar.4 -p. Afterwards, the maps were visualized using the 'd.rast' module (here, the example is given for NDVI): d.rast lsat8_2023.ndvi".Then the map legend was added using the GRASS GIS module "d.legend" with the map elements and adjustments as follows: d.legend raster=lsat8_2023.ndvi range=-1,1 title="NDVI-2023" title_fontsize=14 font=Helvetica fontsize=12 -t -s -b border_color=white thin=12 label_step=0.1 -d d.out.file output=Angola_NDVI_2023 format=jpg –overwrite.

Using the methodology with technical details described above the 10 maps were generated showing vegetation indices. The difference between indices lies in the approach of the formulas used for calculation.

RESULTS

Vegetation indices are based on characteristics of leaf spectral reflectance and their values in the red/near infrared (NIR) bands in multispectral data. Besides, they are widely used to identify and monitor landscape dynamics as a reliable source of information. Such maps are used for biophysical characteristics: biomass, leaf area index, photosynthetic radiation fraction in canopy. Figure 4 shows the NDVI and DVI computed for the 2013 and 2023 Landsat OLI/TIRS images which illustrate land cover and vegetation changes over the territory.

In order to get the best results, we tested with several vegetation indices utilizing GRASS GIS's i.vi algorithms. The NDVI and DVI hues in this figure correspond to the light vegetation, respectively. The NDVI presents a general approach with fixed variation range: from -1 to +1. The formula for NDVI=(NIR-Red)/(NIR+Red) uses the red and near infrared bands of Landsat, Figure 4. The raw data were pre-processed to convert pixel values to radiance in order to compare the NDVI changes between 2013 and 2023. Every NDVI image and computation receives the application of the mapping. Greater values signify lush, green vegetation. In central Angola, the comparison between 2013 and 2023 shows a loss in vegetation. DVI is calculated using the difference between the maximum absorption in the red, which is dependent on chlorophyll pigments, and the maximum reflection in the infrared (IR), which is due to the leaf's cellular structure. Though not normalized, this indicator is comparable to the NDVI but has more stable results. Red and infrared (NIR) bands are available in most RS data, including Landsat, and are used in NDVI. The drawback of NDVI is its sensitivity to noise and climatic factors like humidity and cloudiness.

The ARVI and EVI indices have been computed and are displayed visually in Figure 5. ARVI was first created for the MODIS EOS sensor, but as it uses the blue, red, and NIR bands for computation, it may also be applied to Landsat data. The ability to adjust for atmospheric effects on vegetation detection is its principal benefit. The deciduous forests of central Angola or mixed deciduous and evergreen forests with needle-leaving trees, such as cone or scale forests, are represented by the highest ARVI values in this region, which range from -0.60 to 0.70. Figure 5 of the ARVI and Figure 6 of GEMI exhibit comparable changes.

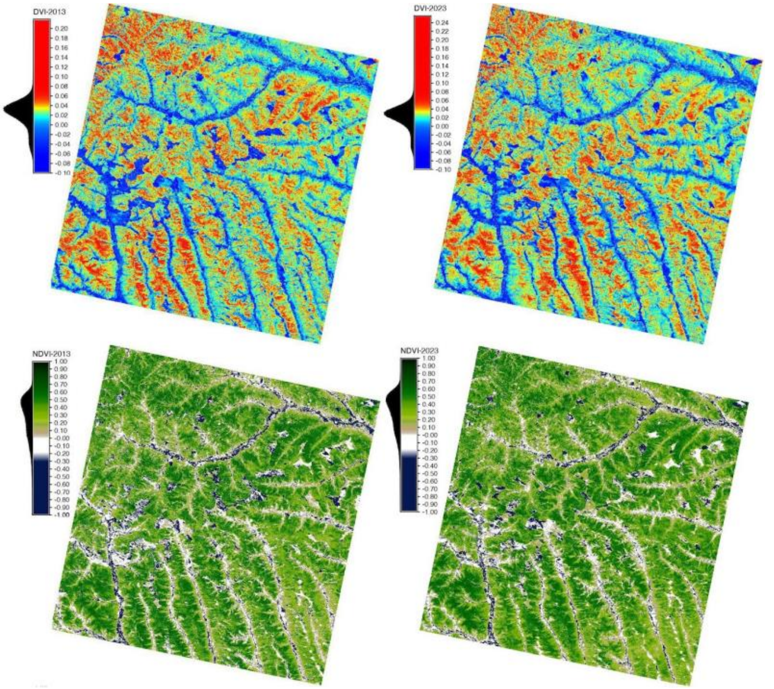

Figure 4: DVI and NDVI for 2013 and 2023.

