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Review paper

POSSIBILITIES OF CABBAGE PRODUCTION UNDER CLIMATIC CHANGES

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Abstract: Cabbage growth and development require temperatures between 15 and 18ºC. However, for most of the growing season, cabbage is exposed to temperatures above 20ºC. To obtain the highest possible prices on farmer's markets, cabbage producers start cabbage planting at the earliest possible dates (early or late autumn). Cabbage growth is "pushed forward" by more abundant irrigation, planting density, cultivation, and protection, to form a marketable (technologically mature) head in the shortest possible time and achieve the highest possible profit on the market. To improve the efficiency of water use and adapt cabbage to warmer and drier conditions, it is recommended to use modified production systems, with an emphasis on postponing the date of sowing or planting to mitigate the effects of temperature increase and drought during cabbage production. This paper aims to point out the effects of temperature and water stress and to provide solutions that can be practically applied to mitigate the negative impacts of these stress factors on cabbage production. Priority should be given to the development of production systems that improve the efficiency of water use adapted to the conditions of hot and dry weather. Irrigation of cabbage should be organised as drip irrigation, as this is a more rational system, with the possibility of comparative feeding and protection.

Key words: temperature stress, water stress, cabbage, irrigation, mulching.

Introduction

The geographic position of Serbia allows for a year-round open-field or greenhouse production of cabbage. The production of early cabbage starts in January and February, while late cabbage is harvested at the end of November or

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even later, until the occurrence of severe frosts. Cabbage is produced all year round, as early cabbage for fresh consumption and as late cabbage for preservation. The cultivation of cabbage is most often defined by the way it is used, i.e. fresh, cooked or pickled. The specificities of the production methods mentioned are closely related to the weather conditions in the part of the year when the production takes place. Although it is adapted to different climatic and soil conditions, cabbage is better suited to cooler and wetter growing areas (Osher et al., 2018). This rule also applies to parts of the year with cooler temperatures, to which cabbage responds very well at all stages of development (Červenski et al., 2022).

Environmental factors have been changing more rapidly, affecting vegetable production and quality. Reduced vegetable production is likely to be caused by a shortened production period, which will negatively affect growth and development, especially under conditions of heat stress and reduced water availability. Climate change causes additional uncertainties and risks, increasingly limiting production, which leads to an increase in the price of vegetables. These changes encourage the spread of pathogens and the development of new pest species and pathogen strains (Malhotra and Srivastva, 2014). The challenges to be faced in the coming years are sustainability and competitiveness, achieving targeted production that can meet the growing demands despite the reduction in arable land and water shortages. New models are needed in order to improve the production of vegetables, with special interventions in certain areas that require experience, knowledge, and the application of new techniques and technologies (Malhotra and Srivastva, 2015).

Cabbage growth and development require temperatures between 15 and 18ºC (Hara and Sonoda, 1982; Criddle et al., 1997; Žnidarčič et al., 2007; Kołota and Chohura, 2015; Lešić et al., 2016; Červenski and Medić-Pap, 2018). However, for the most part of the growing season, cabbage is exposed to temperatures above 20ºC. In the Republic of Serbia, such temperatures occur from the beginning of April to the end of September. In Serbia, the production of early cabbage in the open field, the production of summer cabbage, and the first stage of the autumn cabbage production take place during these months. So as to obtain the highest possible prices on the farmer's markets, cabbage producers start planting cabbage at the earliest possible dates (early or late autumn). Cabbage growth is "pushed forward" by more abundant irrigation, planting density, cultivation and protection, in order to form a marketable (technologically mature) head in the shortest possible time and achieve the highest possible profit on the market.

The aim of this paper was to point out the effects of temperature and water stress and to provide solutions that can be practically applied in order to mitigate the negative impacts of these stress factors on cabbage production.

The area under cabbage cultivation in Serbia declined steadily from 2010 to 2023. In the observed period, cabbage yields mostly increased (Table 1), possibly due to the application of improved cultivation practices, such as the selection of high-yielding hybrids, sowing/planting a larger number of plants per unit area (denser planting), irrigation, control of weeds, disease-causing and harmful insects.

Table 1. Cabbage production areas (ha) and yield (t/ha) in Serbia from 2010 to 2023 (https://www.stat.gov.rs).

Year	Production area (ha)	Yield (t/ha)	Year	Production area (ha)	Yield (t/ha)
2010	20891	16.1	2017	10213	25.7
2011	20581	15.3	2018	8251	25.4
2012	20441	12.2	2019	7957	22.4
2013	19422	14.3	2020	7547	23.8
2014	11116	23.5	2021	7513	24.7
2015	12061	27.9	2022	7335	22.6
2016	10804	26.8	2023	7111	22.3

Thermal and precipitation characteristics of the vegetation period (April–September) in the Republic of Serbia from 2010 to 2024

For a better understanding of the effects of temperature and water stress on the production of cabbage, the thermal and precipitation characteristics of the growing season (April–September) from 2010 to 2024 in the Republic of Serbia are presented in this paper.

The number of days with a maximum air temperature > 20ºC (Tmax >20ºC) in the growing season (April–September) in the period 2010–2024 ranged between 131 (2019) and 169 days (2018) (Figure 1).

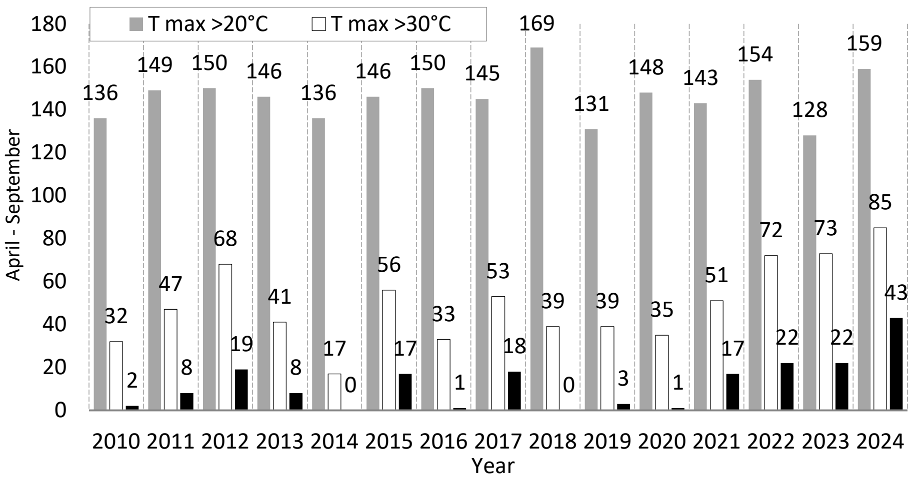

Figure 1. Number of days with T-max>20ºC; T-max>30ºC T-max>35ºC during the vegetation period (April–September) in 2010–2024 in Serbia. (https://www.hidmet.gov.rs/)

The number of days with a maximum air temperature > 30ºC (Tmax >30ºC or summer days) in the growing season (April–September) in the period 2010–2024, ranged between 17 (2014) and 85 days (2024) (Figure 1).

The number of days with a maximum air temperature > 35ºC (Tmax >35ºC or tropical days) in the growing season (April–September) in the period 2010–2024 ranged between 0 (2014 and 2018) and 43 days (2024) (Figure 1).

In July and August, tropical temperatures were recorded at night (morning temperatures were above 20°C), 26ºC in July 2023, as well as 17 days in 2024 (https://www.hidmet.gov.rs/).

The number of rainy days during the vegetation period (April–September) in 2010–2024 varied from 29 (2023) to 70 (2014) (Figure 2).

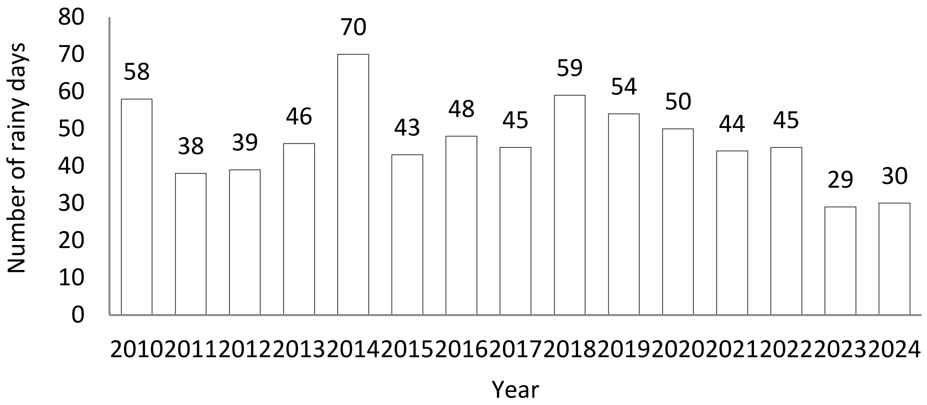

Figure 2. Number of rainy days during the vegetation period (April–September) in 2010–2024 in Serbia. (https://www.hidmet.gov.rs/)

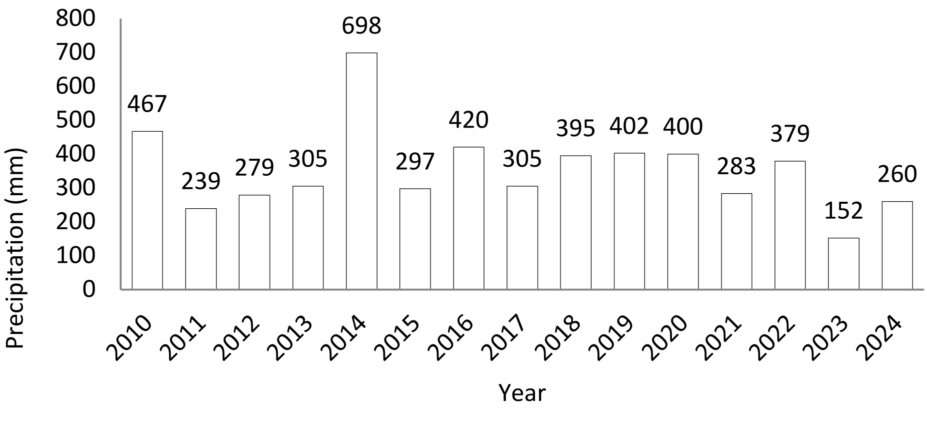

Figure 3. Precipitation in mm during vegetation (April–September) in 2010–2024 in Serbia. (https://www.hidmet.gov.rs/)

Precipitation in mm during the vegetation period (April–September) in 2010–2024 in Serbia varied from 152 mm (2023) to 698 mm (2014) (Figure 3).