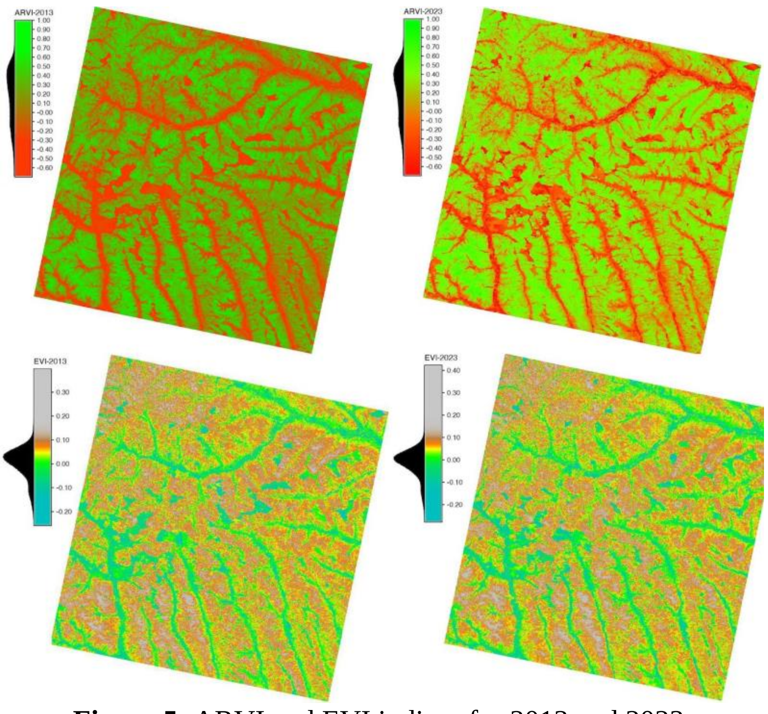

Figure 5: ARVI and EVI indices for 2013 and 2023.

For vegetation mapping in Angola's tropical mountainous regions, ARVI is more accurate than GEMI as it is less susceptible to atmospheric impacts. Bright green shows vegetation in ARVI, whereas bright red indicates river valleys. This index can be used for hydrological and geomorphological mapping since it makes a distinction between densely covered forests and heavily vegetated river valleys that have scant or nonexistent vegetation. The major range of values for the EVI index is between -0.10 and +0.10, as shown on data distribution in the histogram, whereas local minima with a constant of values below -0.20 are filled.