Possibilities of mitigating the negative impacts of temperature stress on cabbage production

When producing early cabbage in the open field or in the greenhouse, it is necessary to react adequately and timely so as to reduce the impacts of low temperatures. Low temperatures during spring can extend the planting time of early cabbage and have a negative impact on early cabbage sown or planted in an unheated greenhouse or in the open field, which can result in partial damage to individual plants or complete failure of production. In the winter/spring period, low temperatures in the range of 0°C and even below -10°C, with weaker or stronger frosts, are possible. Under the conditions of low temperatures, the plant primarily defends itself by closing the stomata, which reduces the intensity of one of the basic physiological processes – photosynthesis. In order to strengthen the cabbage seedling as much as possible, it is necessary to gradually expose it to external weather conditions, unpredictable situations and temperature fluctuations (Červenski and Medić-Pap, 2018; Červenski et al., 2022).

Early cabbage production in the greenhouse takes place in tunnels without additional heating at a height of 1.8–2.5 m (Ilin et al., 2017), and is often accompanied by the use of several polythene sheets to prevent vernalisation of the early cabbage plants due to prolonged exposure to low temperatures.

At a temperature of 4–5ºC, the vegetative cabbage head can undergo vernalisation, while the most intensive processes occur at a temperature of 5 to 6ºC. This is an undesirable phenomenon in early production. Longer exposure to low temperatures of 5 to 7ºC in more sensitive cabbage varieties and hybrids leads to vernalisation and flowering. Length of the vernalisation stage ranges from 30 to 70 days (Amasino, 2004). With a day length of 12 hours and temperature conditions of 6°C, vernalisation lasts about 7 weeks. In early spring production, cabbage seedlings can vernalise in 12–14 days during periods of low temperatures (Adžić et al., 2012; Červenski and Medić-Pap, 2018).

On the one hand, quality polyethylene films in early production preferably have a high surface tension of the inner layer, so that the hanging drops do not reflect a significant amount of direct sunlight and thus reduce the length and intensity of photosynthetic activity. On the other hand, direct sunlight transports more heat than diffused sunlight and the temperature under such foils is significantly higher. If such foils also contain a supplementary thermo-protective barrier against the radiation of heat accumulated in the soil and in the young plants during the sunny days, then in addition to exceptional earliness, larger and more beautiful heads, higher yields and significantly higher profits can be expected (Momirović, 2004, Kim et al., 2022).

The maximum film thickness acceptable from the point of view of thermal and mechanical properties depends on the width of the film, which is about 80 microns for tunnels covered with an 8 m wide film. Tests have shown that the non-drip, thermo-protective foil of 80 microns ensures a temperature higher by 4–5ºC after weaker morning radiation frosts compared to the standard foil of 160 microns, which is mainly used by our manufacturers (Červenski and Medić-Pap, 2018).

Mulching is also a key factor for the successful cabbage production (Ilić and Milenković, 2022). Natural mulch contributes organic matter to the soil, whereas synthetic mulch raises soil temperature, retains moisture, and minimises weed competition. The impact of seeding varies with the growing season. Using black polyethylene film to cover the soil is advisable when the spring is cold and wet, but its benefits may be diminished at higher temperatures (Adamović et al., 2023). Black polyethylene mulch absorbs heat from solar radiation, raising soil temperature, which helps boost cabbage production, particularly during the winter season (Farjana et al., 2019; Adamović, 2020; Ponjičan et al., 2021).

Production of cabbage in greenhouses during the winter-spring period requires additional heating and the use of double covering foils and agro-textiles inside the greenhouse. Fleece films, categorised as non-woven agro-textiles, are made with specific technology from UV-stabilised polypropylene and serve as a thermal barrier that mitigates temperature extremes in the crops. They do not contain any chemical agents, but the fibres are connected by a specific thermal process. They have high light transparency and permeability to water and air, and are applied by directly covering young, sprouted or transplanted plants (Khramov et al., 2022).

In cabbage production, they are used at low temperatures, rarely at extremely high temperatures, or when additional protection against harmful insects, vectors, dangerous viral diseases, or in the organic growing system is required. These light thermal protection barriers usually weigh 17–60 g/m^2, but when they are slightly thicker, they provide an ideal environment for accelerating the growth of cultivated cabbage, ensuring earlier arrival and higher yields, and protecting crops in the coldest periods. They are especially effective during light radiation morning frosts when, thanks to the retention of long-wave thermal radiation, the temperature of the ground layer is 4 to 5ºC higher. The effect of previously accumulated heat in the soil and in the plants does not last longer than a few days if very cold weather accompanied by weak daylight lasts longer. Therefore, the best effect of covering the plants directly with agro-textiles is related to transitional seasons. In addition, it is notable that due to the high permeability of agro-textiles, we can easily irrigate the cabbage using micro-irrigation without removing the foil beforehand. However, the application of pesticides or water-soluble fertilisers is not allowed, because it can drastically reduce the duration that extends over several seasons of vegetable growing (Ilin et al., 2017; Stanciu, 2023). In the study by Stanciu (2023), covering cabbage with agrotextile in early production in the greenhouse and in the open field resulted in a 4–7-day earlier harvest as well as a higher rosette diameter, plant height, number of leaves in the rosette, head weight, leaf weight.

For cabbage production in greenhouses during spring and autumn, the average daily temperature should range between 15 and 25°C, i.e. 10 and 30°C (Xianbing et al., 2020). In warmer years, plastic mulching films can increase the soil temperature around the root system, which can negatively impact cabbage growth and yield. Therefore, they should be avoided in areas with high levels of solar radiation (Djigma and Diemkouma, 1986, Díaz-Pérez and Batal, 2002, Adamović et al., 2023). Some authors (Hou et al., 2010) recommend removing plastic films at a certain stage of plant development.

July and August are usually the hottest months of the year. These two summer months are characterised by temperature stress with high temperatures over 32–33°C, and often 34–35°C. Chang et al. (2016) reported that daily temperatures exceeding 30°C during summer reduce the yield and quality of cabbage. A larger number of leaves and a larger leaf area were recorded when growing cabbage at 20°C, compared to 25°C, while no significant differences were observed between 20 and 22.5°C (Suh et al., 2012). The same authors state that the decrease in yield under the influence of a temperature of 22.5°C amounts to 4%, while the decrease in yield under the influence of a temperature of 25°C amounts to 15%.

Optimum temperatures for cabbage are 20°C during the day and 15°C at night (Kalloo and Bergh, 2012). The occurrence of high temperatures also represents stress. Temperature stress can have a negative effect on the final stage of the vegetation period of summer cabbage and the initial phase of the vegetation period of late or autumn cabbage intended for preservation. Extended periods of higher temperatures lead to an increased number of plants that do not form heads, premature flowering, smaller heads (Kalloo and Berg, 2012), firmer leaf texture, curling, scalding, and damage to the leaves of the rosette and heads, as well as slower growth (Yue et al., 2021), loose heads, and the appearance of multiple smaller heads on a single plant (Figures 1 and 2).

Due to the high temperatures caused by climate change, plants adjust their metabolism, which can affect their nutritional value. High temperatures trigger physiological and biochemical changes in plants that influence their nutritional potential (Gmižić et al., 2023). The same authors note that high temperatures significantly increased the content of total phenols, soluble sugars, carotenoids, potassium, sodium, and antioxidant capacity as measured by the ABTS and FRAP assays.

In order to maximise the profitability of production, producers should know the cultivated cabbage variety/hybrid and, above all, its vegetation period. In spring production, knowing the vegetation period of the cultivated cabbage variety/hybrid can ensure that negative effects of high temperature stress, which is more common in June and July, are avoided. Stanciu (2023) emphasises the importance of hybrid selection for extra-early cabbage production.

Figure 4. Loose head.
(Adamović B., 2024)

Figure 5. Multiple heads of cabbage.
(Adamović B., 2024)

Knowing the length of late cabbage vegetation from the autumn production, the aforementioned practices can be organised with a time shift in planting dates towards August or September. Temperatures are more optimal for the production of autumn cabbage during September, October and November. With this system of organising cabbage production, the priority to bringing fresh autumn cabbage to the market, when the prices are at their highest, is lost. However, producers get a safer production of better-quality cabbage, which should be the main goal of any production.

One way to maximally compensate for the impacts of high temperatures on cabbage production is to cultivate locally grown cabbage varieties. Cabbage varieties and populations that originated or became domesticated in a particular area should be cultivated as much as possible for several reasons. These varieties are well adapted to local climatic conditions, tolerate new climatic changes well, often have better quality (sugar content and lower leaf thickness) than other cultivated cabbage hybrids, and are part of the culinary tradition in a particular area.

Possibilities of mitigating the negative impacts of water stress on cabbage production

Cabbage requires high soil moisture throughout the growing season, which makes it a hydrophilic plant. Shock and Wang (2011) state that the plant species *Brassica oleracea* is one of the most sensitive to soil water deficiency, while Bute et al. (2021) claim that cabbage is moderately sensitive to water stress. If soil moisture exceeds desirable levels, cabbage will consume it inefficiently and uneconomically (Červenski and Takač, 2012). Cabbage forms a large above-ground mass with large leaves and has a shallow but extensive root system, with a depth of up to 30 cm, where the greatest root mass is concentrated in the top 10-cm layer (Yamamoto et al., 2015). The root surface area through which it absorbs water and nutrients is up to 25 cm^2 (Cobos-Tores et al., 2021). The visible leaf area at the beginning of head formation ranges from 0.59 to 0.91 m^2, and during the head formation stage, it ranges from 0.57 to 0.85 m^2 (Lüling et al., 2022), which affects the high transpiration and water requirements, while the cabbage leaf area index is around 3.96. The high leaf area index and the high transpiration lead to rapid soil drying (Seidel et al., 2017).

The accurate estimation of crop water requirements is an important prerequisite for high yields and crop productivity (Seidel et al., 2017). Water requirements are also determined by the growth phase, and the greatest is during the seedling stage, intensive growth of the rosette leaves and head formation (Karagić, 1998). In addition, water requirements are determined by the time and location of crop growth. The water requirements are the highest during the hot days of July and August, and higher than during the head growth at lower temperatures and higher humidity, which reduces transpiration and evaporation of water from the soil (Bošnjak, 2003). Similarly, Seidel et al. (2017) state for the agroecological conditions in Germany that plants expirience drought stress for up to 62 days after transplanting, and that rainfall or irrigation in later stages can compensate for the stress caused by early water deficiency.

Multi-year research by Domuta et al. (2017) shows that autumn cabbage requires a substantial amount of water. This is supported by Büyükcangaz (2018), who states that cabbage requires 380 to 500 mm of water depending on the climate and length of vegetation. Domuta et al. (2017) found that irrigation significantly increased the average cabbage yield by 153%.

It is also important to take into account precipitation throughout the entire growing season and the climate of the region where cabbage is grown, as well as to ensure critical irrigation stages during the 6–7-leaf stage and the beginning of head formation are not missed (Bute et al., 2021). The absence of irrigation during the head formation stage reduces head mass by more than 50%, leading to unacceptable market losses (Radovich et al., 2004). The same authors note that, in addition to lower yields, the value of the crop also decreases due to deviations in the shape and size of the heads.

Lower soil moisture slows down the formation of heads, which remain small and soft, while irrigation during head development results in larger and heavier heads, with a lower dry matter content (Radovich et al., 2005). Cabbage is sensitive to changes in soil moisture, especially in the stage of head formation and technological ripening. A water deficit 3 to 4 weeks before harvest lowers yield and quality. Low soil moisture stops the growth, while the leaves and heads are small and loose. If drought stress occurs in the earlier stages of plant development, it slows down the growth of the above-ground biomass, resulting in reduced plant height and smaller leaf area (Seidel et al., 2017). On the other hand, excessive humidity during this period can cause cracking in the heads. This occurs when the soil is dry for a prolonged period and when rewetting occurs through precipitation or watering (Červenski and Medić-Pap, 2018).

Irrigation and watering norms depend primarily on weather conditions and the amount and distribution of precipitation. Irrigation norms, i.e. the amount of water applied expressed in mm or litres, range from 3 to 8 or even more. For every 5 mm of natural rainfall, irrigation should be postponed by one day and conducted at every 6 to 8 days with about 30 mm of water (Dragović et al., 2006).

A decrease in soil moisture leads to an increase in soil temperature, which is another key factor affecting cabbage growth. Hamad et al. (2022) indicate that cabbage growers frequently apply high amounts of nutrients and water to increase yields, while Bute et al. (2021) state that excessive water usage become a standard practise in order to achieve higher yields. Excessive and frequent irrigation can negatively affect yield, but not as much as insufficient irrigation (Cripps et al., 1982). In addition to increasing overall costs, excessive irrigation can lead to nitrogen leaching (Imtiyaz et al., 2000; Seidel et al., 2017).

Irrigation can be performed using sprinklers (Imtiyaz et al., 2000; Maršić et al., 2012; Seidel et al., 2017) or a drip irrigation system (Radovich et al., 2004; Žnidarčič et al., 2007; Maršić et al., 2012; Adamović et al., 2023). Sprinkler irrigation produces a favourable effect on the microclimate, lowering the temperature of the leaves by 7–12ºC compared to the air temperature. Additionally, sprinkler irrigation increases the relative humidity, which should range between 85% and 90% for cabbage (Bute et al., 2021). Subsurface drip irrigation is a more efficient method of water management compared to other irrigation techniques. It offers several benefits, including water conservation, easy fertigation, and reduced surface runoff and deep percolation (Hamad et al., 2022). The main advantage of the drip irrigation system is that it reduces the occurrence of unfavourable conditions (wetting of leaves and heads), so the intensity of pathogen infestation in cabbage is much lower compared to irrigation systems using artificial rain (Vlajić et al., 2017).

If the cabbage is produced during the dry season at temperatures above 30ºC (during July and August), irrigation should be carried out every 8–12 days with a water rate of 30–40 mm. Frequent watering (every 4–6 days) at high temperatures (above 30ºC) can contribute to the formation of loose cabbage heads, which reduces the market value of cabbage (Červenski et al., 2008; Červenski and Medić- Pap, 2018).

The main element of rational irrigation in cabbage production is the knowledge of the potential evapotranspiration, i.e., plant water requirements, or consumption of water that contributes to the highest yield of good quality (Karagić, 1998). Cabbage has a lower evapotranspiration than other vegetable crops, because its leaves are covered with a thin wax coating and have a lighter colour, but it has higher water requirements. Evapotranspiration depends on the environment, the amount and distribution of precipitation during the growing season and water requirements of the plant. All these parameters are significantly changed by postponing the date of planting. Cabbage evapotranspiration decreases with later planting due to changes in the environment, i.e., a decrease in the sum of temperatures at later planting dates (Karagić et al., 2001).

Conclusion

The weather conditions in Serbia are gradually exceeding the levels required for the production of cabbage. The effects of temperature and water stress on cabbage production are increasing. Average air temperatures are the highest in July and August, causing temperature and water stress, which increases the cost of production and affects the final price of technologically mature heads.

To improve the efficiency of water use and adapt cabbage to warmer and drier conditions, it is recommended to use modified production systems, with an emphasis on postponing the date of sowing or planting in order to mitigate the effects of temperature increase and drought during cabbage production.

The avoidance of temperature and water stress must be adjusted with the aim of achieving maximum quality / technological compaction of the head, and the time needed for obtaining the highest price on the market.

The development of production systems that improve the efficiency of water use adapted to the conditions of hot and dry weather should be prioritised. The irrigation of cabbage should be organised as drip irrigation, as this is a more rational system, with the possibility of comparative feeding and protection.

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UTICAJ KLIMATSKIH PROMENA NA PROIZVODNJU KUPUSA

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R e z i m e

Za rast i razvoj kupusa, optimalne temperature su od 15 do 18ºC. Međutim, veći deo vegetacionog perioda kupusa je izložen temperaturama preko 20 ºC. Iz razloga dobijanja što veće cene na zelenoj pijaci, proizvođači kupusa kreću sa što ranijim rokovima sadnje kupusa (ranog ili kasnog jesenjeg). Na sam kupus se „vrši pritisak" kroz obilnije navodnjavanje, gušći sklop, prihranu i zaštitu da bi u što kraćem roku formirao tržišno zbijenu glavicu (tehnološki zrelu), koja bi ostvarila što bolju dobit na tržištu. U cilju poboljšanja efikasnosti korišćenja vode i prilagođavanja kupusa toplijim i suvljim uslovima, za preporuku je korišćenje modifikovanih proizvodnih sistema. Akcenat možemo staviti na pomeranje datuma setve ili sadnje u cilju borbe protiv sve prisutnijeg povećanja temperature i perioda nedostatka vode tokom sezone uzgoja kupusa. Cilj ovog rada je ukazati na uticaj temperaturnog i vodenog stresa, te dati određena rešenja koja se mogu praktično primeniti radi ublažavanja negativnog uticaja pomenutih stresova na proizvodnju kupusa. Prednost bi trebalo dati razvoju proizvodnih sistema za poboljšanje efikasnosti korišćenja vode prilagođenih uslovima toplog i suvog vremena. Navodnjavanje kupusa bi trebalo organizovati sistemom kap po kap. Ovim sistemom navodnjavanja racionalnije trošimo vodu, uz mogućnost uporednog prihranjivanja i zaštite.

Ključne reči: temperaturni stres, vodni stres, kupus, navodnjavanje, malčiranje.