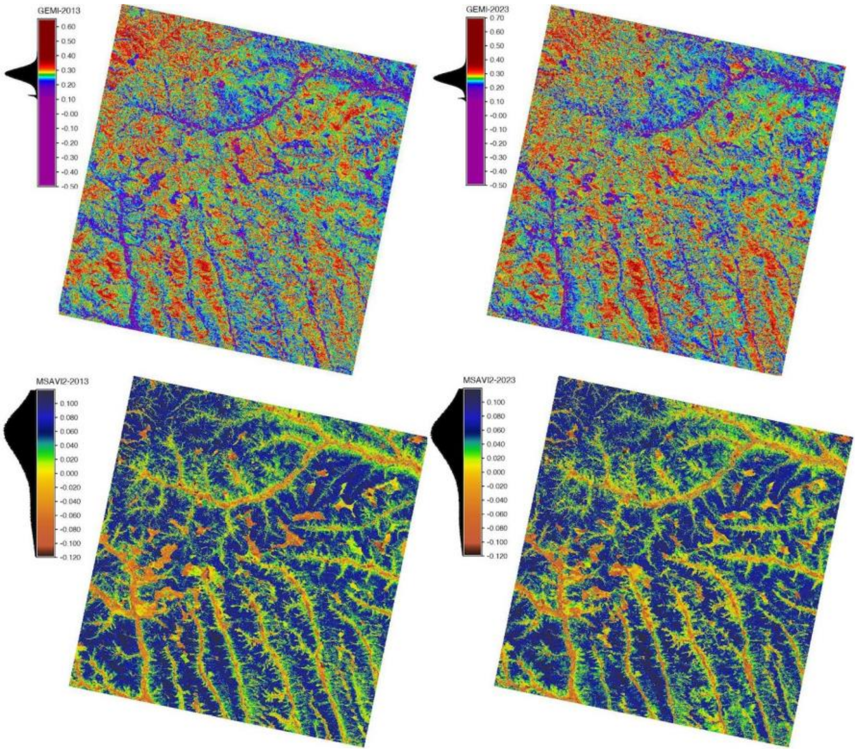

Figure 6: GEMI and MSAVI2 for 2013 and 2023.

Figure 6 displays the computed indices for MSAVI and GEMI. MSAVI accounts for soil reflectance, so its range of values is limited to -0.12 to 0.1. This makes it possible to separate soil from vegetation. The drawback of this method is that it needs a lot of vegetation cover. Due to the soil effects, the reflectance values may be incorrect if vegetation cover is sparse, and due to similar spectral reflectance, plants with comparable photosynthetic properties may be mixed. Conversely, GEMI values are between -0.5 and 0.6, with 0.15 to 0.40 being the most notable numbers. The graphic displays the brilliant plant cover as a black side histogram on the map legends, which indicates data distribution. To reduce the impact of the atmosphere on the measurement of the vegetation index, GEMI takes into account a non-linear connection.

Figure 7 displays the computed values for the PVI and NDWI indices. The amount of biomass, the green area, the health of the crop, and vegetation with active photosynthetic activities are all closely correlated with the plant water content, as the NDWI shows. Accordingly, this index's lowest values in the research region are -0.7-, and its highest values are reached up to 0.20, at which point the values settle. Higher results, over 0.2 and above, correlate to areas of shrubs or natural deciduous and broadleaf forests in high mountains. Very low values, of negative order, depict rocky, sandy, or bare places.

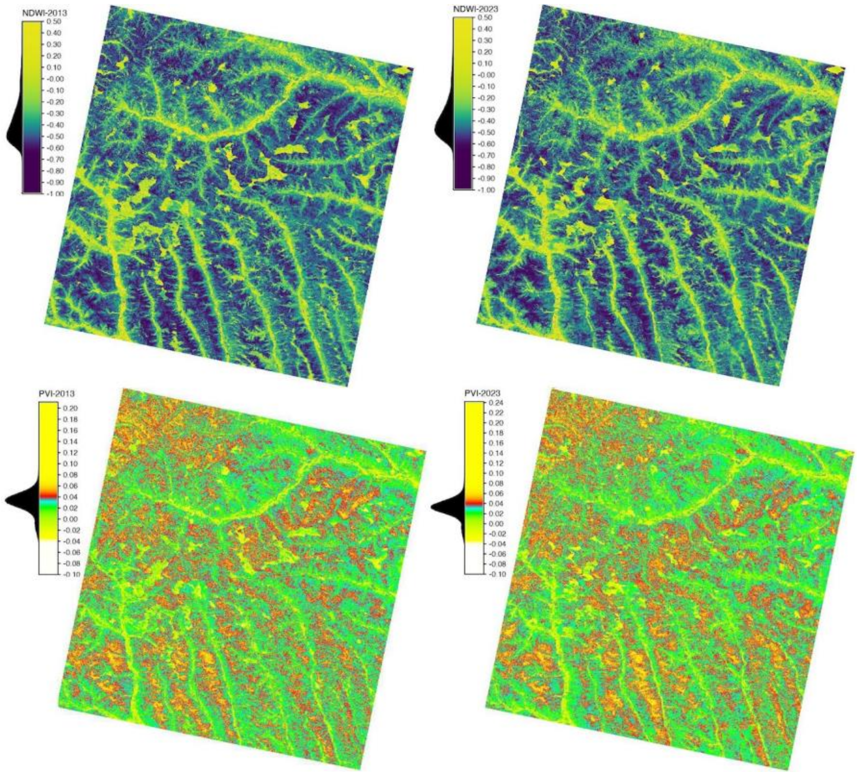

Figure 7: NDWI and PVI for 2013 and 2023.