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AMMI, GGE-BIPLOT, AND JOINT REGRESSION TECHNIQUE AS A TOOL IN MEASURING G × E INTERACTION IN 3-WAY CROSS MAIZE (*ZEA MAYS* L.) HYBRIDS

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Abstract: Genotype by environment (GE) interaction has a large impact on selecting adapted and predictable genotypes. Therefore, it is necessary to evaluate maize genotypes across different environments, seasons or locations for a successful selection. Twelve 3-way cross maize hybrids obtained from the International Institute of Tropical Agriculture (IITA) were evaluated on the field of the Federal University of Agriculture, Abeokuta, Nigeria (latitude 7º 15º N and longitude 3º 25º E) across three growing seasons of 2021 and 2022. The experiment was laid out with three replicates. Additive main effect and multiplicative interaction (AMMI), genotype (G) plus GE (GGE) biplot and joint regression techniques were used to identify stable and high-yielding genotypes. The AMMI analysis showed that the total variances in the yield of the three-way maize hybrids accounted for by G, environment (E) and GE interaction were 30.6%, 44.19% and 25.31%, respectively. Based on the AMMI biplot, the genotypes LW1701-10 and OBA SUPER-9, which combined high yield with stability, were the most desirable. The GGE biplot showed that hybrids LW1701-10, OBA SUPER-9 and LW1701-6 were the most stable and desirable genotypes. The joint regression technique showed that the performance of the genotypes could not be revealed in a linear manner as the deviation component variance accounted for 81.05% and identified LW1701-6, LW1701-16, LW1701-12, LW1701-21, LW1701-4 as stable and desirable genotypes. The study revealed that the GGE and AMMI models were more effective than the joint regression technique in examining yield stability of maize hybrids. The study deals with the comparison of AMMI, GGE biplot and joint regression techniques.

Key words: genotype by environment interaction, stable, AMMI, GGE biplot, joint regression technique.

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Introduction

Maize (*Zea mays* L.), a well-known grain, is important in Nigeria, as it is widely cultivated by a large number of farmers and has high economic value (Adetimirin et al*.,* 2008). However, its productivity remains low across sub-Saharan African countries when compared to the global average production (FAO, 2023). This is due to several abiotic and biotic factors, including low soil fertility, drought and heat stresses, striga infestations, use of low-yielding and un-adapted varieties. High-yielding varieties/hybrids with reliable performance under different environmental conditions are of great importance for sustainable production and productivity. Grain yield is an important agronomic trait that is inherently quantitative and influenced by genes in the crop, the environment and soil management as well as their interactions (Messina et al*.,* 2009).

Multi-environment trials (MET) are conducted worldwide for different crops to identify high-yielding genotypes and the environment(s) that best represent the test environments. In MET, genotypes are tested in different environments, seasons, and years to determine the adaptation of crops. However, the interaction of the genotypes with the environment (GE) makes the selection of the best performing and most reliable genotypes a difficult task. There are numerous stability statistics available to plant breeders which provide different approaches for dealing with GE interactions. These include combined ANOVA, regression analysis and multivariate methods, the additive main effects and multiplicative interaction (AMMI) proposed by Gauch and Zobel (1996) and Gauch (1992), and genotype plus GE interaction (GGE biplot analysis) proposed by Yan et al. (2000).

The AMMI model is one of the most widely used statistical tools in the analysis of different environment trials. It has two uses, namely for understanding complex GE interactions and for accuracy. The model has been used to study grain yields of different crops, select stable genotypes, and investigate GE interactions (Crossa, 1990). The complex relationships among the environments or among genotypes can be adequately represented in a scattered diagram that shows both the genotypes and environments simultaneously. This scattered diagram is called an AMMI biplot (Mohammadi et al., 2007).

The GGE biplot is based on environment-centered data, which removes the main effects of environments (because environments account for a large percentage of variation and are not useful in genotype selection) and integrates the genotypic main effect with the GE interaction effect of a genotype by environment data set (Yan et al., 2000). The GGE biplot is a useful tool for mega-environment analysis ("which-won-where" pattern), whereby specific genotypes can be recommended for specific mega-environments (Yan and Kang, 2003), genotype evaluation (the mean performance and stability), and environmental evaluation (the ability to discriminate among genotypes in the target environments).

Joint regression analysis is an important model for analyzing and interpreting the GEI of two-way classified data and continues to support traditional statistical analysis in genetics and plant breeding in determining the yield stability of different genotypes or agronomic treatments across varying environments (Crossa, 1990). A recent study by Dias et al. (2023) provides a comprehensive overview of joint regression techniques in the context of genotype × environment interaction (GEI) analysis. The authors discuss the application of joint regression models, emphasizing their utility in assessing yield stability and adaptability across diverse environments. This work highlights the continued relevance of joint regression methods in modern crop evaluation and breeding programs. Joint regression analysis combines additive and multiplicative components and thus analyzes both main effects and interaction with main effects, reducing its power for general significance testing (Farshadfar and Stuka, 2006).

Material and Methods

Twelve three-way cross maize hybrids were sourced from the germplasm of the Maize Breeding Unit of the International Institute of Tropical Agriculture (IITA), Ibadan, and were used for this study (Table 1). The experimental sites for the study were the upland and inland valley located at the Teaching and Research Farm of the Federal University of Agriculture, Abeokuta (FUNAAB), Ogun state, Nigeria (Lat 7^o 10^o N and 7^o 58 ^{o}N and Long 3^o 20^o E and 4^o 37^o E). The study was conducted in two different cropping seasons, the dry and rainy season of 2021 and 2022. The agro-meteorological data of the FUNAAB experimental sites for the period in which the study was conducted are shown in Table 2.

Table 1. List of genotypes and sources.

Genotypes	Source
LW1701-4	IITA
LW1701-6	IITA
LW1701-7	IITA
LW1701-10	IITA
LW1701-12	IITA
LW1701-16	IITA
LW1701-21	IITA
OBASUPER -7	IITA
OBASUPER-9	IITA
SAMMAZ-23	IITA
SAMMAZ-22	IITA
OSIELE (CHECK)	FUNAAB

Source: IITA: International Institute of Tropical Agriculture, Ibadan.

Table 2. The hydrothermal parameters for 2021 and 2022.

2021	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
Rainfall (cm)	----	4.8	20.02	19.72	8.8	4.94	22.56	13.16	9.35	9.93	10.52	30.85
Max temp°C	34.21	36.21	34.2	33.6	34.02	30.92	30.9	31.02	30.4	32.37	32.85	23
Min temp°C	24.11	25.55	23.3	23.4	24.40	23.12	22.6	22.70	22.55	21.97	22.75	26.93
R.H (%)	62.99	63.05	69.68	80.09	77.36	77.83	74.25	82.76	77.98	78.15	78.18	78.94
Sunshine (hr)	5.15	4.94	5.33	6.3	7.25	4.86	4.6	3.20	6.20	5.69	5.9	4.6
Evaporation (mm)	----	3.5	4.05	3.31	4.7	3.9	3.18	1.90	3.06	3.41	3.43	3.80
2022	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEPT	OCT	NOV	DEC
Rainfall(cm)	-----	------	11.83	2.3	2.49	15.59	1.95	0.81	11.58	2.79	11.62	
Max temp°C	31.77	33.46	31.23	30.6	30.90	29.36	29.44	26.7	29.7	31.1	32.45	
Min temp°C	22.77	22.2	22.55	22	21.94	21.83	22.33	20.61	21.2	21.4	21.2	
R.H (%)	88.88	75.32	79.25	83.7	80.33	80.74	84.42	83.45	82.7	79.3	77.93	
Sunshine (hr)	4.44	5.19	4.72	3.9	4.92	3.88	4.25	2.76	3.7	4.02	3.92	
Evaporation (mm)	4.36	3.91	3.64	3.4	3.71	3.01	3.16	2.05	2.8	3.3	3.31	

The first and second plantings were carried out during the 2021 rainy season (20^{th} June, 2021 – 30^{th} August, 2021) and the 2022 rainy season (3^{rd} February, 2022 – 26^{th} May, 2022) at upland area, while the third planting was carried out at in-land valley during the 2022 dry season (3^{rd} August, 2022 – 24^{th} November, 2022). Each planting was laid out in a randomized complete block design in three replications. Two seeds of each of the 12 genotypes were sown at the spacing of 75 cm between rows and 50 cm within rows. The blocks were separated by a 1 m wide alley. Weed control was carried out manually if necessary.

Data collection

Data were collected from five (5) plants from the center of each row on the following traits:

i. Days to 50% tasseling: this was determined by counting the number of days from planting to the time when 50% of the plants were tasseling.

ii. Days to 50% silking: this was determined by counting the number of days from planting to the time when 50% of the plants have developed silks.

iii. Plant height (cm): the height of the selected plants was measured from the base to the beginning of tassel branching using a meter rule.

iv. Ear height (cm): this was determined by measuring the height from the base of the plant to the node bearing the upper ear with a meter rule.

v. Field weight (kg): this was measured by weighing the cobs per plot with a weighing scale.

vi. Grain yield per plot (kg): this was done by measuring the total yield per plant at 15% moisture content with a weighing scale.

vii. 1000-seed weight (g): this was determined by counting 1000 seeds and weighing with a weighing scale.

Data analysis

The data collected were subjected to separate and combined analysis of variance (ANOVA) across environments using SAS 9.2 software package to determine the effects of genotype (G), environment (E) and GEI. The means were separated using the least significant difference (LSD) at a 5% probability level.

The yield data were subjected to the additive main effect and multiplicative interaction (AMMI) model according to Gauch and Zobel (1997). The linear model of the technique is:

$$Y_{ger}=\mu + \alpha_g + \beta_e + \sum_n \lambda_n \Upsilon_{gn} \delta_{en} + \rho_{ge} + \epsilon_{ger} \quad (1)$$

Y_{ge} is the trait of genotype g in environment e;
μ is the grand mean;
α_g is the deviation of the genotype from the grand mean;
β_e is the environment deviation from the grand mean;
λ_n is the eigen value of PCA axis n;
$\Upsilon_{gn}\delta_{en}$ are the genotype and environment PCA scores for PCA axis n;
ρ_{ge} is the residual of the AMMI model.

The AMMI stability value (ASV) was calculated using the formula proposed by Purchase et al. (2000) as follows:

$$ASV=\sqrt{\left[\frac{IPCA\ 1SS}{IPCA\ 2SS}(\text{IPCA 1scores})2\right] + (\text{ IPCA scores})2} \quad (2)$$

where IPCA 1 SS/IPCA 2 SS is the weight given to the IPCA 1-value.

The IPCA is an indicator of stability to measure the response of both genotypes and environments. The absolute value of the first IPCA score represented the simplest measure of yield and stability.

Genotype plus genotype × environment interaction (GGE) following the procedures of Yan (2001), Yan and Kang (2003), Yan et al. (2007) was used to decompose the GEI into mega-environments and the best-performers according to their visual characteristics. The mean performance and stability of the genotypes were determined, and the discriminatory power and representativeness of the test environments were revealed (Yan, 2001). The GGE biplot was used to identify the ideal genotypes and locations.

The equation of the GGE biplot is as follows (Yan and Kang, 2003):

$$\bar{Y}_{ij} - Y_J = \lambda_1 \Sigma_{I1} \Pi_{ji} + \lambda_2 \Sigma_{I2} \Pi_{j2} + \epsilon_{ij} \quad (3)$$

$\bar{Y}_{ij}$ is the mean yield of genotype I in environment j;

Y_J is the mean yield across all genotypes in environment j;

λ_1 and λ_2 are the singular values for PC1 and PC2, respectively;

Σ_{i1} and Σ_{i2} are the PC1 and PC2 scores for genotype I;

Π_{jI} and Π_{j2} are the PC1 and PC2 scores for environment j;

ϵ_{ij} is the error associated with genotype i in environment j.

Genotype stability index: a stability index recommended by Farshadfar (2008) was calculated for each genotype by adding the overall mean performance and ASV for each trait as follows:

$$GSIi = RASVi + RYi \quad (4)$$

where GSIi = the genotype stability index for the i^{th} genotype across the environment for each trait;

RASVi = the rank of the i^{th} genotype across the environments based on ASV;

RYi = the rank of the i^{th} genotype based on the mean yield across the environment. The genotype with the lowest GSI was considered the best for a particular trait.

Joint regression analysis was carried out according to the procedure of Perkins and Jinks (1968) to identify and select stable genotypes, using a linear regression coefficient (b) and the mean square of deviation (S^2di). The means of the three characters were regressed individually on the means of each environment to determine the response pattern. The GEI was partitioned by the use of variance components to determine the relative magnitude of the variances due to heterogeneity and deviation mean squares according to Breese (1969).

The linear model of Perkins and Jinks (1968) is as follows:

$$Y_{ij} = \mu + d_i + E_j + g_{ij} + e_{ij} \quad (5)$$

Y_{ij} is the yield variable of the i^{th} genotype at the j^{th} environments;

μ is the general mean over all genotypes and environments;

d_i is the additive effects of the i^{th} genotype;

E_j is the effects of the j^{th} environment;

g_{ij} is the GE interaction of the i^{th} genotype with the j^{th} environment;

eij is the error term.

The deviation of bi values from 1.0 was tested using the *t* test for the null hypothesis.

$$t = \frac{(bi-1)}{S.E.}$$

where S.E. = standard error.

If bi is significant, its significant deviation from unity is tested by the following formula:

$$t = \frac{(b-1)}{S.E.}$$

The deviation (S^2di) from zero was tested by dividing the stability values by the pool error M.S.

Results and Discussion

The combined ANOVA of maize genotypes in three environments is presented in Table 3. The result showed that genotype and environments effects were highly significant ($p<0.001$) for all traits evaluated. Similarly, GEI was equally significant for plant height, ear height, 1000-seed weight and grain yield. The maize genotypes evaluated differed significantly in all the characters, suggesting that all twelve genotypes differed from each other. The significant differences observed across the three seasons provided an opportunity to identify reliable genotypes. The environment accounted for more of the total variation than the genotype. This opines with the results of Ojo et al. (2021) and Komolafe et al. (2022).

The result of the AMMI analysis for grain yield of twelve genotypes of 3-way cross maize hybrids is presented in Table 4. Genotype accounted for 30.6% of the total variation, while environments and genotype by environment interaction accounted for 44.19% and 25.13%, respectively. The GEI was divided into the first two IPCA axes and the residual. IPCA 1 and IPCA 2 explained 67.8% and 32.10% of the total GEI variance, respectively. Crossover is present when a genotype performs better in one environment but poor in another, while non-crossover is present when a genotype continues to lead to a change in the IPI values. The AMMI analysis has been used by several authors as a tool to identify the superior and stable genotypes, as well as favorable and high-yielding environments (Tekdal and Kendal, 2018). Based on the results of this study, the AMMI model accounted for a substantial part of the total sum of squares, suggesting that the model was appropriate to explain the GEI. The differences in the amount of rainfall and other climatic conditions in the environments used for the study may have been responsible for the variability in the performance of the genotypes. This is consistent with the report by Komolafe et al. (2022). The significant sum of squares for environment and GEI obtained in the AMMI analysis indicated that the environments used for this study were different from each other, and thus, genotypes responded differently to each environment. The results agree with the findings of Naroui Rad and Bakhshi (2021).

The mean grain yield of 12 genotypes of 3-way cross maize hybrids grown in three environments and their first IPCA scores are presented in Table 5. The results show that the grain yield per plant ranged from 519.94 g/plant to 1011.11 g/plant. SAMMAZ-22, LW1701-10, LW1701-7, OBASUPER-9, LW1701-21 and LW1701-6 performed above average during the 2021 rainy season (E1). In the 2022 dry season (E2), LW1701-10, LW1701-12, LW1701-16, LW1701-6, OBA SUPER-7, and OBA SUPER-9 performed above average. However, in the 2022 rainy season (E3), only 4 genotypes LW1701-10, LW1701-21, LW1701-6, and OBA SUPER-7 performed above average. Environment 3 had the highest mean yield of 881.94 g/plant, while environment 2 had the lowest mean yield of 579.44 g/plant. Osiele Check had the highest IPCA score of 8.11, while Sammaz-22 had the lowest IPCA score of -12.65.

Table 3. Combined analysis of variance for grain-yield and related traits of 12 maize genotypes in three environments.

Source of variation	df	Days to tasseling	Days to silking	Plant height	Ear height	Field weight	1000-seed weight	Grain yield
Environment (E)	2	363.36**	253.48**	115002.96**	17682.06**	2.99**	16893.14**	1047689.58**
Block/E	6	18.19	4.39	1183.45**	217.07*	0.36**	148.28	13736.81
Genotype (G)	11	29.99**	31.42**	1030.21**	290.20**	0.36**	3961.81**	132223.80**
G x E	22	14.41	8.93	1152.45**	539.94**	0.18	1575.15**	54691.60**
Error	66	10.08	7.54	382.52	97.85	0.11	684.16	19248.93

**,* significant at 0.01 and 0.05, respectively.

Table 4. AMMI ANOVA table for grain yield of the twelve genotypes of 3-way cross maize hybrids evaluated across three environments.

Source of variation	Df	SS	MS	% of treatment	% G×E
Total	107	6105906	57065		
Treatment	35	4753056	135802**		
Genotype	11	1454462	132224**	30.6	
Environment	2	2095379	1047690**	44.19	
Block	6	82421	13737		
Interaction	22	1203215	54692**	25.31	
IPCA 1	12	816952	68079**		67.89
IPCA 2	10	386264	38626*		32.10
Residual	0	0	*	*	*
Error	66	1270429	19249		

**, * and IPCA mean significant at 0.01, 0.05 and interaction principal component axes, respectively.

Table 5. Mean and the first IPCA scores of the AMMI analysis of grain yield for the 3-way cross maize hybrids across three environments.

S/N	Environment Genotype	E1	E2	E3	Mean yield	First IPCA
1	Osiele Check	475.00	450.00	633.33	519.94	8.11
2	LW1701-10	1033.33	800.00	1200.00	1011.11	2.37
3	LW1701-12	800.00	583.33	850.00	744.44	2.18
4	LW1701-16	766.67	833.33	866.67	822.22	10.32
5	LW1701-21	900.00	450.00	983.33	777.78	-4.11
6	LW1701-4	866.67	470.00	800.00	712.22	-3.48
7	LW1701-6	966.67	600.00	950.00	838.89	-2.36
8	LW1701-7	1033.33	453.33	766.67	751.11	-9.69
9	OBA-SUPER-7	700.00	583.33	1033.33	772.22	6.55
10	OBA-SUPER-9	1000.00	850.00	833.33	894.44	2.80
11	SAMMAZ-22	1133.33	450.00	850.00	811.11	-12.65
12	SAMMAZ-23	733.33	430.00	816.67	660.00	-0.04
	Mean	867.36	579.44	881.94	776.25	
	First PCA	-17.42	14.48	2.95		

E1= First rainy season of 2021, E2 = Dry season of 2022, E3 = Second rainy season of 2022.