In central Angola, the PVI ranges from -0.04 to 0.08 (Figure 7). The perpendicular distance between each pixel and the ground line that gives this index its name is used in the calculation. Each pixel's distance from the ground line determines whether it is land cover or vegetation cover. Identification of vegetation is made possible by this index's adjustment to soil reflectivity. Its data distribution range is different from the NDVI's since its values are determined using perpendicular distances to the ground line, represented in reflectivity units. Thus, when compared to the NDVI, this index illustrates better results for differentiating vegetation.

Normalization by the sum of the 2 bands calculated in the IPVI shown in Figure 8 reduces the effects of light and results in values between -0.1 and 0.90. The IPVI maintains values regardless of the illumination. This differentiates it from simple vegetation indices which are sensitive to changes in illumination. The GARI values are between -1 and +1, however, negative values corresponding to non-vegetated surfaces are reduced to -0.50, as land use types corresponding to negative values, such as snow or dense clouds, are absent. The reflectance in the red is higher than that of the NIR, which explains the values. The increase of values to 0.40 means the increase of shrubs and mixed vegetation.

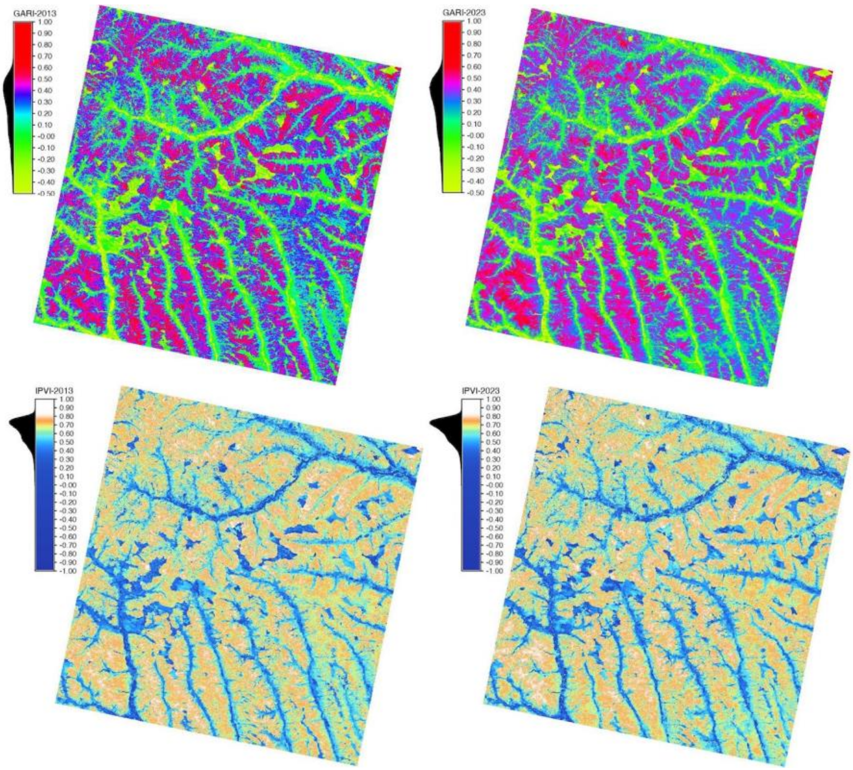

Figure 8: GARI and IPVI for 2013 and 2023.