In addition, environment 2 had the largest IPCA score of 14.48, while E1 had the lowest IPCA score of -17.42. Thus, when a genotype and an environment have the same sign on their respective first IPCA axes, their interaction is positive, if different, their interaction is negative. The AMMI stability values revealed variations in grain ranking yield among the twelve genotypes. G12 (Sammaz-23) and G3 (LW1701-12) were considered stable according to ASV, but unstable according to GSI. This agrees with the report by Komolafe et al. (2022), who found some genotypes stable according to ASV but slightly unstable according to GSI. The AMMI biplot enables the simultaneous selection for yield and stability of genotype performance. This selection is always based on genotype yield responses to the environments, adaptability of the genotypes to the environments and the minimum deviation from zero score on IPCA 1 (Ariyo and Ayo-Vaughan, 2000).

The polygon view of the GGE biplot of twelve genotypes of maize grown in three environments is presented in Figure 1. The PC1 explained 55.5% of the total variation, whereas PC2 explained 30% of the variation in grain yield. The two principal axes accounted for 85.5% of the total variation in grain yield across three environments. The polygon view grouped the three environments into two mega-environments, with environment 1 in one mega-environment and environments 2 and 3 in the second mega-environment. The genotypes at the vertex of each sector denote the highest yielding genotype in the environments that fall within that sector. The vertex genotypes are G2 (LW1701-10) for mega-environment 1 and G11 (SAMMAZ-22) for mega-environment 2. G2 (LW1701-10) was the best in environment 3 and G11 (SAMMAZ-22) was the best in environment 1. The genotypes that fell out of the mega-environments are low-yielding and therefore not desirable in any of the environments and probably the poorest in some or all environments (Yan, 2001). The GGE biplot ranks the genotypes according to the average yield and stability. G2 (LW1701-10), G10 (Oba Super-9), and G7 (lw1701-6) combined high yield with stability, indicating that these genotypes possess the ability to maintain constant performance in terms of yield over a range of environmental conditions. This is in agreement with Kusmiyati and Setiawan (2022), who defined yield stability as the ability of a genotype to avoid significant yield fluctuation over a range of environmental conditions.

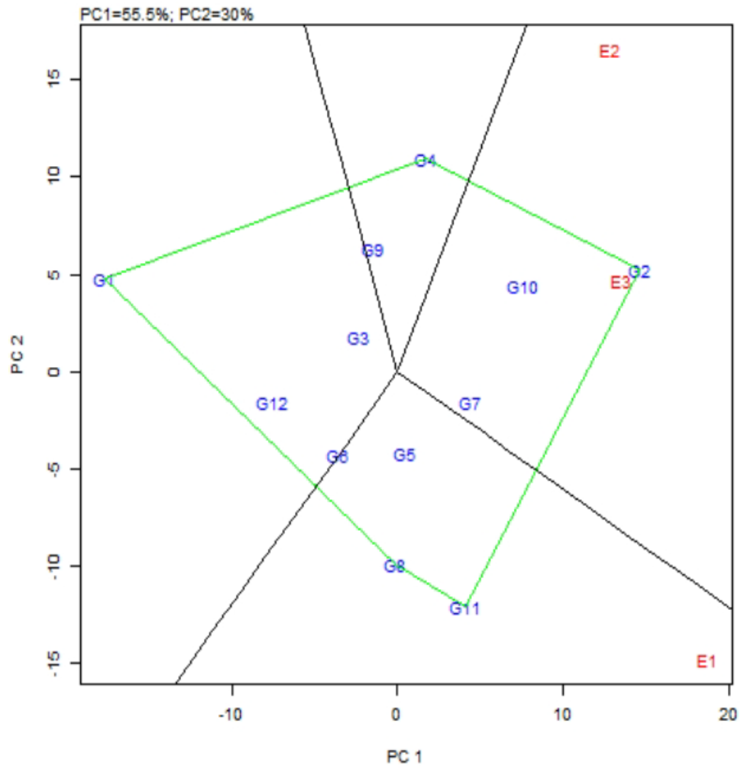

Figure 1. Polygon view of GGE biplot for grain yield data of twelve 3-way cross maize hybrid genotypes evaluated across 3 environments between 2021 and 2022.
E1=Rainy; June 2021 – Aug 2021, E2=Dry; Feb 2022 – May 2022, E3=Rainy; Aug 2022 – Nov 2022. G1=Osiele Check, G2=LW1701-10, G3=LW1701-12, G4=LW1701-16, G5=LW1701-21, G6=LW1701-4, G7=LW1701-6, G8=LW1701-7, G9=OBA SUPER-7, G10=OBA SUPER-9, G11=SAMMAZ-22, G12=SAMMAZ-23.

Though G11 (Sammaz-22) and G4 (LW 17101-16) were high-yielding, they were not stable and therefore unpredictable. G12 (Sammaz-22) and G3 (LW 1701-12) were stable but low-yielding genotypes, making them undesirable for selection. G1 (Osiele Check), G8 (LW 1701-7) and G9 (Oba- Super 7) were low-yielding and unstable, making them the most undesirable. Yan and Kang (2003) defined an ideal genotype on the basis of mean performance and stability. Kaya et al. (2006) have also reported that a genotype is only stable when it translates to high yields. This ideal genotype rarely occurs in nature, thus the genotype closest to the small circle is called ideal. Based on this information, G2 (LW 1701-10) was classified as the ideal genotype in terms of grain yield, as it combines yield and stability. This is in contrast to Jalata (2011), who have argued that there is no ideal genotype.

The discriminatory and representative view of the environments and ranking of the test environment relative to an ideal environment is presented in Figure 2. An environment is called discriminative when it is able to identify the best genotypes, while representativeness refers to the ability of a test location to represent the test environments (Kassaye et al., 2024). The length of the environment vectors (which approximates the standard deviation within each environment) from the biplot origin and the angle formed with the abscissa of the AEC reveal the discriminatory ability and representativeness of the test environment, respectively. A test environment that has the longest vector is more discriminatory when compared with other test environments. Hence, environment 1 was the most discriminatory environment. A test environment is more representative when it has the smallest deviation angle with the AEC abscissa compared with the other test locations. Thus, environment 3 was identified as the most representative. An ideal environment is the most discriminatory of genotypes and yet representative for the other test environments. Hence, the dark circle in the ring of circles determines the ideal environment and the environment closest to the best environment. This biplot shows that E3 comes closest to the ideal environment. The GGE biplot evaluates the test locations through their discriminatory power and representativeness. The environment with the highest discriminatory power is the environment that provides the best information on the genotype differences among the tested genotypes, while the ability to represent the average environment confers representativeness (Kassaye et al., 2024). Kassaye et al. (2024) defined an ideal test environment as a virtual environment that has the longest vector of all the test environments (most discriminating) and located on the AEC abscissa (most representative). In terms of grain yield, the 2021 rainy season was the least representative and most discriminatory among the environments. This is true as the means of genotypes in the 2021 rainy season were not significantly different from each other.

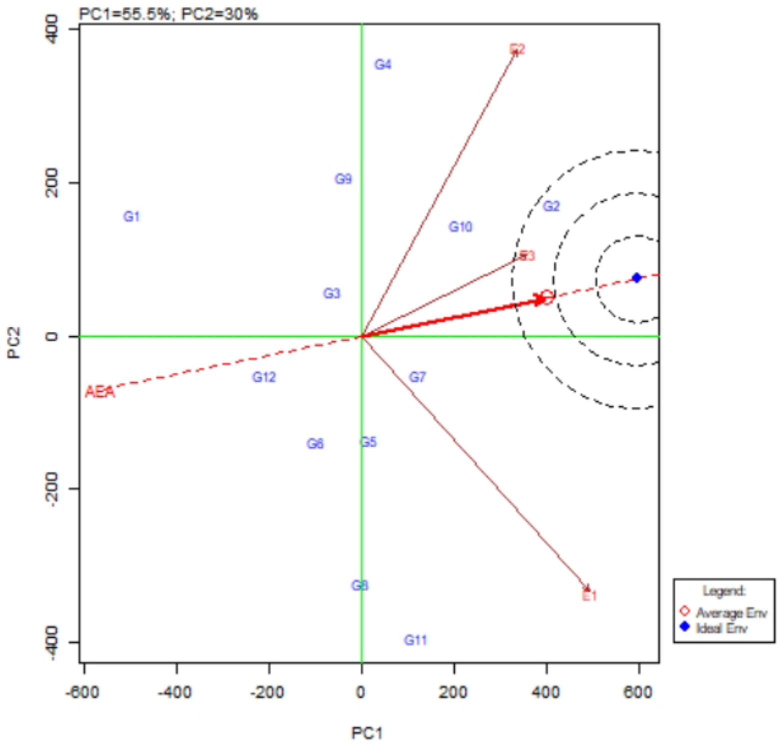

Figure 2. The biplot showing discrimination and representativeness based on genotype by environment grain yield data of twelve genotypes of 3-way cross maize hybrids evaluated across 3 environments between 2021 and 2022.
E1=Rainy; June 2021 – Aug 2021, E2=Dry; Feb 2022 – May 2022, E3=Rainy; Aug 2022 – Nov 2022, G1=Osiele check, G2=LW1701-10, G3=LW1701-12, G4=LW1701-16, G5=LW1701-21, G6=LW1701-4, G7=LW1701-6, G8=LW1701-7, G9=OBA SUPER-7, G10=OBA SUPER-9, G11=SAMMAZ-22, G12=SAMMAZ-23.