The results are encouraging for mapping rainforest ecosystems and tropical vegetation in Africa. Therefore, Landsat-8 OLI/TIRS data can be used in similar studies using the presented programming codes as a continuous data source for vegetation monitoring. The presented findings indicate the value of RS data as a source of information for environmental monitoring. Using multi-temporal images, this data can be used to map vegetation cover at all time and spatial scales, hence facilitating the mapping of landscape dynamics. Changes in the terrain and vegetation cover are becoming more obvious in Central and Southern Africa due to the effects of climate change and human activities associated with deforestation. This leads to a complicated alteration in the patterns of land cover, including the fragmentation of landscapes, which lowers biodiversity. As this study demonstrates, research on tropical forest vegetation can advance thanks to the wealth of spectral, resolution, and temporal data offered by satellite imaging and advancements in programming.

Vegetation index computation is essential for environmental monitoring. More suitable features that adapt to various transboundary zones with varied vegetation types (savannah, tropical forests, deciduous forests, mosaic grasslands, or shrubs) should be the focus of future research. Finding places with complex and shifting landscape structures would be made easier by adapting regional characteristics of land cover classes. The GRASS GIS method uses scripts to automate image processing in order to define the cartographic workflow. This makes it possible to identify vegetation traits and distinguish between areas that are diverse or those are homogeneous sections of the landscape.

The tropical forests of Angola are surrounded by agricultural fields, cultivated plantations and urban settlements. They are clearly distinguished from built-up areas and cultivated areas during the growing season. For this reason, we tested several vegetation indices that differ from each other by several technical parameters (use of different Landsat channels and their combination in equations) and adjustments for environmental parameters and atmospheric characteristics.

DISCUSSION

Revealing the geographical distribution and structural properties of vegetation has become increasingly dependent on satellite pictures due to their effectiveness, affordability, and wide coverage (Goodchild, 1994, Altobelli et al., 2007, Lemenkova, 2024d, Burstein et al., 2023). Nevertheless, there are still a lot of difficulties in integrating programming techniques with data from remote sensing to enable vegetation research and modeling (Kopecký and Čížková, 2010; Deepthi Murthy et al., 2023). For various vegetation mapping applications, many image types that differ in their spectral, spatial, radiometric, and temporal features are appropriate. Furthermore, a variety of image interpretation processes and techniques can be used when mapping vegetation using remotely sensed images. Therefore, employing remote sensing data to categorize and map plant cover continues to be a difficult endeavor (Whig et al., 2024).

Satellite images have been successfully utilized by numerous researchers to continuously monitor vegetation at different scales using dynamic processes. For instance, Zhang et al. (1999) evaluated crop condition and variability at different growth phases by monitoring and evaluating vegetation crop management using remotely sensed imagery. Kawamura et al. (2005) monitored seasonal vegetation changes and short-term vegetation phenology with respect to forage quantity and quality using data from sensors that measure the Advanced Very High Resolution Radiometer (AVHRR) and Moderate Resolution Imaging Spectroradiometer (MODIS). Papeş et al. (2012) quantified the impact of vegetation canopy on seasonal migration and behaviour of birds using remotely sensed data.

Moreover, specific soil-vegetation indices are developed for agricultural characteristics. For instance, Herbei et al. (2022) showed that agrochemical indices of pH, humus (H), saturation in bases (V), nitrogen index (NI), phosphorus (P) and potassium (K) content, can be applied for analysis of soil. Finally, vegetation indices, such as NDVI, are used for conservation of biodiversity and ecosystem services using analysis of temporal analysis of vegetation cover (Rios et al., 2024).

Both GIS and remote sensing are limited to approximations of the truth and the geographical continuum can be discretized in a variety of ways, they exhibit inaccuracy and uncertainty (Atkinson and Foody, 2002). The accuracy of differentiating vegetation from other land cover types can be diminished due to mixed pixel difficulties resulting from the spatial resolution limitations of satellite photography (Fairbanks and McGwire, 2004). Spatial uncertainty requires attention in order to facilitate spatiotemporal dynamics through data analysis (Bouaziz et al., 2020). Furthermore, the application capabilities of satellite RS are limited by elements like weather (Khillare and Patil, 2023; Niraj et al., 2023), revisit intervals, and fixed orbits (Tůma et al., 2022).

Compared to other studies that also used vegetation indices (Coles-Ritchie et al., 2007; Mutiibwa and Irmak, 2013; Patra et al., 2024; Zeydan et al., 2023), the obtained results reveal the following advantages. The approach demonstrated in this paper was developed by integrating GIS techniques, RS data, and programming algorithms for satellite image processing. It presented an example of mapping spatial ecology for computing vegetation indices, and demonstrated the usefulness and efficiency of integration of RS data, GIS methodology, and programming techniques. Specifically, we demonstrate the variety and difference of vegetation indices calculated using the GRASS GIS programming approach for the central region of Angola. The computation and visualisation of vegetation indices in tropical rainforests of Angola is important to monitor health vegetation and detect deforestation.

A GRASS GIS script-based method was used for extracting information on distribution of healthy or sparse vegetation and distinguished them from the bare land. The advantage of this method is that it considers band-to-band relationships between pixel spectral reflectance and vegetation cover identification. This is useful for environmental monitoring using Landsat multispectral image processing. Several vegetation indices were computed using formulas based on Landsat bands: NDVI, ARVI, GARI, and GVI. GRASS GIS operations operated excellent with satellite images.

CONCLUSIONS

Landscape dynamics can be examined in greater detail from a spatiotemporal perspective by using image sequences. This data's content can be obtained by analyzing appropriate and adequate features for landscape monitoring using satellite photography. In order to distinguish between land plots with drastically different textural and structural characteristics for the purpose of identifying deforestation, vegetation shrinkage, or other examples of land cover change, it is therefore possible to take into consideration the exact classification of land cover types based on vegetation indices.

This paper illustrated the importance of programming in RS data processing using spectral and texture feature analysis of satellite images for analysis of vegetation indices. Vegetation indices can be applied as characteristics for analysis of landscape heterogeneity. Landscape diversity can manifest itself in different ways depending on the data categories and quantification methods. It is a key feature of the environment and is particularly evident at the landscape scale in regions as complex as in Angola, southern Africa. Therefore, vegetation indices can reveal information on spatial distribution of landscapes which can be studied to analyse spatial heterogeneity of the territory. In turn, landscape diversity at the spatio-temporal scale shows a gradient that illustrates the dynamics of ecosystems in space and time.

Using this information, landscape structure can be analysed through mapping constituting elements which have distinct and complex boundaries and reflected as different patches in the mosaic of vegetation indices on the maps. Automated interpretation of the vegetation distribution through index calculation yields better results than traditional classification. Adaptive vegetation type determination by computed indices is a novel aspect of this study. Landscape monitoring using RS data is an important research topic for climate-environment monitoring in Africa, as automatic processing of satellite images using computer vision algorithms is a challenging task. Detecting vegetation on satellite data using computed indices has shown excellent results on Landsat-8 OLI/TIRS images with simple color texture covering tropical Angola.

The presented maps demonstrated landscape dynamics in Angola. The tropical forests of the country are known for their rich biodiversity and exceptional value to the planet. Nevertheless, the decrease of natural vegetation endangers ecosystems. Cultivated plantations are gradually replacing natural tropical forests for commercial reasons. Landscape mapping and vegetation analysis of landscape units were conducted for the mountainous region of Angola for 2013 and 2023. The consequences of human activities (agricultural practices and commercial deforestation) and climate change (increasing temperatures, periods of drought, erratic rainfall) have strongly contributed to the loss of tropical forests and increased fragmentation of landscapes. The difference in areas occupied by woodlands in 2013 and 2023 is visible on the presented maps.

The dynamics of spatial and temporal landscape diversity allows to measure their heterogeneity. Nevertheless, the contribution of this work and its scope is not only a technical mapping and processing of satellite images but also the assessment of deforestation in Angola for the environmental monitoring of its landscapes. Therefore, this study integrates technical methods and satellite data for environmental monitoring objectives in West Africa based on the vegetation indices computed for selected region of Angola, southern Africa. In future work, we suggest modeling changes of land use types. It might, for instance, investigate segmentation techniques that are applicable to multi-scale satellite picture analysis. Based on the determined vegetation indices, this method's advancement could incorporate automatic segmentation, object classification, and feature extraction from images. The emphasis would be on employing computer vision techniques to automatically recognize different forms of land cover with comparable spectral reflectance.

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