According to Yan and Tinker (2006), such an environment gives little or no encouragement to good genotypes with the potential to upgrade poor genotypes. The rainy season (2022), which had the smallest angle with the AEC abscissa, is the most representative environment and is ideal for selecting superior genotypes. This is true as the test location is the closest to the ideal environment. The most representative environment can therefore be used to select sample environments with wide adaptation, lowering the cost of multi-environment trials while the non-representative environments are useful for selecting specifically adapted genotypes.

The performance of twelve maize genotypes based on the mean grain yield/plant and stability across the three environments is presented in Figure 3. The single-arrowed line, called the Average Environment Coordinate (AEC) abscissa, indicates the direction of increasing mean genotype performance across test environments. The double-arrowed line, orthogonal to the AEC abscissa and passing through the origin, is the AEC ordinate**.** This axis divides the genotypes into two groups: those on the right performed above the overall mean**,** while those on the left yielded below the average**.** G2 (LW1701-10) was the best performer across the three environments based on yield. The projections on the ordinate are measures of the stability of the genotypes. The shorter the vector, irrespective of the direction, the more stable is the associated genotype. A short vector denotes high stability (Yan and Kang, 2003). LW1701-10 (G2) was identified as the most stable, followed by LW1701-6 (G7), OBA-SUPER-9 (G10), while Osiele-Check (G1), LW1701-7(G8), SAMMAZ-22(G11), LW1701-16(G4), OBA-SUPER-7 (G9), LW1701-21(G5) had long vectors and were unstable. An ideal genotype is the genotype that combines high yield and stability. It is represented by the small circle with the arrow pointing to it. LW1701-10 (G2) was identified as the most desirable genotype near the ideal genotype.

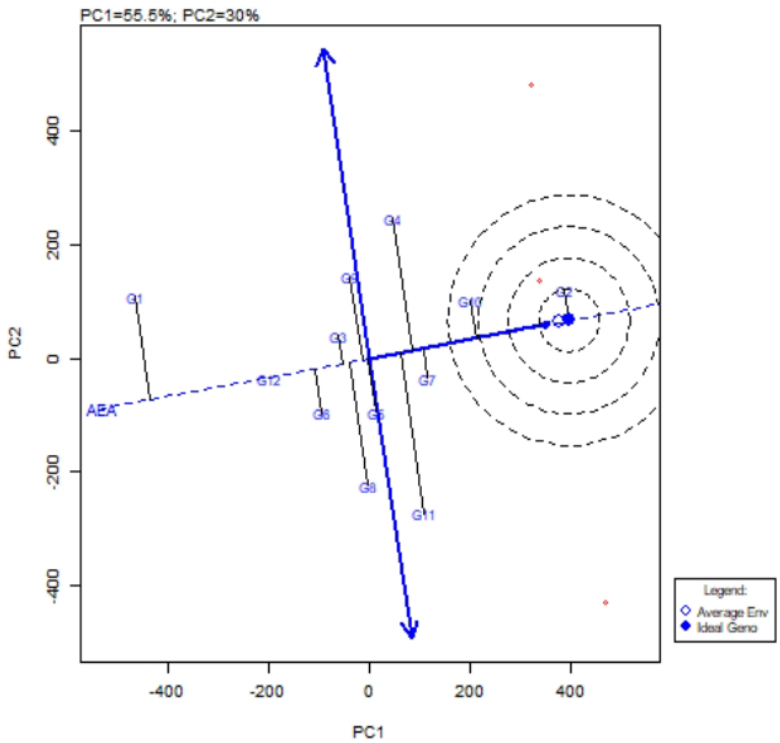

Figure 3. The GGE biplot showing the performance of the 3-way cross maize hybrid genotypes for both grain yield and stability evaluated across 3 environments between 2021 and 2022.

E1=Rainy; June 2021 – Aug 2021, E2=Dry; Feb 2022 – May 2022, E3=Rainy; Aug 2022 – Nov 2022, G1=Osiele check, G2=LW1701-10, G3=LW1701-12, G4=LW1701-16, G5=LW1701-21, G6=LW1701-4, G7=LW1701-6, G8=LW1701-7, G9=OBA SUPER-7, G10=OBA SUPER-9, G11=SAMMAZ-22, G12=SAMMAZ-23.

Using the procedures described by Perkins and Jinks (1968), analysis of variance for performance stability revealed that genotype, environment and GEI were significant for grain yield (Table 6). The significant remainder mean square also indicated that a significant portion of the variation was non-linear due to the genotype × environment interaction. However, the variance component was used to examine the relative contribution of heterogeneity and deviation from regression to GEI. It was found that the larger proportion of GEI was accounted for by the deviation component (81.05%) and the heterogeneity between regressions accounted for 8.53%.

Table 6. Mean squares from the stability analyses of variance of 3-way cross hybrid maize (Perkins and Jinks, 1968).

Source	DF	Grain yield	Component of GEI variance
Genotype	11	44074.59*	
Environment (joint regression)	2	349231.83**	
Genotype × environment	22	18230.31**	
Heterogeneity between regression	11	19015.75	8.53
Remainder	12	15991.14**	81.05
Pooled error	72	6416.31	

The average grain yields per plant and the regression coefficient of each genotype are presented in Table 7. Since the regression coefficients measure the responses of the genotypes to an increment in an improving environment, G11 (SAMMAZ-22), G5 (LW1701-21) and G8 (LW1701-7) showed above average responses with regression coefficients greater than 1.00 and were considered good yielders in above average environments. G2 (LW1701-10) and G9 (OBA – SUPER-7) with regression coefficients averaging 1.09 and 1.00 were also consistent in all environments. Other genotypes such as G1 (OSIELE CHECK), G3 (LW1701-12), G4 (LW1701-16), and G10 (OBA-SUPER-9) had regression coefficients smaller than 1 and only performed well in below-average environments. Six genotypes were found stable among the twelve genotypes: LW 1701-12(G3), LW 1701-16(G4), LW 1701-21(G5), LW1701-4 (G6), LW1701-6 (G7) and SAMMAZ-23 (G12). LW 1701-16(G4), LW1701-6 (G7) and LW1701-21(G5) were high-yielding and stable. The non-significant heterogeneity between the regressions in this study indicated that the response of the genotypes was not different and therefore the relative performance of maize grain yields cannot be accurately predicted. Genotypes are regarded as being stable based on t of their stability deviation being zero. The following genotypes LW 1701-12(G3), LW 1701-16(G4), LW 1701-21(G5), LW1701-4 (G4), LW1701-6 (G7) and SAMMAZ-23 (G12) were regarded as stable across the test location. LW1701-16 (G4), LW1701-6 (G7) and LW1701-21(G5) combined both high yield and stability, while SAMMAZ-23 had a low yield and was stable. Differences in environments are an important factor and largely determine the usefulness of b_i values (Pfahler and Linskens, 1979). The significance and non-significance of pooled deviation and heterogeneity between regressions in the joint regression indicated the presence of a GE interaction and that the response of genotypes to the maize grain yield was non-linear.

Table 7. Mean yield for genotypes, regression coefficient and stability value of each maize hybrid.

S/N	Genotypes	Mean grain yield/plant (g)	Regression coefficients b_i	Stability
1	OSIELE CHECK	519.4	0.37 ± 0.45	5294.96**
2	LW1701-10	1011.11	1.09± 0.44	4967.40**
3	LW1701-12	744.44	0.82 ± 0.11	-5693.29
4	LW1701-16	822.22	-0.04 ± 0.30	-1342.96
5	LW1701-21	777.78	1.67 ± 0.17$^+$	-4676.53
6	LW1701-4	712.22	1.22 ± 0.25	-2842.68
7	LW1701-6	838.89	1.21 ± 0.10$^+$	-5826.68
8	LW1701-7	751.11	1.48 ± 0.85	35188.75**
9	OBA-SUPER-7	772.22	1.00 ± 0.94	44477.40**
10	OBA-SUPER-9	894.44	0.20 ± 0.50	8001.06**
11	SAMMAZ-22	811.11	1.80 ± 0.91	41573.22**
12	SAMMAZ-23	660.00	1.18 ± 0.19	-4222.68
	Average	776.25	1.00	

$^+$Regression coefficients (b_i), significantly greater than 1.0, the other genotypes have regression coefficients not significantly different from 1.0 or less than 1.0. **Stability, significantly greater than 0.

Further partitioning of the GE interaction into components according to Breese (1969) revealed that the deviation from linearity was larger than the heterogeneity between regressions. The present study on maize clearly demonstrates that it is not reasonable to simply assume that GEI may always be explained by a linear function of the environment. The result of this study is in agreement with the findings of Perkins and Jinks (1968), where the remainder M.S. is significant against the error M.S. This result also aligns with recent findings that report highly significant deviation components for yield-related traits, highlighting the presence of substantial genotype × environment interactions. Such findings underscore the need to assess genotypic stability and adaptability in crop breeding programs (Adu et al., 2019; Ajala et al., 2021). This result is in contrast to the findings of Perkins and Jinks (1968), who showed that there was a significant linear regression which accounted for the entire GEI. This result is also in contrast to the results of Anil et al. (2013) and Khathod et al. (2006), who observed a significant linear and nonlinear response of genotype to environment. In contrast, Breese (1969) found a linear response to yield in the five grass populations studied.

The relative effectiveness of joint regression, AMMI and the GGE biplot in discriminating desirable genotypes is presented in Table 8. AMMI identified four genotypes as desirable, while GGE biplot and joint regression identified three (3) and five (5) genotypes, respectively, as the desirable genotypes. These three techniques identified LW1710-6 as the desirable genotype. AMMI and GGE biplot also agreed on LW1701-10, LW1701-6 and OBA SUPER-9 as high-yielding and stable and therefore the most desirable. In the order of the superiority, the GGE biplot was found to be the most sensitive, while the AMMI was superior to the joint regression. This result is in agreement with the findings of Yan et al. (2007), who observed the superiority of the GGE biplot over the AMMI analysis.

Table 8. Relative effectiveness of AMMI, GGE biplot, and joint regression in discriminating desirable maize genotypes in terms of grain yield.

S/N	AMMI	GGE BIPLOT	Joint regression
1	LW1701-10	LW1701-10	LW1701-6
2	LW1701-21	LW1701-6	LW1701-16
3	OBA SUPER-9	OBA SUPER-9	LW1701-12
4	LW1701-6		LW1701-21
5			LW1701-4

The GGE biplot was found to be more informative in the “which-won-where’’ view, mega-environment evaluation and identification of ideal genotypes and environment than the AMMI model. Yan et al. (2010) suggested that the GGE biplot analysis should be supported by conventional statistical analysis. This is because the GGE biplot use is only justified when either G or G × E in the data is statistically significant and an analysis of variance should be conducted to see if it is worthwhile to perform a GGE biplot analysis. The AMMI analysis combines the analysis of variance and principal component analysis (PCA) into a unified approach (Gauch and Zobel, 1988). Using this tool, larger sum of squares was recorded for the interaction components when compared to the joint regression analysis. A similar result was found by Ariyo and Ayo-Vaughan (2000). This study suggests that the AMMI model has been found superior to regression techniques by being more effective in explaining the GEI. Zobel et al. (1988) reported that AMMI analysis significantly improved the probability of successful selection.

Conclusion

The study revealed significant differences among the twelve maize genotypes, indicating the potential for selecting high-yielding and stable genotypes. The AMMI analysis identified LW1701-10 as the most stable genotype with high yields, while LW1701-16, OBA-SUPER-9, LW1701-6, and SAMMAZ-22 also showed high yield performance but were unstable. A specific genotype adaptation was observed: OBA-SUPER-9 was best suited for the 2022 rainy season, Osiele-1 for the 2022 dry season, and SAMMAZ-22 for the 2021 rainy season. GGE biplot analysis further confirmed LW1701-10 as the highest yielding and most stable genotype, particularly well suited to the 2022 rainy season. Among the testing environments, the 2021 rainy season was identified as the most discriminating, while the 2022 rainy season was the most representative.

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Appendix 1: Means of seven traits for 12 three-way cross maize hybrids across three environments.

Genotype	DYTS (days)	DYSK (days)	PLHT (cm)	EHT (cm)	FDWT (g)	1000-SW (g)	GYLD (g/plant)
OSIELE-1	63.67	65.78	182.43	97.72	0.59	248.27	519.44
LW1701-10	58.11	60.67	169.81	90.14	1.18	316.87	1011.11
LW1701-12	57.11	59.89	189.19	91.68	1.16	315.75	744.4
LW1701-16	59.22	61.56	183.08	87.61	1.03	301.12	822.22
LW1701-21	57.44	60.33	187.31	94.35	1.22	304.84	777.78
LW1701-4	59.00	62.44	187.63	96.13	1.14	292.78	712.22
LW1701-6	59.22	61.67	190.30	94.14	1.19	284.52	838.89
LW1701-7	57.67	62.00	185.16	93.43	1.03	283.83	751.11
OBA SUPER-7	58.72	58.94	192.02	84.29	0.68	303.59	772.22
OBA SUPER-9	56.67	58.56	195.36	97.47	1.11	277.93	894.44
SAMMAZ-22	60.00	61.33	162.80	84.10	0.97	326.48	811.11
SAMMAZ-23	59.17	61.44	165.42	80.56	1.06	301.75	660.00
LSD	2.58	2.58	18.41	9.31	0.31	24.62	74.98

DYST=Days to 50% tasselling, DYSK=Days to 50% silking, PLHT=Plant height. HT=Ear height, FDWT=Field weight, 1000-SWT=1000-seed weight. GYLD=Grain yield/plant.

ADITIVNI GLAVNI EFEKTI I VIŠESTRUKA INTERAKCIJA, GLAVNI EFEKAT GENOTIPA I INTERAKCIJE GENOTIP × SREDINA, ZDRUŽENA REGRESIJA U KVANTIFIKACIJI INTERAKCIJE GENOTIP × SREDINA TROLINIJSKIH HIBRIDA KUKURUZA

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R e z i m e

Interakcija genotipa i spoljašnje sredine (engl. *genotype by environment – GE*) ima veliki uticaj pri odabiru adaptiranih i superiornih genotipova. Zbog toga je neophodno ocenjivati genotipove kukuruza u različitim okruženjima, sezonama ili lokacijama radi uspešnog odabira. Dvanaest trolinijskih hibrida kukuruza dobijenih iz Međunarodnog instituta za tropsku poljoprivredu (engl. *International Institute of Tropical Agriculture – IITA*) ocenjivano je na oglednom polju Saveznog univerziteta za poljoprivredu u Abeokuti, Nigerija (7^{o}15′ severne geografske širine i 3^{o}25′ istočne geografske dužine) tokom tri vegetacione sezone 2021. i 2022. godine. Ogled je postavljen u tri ponavljanja. Za identifikaciju stabilnih i visokoprinosnih genotipova korišćeni su aditivni glavni efekti i višestruka interakcija (engl. *additive main effect and multiplicative interaction – AMMI*), glavni efekat genotipa i interakcije genotip × spoljašnja sredina (engl. *genotype and genotype environment interaction*), združena regresija. Analiza AMMI je pokazala da je ukupna varijansa u prinosu trolinijskih hibrida kukuruza bila raspodeljena na genotip, sredinu i interakciju genotip × sredina sa udelima od 30,6%, 44,19% odnosno 25,31%. Na osnovu AMMI analize, genotipovi LW1701-10 i OBA SUPER-9, koji su kombinovali visok prinos sa stabilnošću, bili su najpoželjniji. GGE biplot analiza je pokazala da su hibridi LW1701-10, OBA SUPER-9 i LW1701-6 najstabilniji i najpoželjniji genotipovi. Združena regresija je pokazala da se učinak genotipova ne može prikazati na linearan način, jer je varijansa komponente odstupanja iznosila 81,05% i identifikovala je LW1701-6, LW1701-16, LW1701-12, LW1701-21, i LW1701-4 kao stabilne i poželjne. Studija je pokazala da su modeli GGE i AMMI bili efikasniji od združene regresije u ispitivanju stabilnosti prinosa hibrida kukuruza. Studija se bavi poređenjem modela AMMI, GGE i združene regresije.

Ključne reči: interakcija genotip × sredina, stabilnost, AMMI, GGE biplot analiza, združena regresija.

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COMPONENTS OF PHENOTYPIC VARIANCE AND HERITABILITY OF EARLY VIGOUR TRAITS OF BREAD WHEAT UNDER CONTRASTING WATER REGIMES

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Abstract: Exploring drought-tolerance potential and phenotypic plasticity at early stages of development in root system architecture could be crucial in regard to breeding for drought resistance and for selecting wheat ideotypes under climate change conditions. A total of 11 genotypes from the collection of 101 bread wheat genotypes, with desirable traits related to increased drought tolerance, were selected as parents and eight crosses were performed. The genotypes of the P and F1 generations were grown in hydroponic cultivation under polyethylene glycol 6000-induced osmotic stress. The objective of this research was to assess components of phenotypic variance and broad-sense heritability of early vigour traits (nine root and shoot traits) of bread wheat genotypes under induced drought stress compared to the control, in order to choose appropriate traits for breeding for drought resistance. The effect of the genotype on the variability of the tested root traits was higher (46.6%), compared to the tested shoot traits (25.5%), meaning that the root traits can be taken as a more reliable criterion for selection for drought tolerance compared to the investigated shoot traits. Broad-sense heritability was high (> 82%) for most of the tested traits (primary root length, number of seminal roots, total seminal root length, angle of seminal roots, shoot length, the ratio of root dry mass to shoot dry mass), with low genotype × environment interaction (< 20% of total variation) and breeding for drought tolerance should be focused on these traits.

Key words: *Triticum aestivum* L., drought stress, root system architecture, shoot traits, seedlings, broad-sense heritability, components of phenotypic variance.

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Introduction

Drought can lead to a 50–90% reduction in wheat yield, and genotypes displaying strong early seedling establishment (vigour) in challenging conditions can be identified and selected by screening diverse plant materials on a large scale (Bhandari et al., 2024; Ahmed et al., 2025). Early vigour is a genetically complex trait (Moore and Rebetzke, 2015). Despite the moderate heritability, all the contributing traits are influenced by strong environmental covariates through the maternal effect as well as a potentially significant genotype × environment (GE) interaction (Rebetzke et al., 2022). Experimental approaches aimed at understanding the GE interaction as well as the management of environmental processes is expensive and time-consuming due to the time required for studying climatic factors over a long period of time. There is an opportunity to investigate the effect of early vigour through crop process modelling that integrates crop physiology, climate and soil conditions and their impacts on crop growth and yield. Such approaches enable a rapid assessment of the importance of early vigour under different climatic, edaphic and agro-technical conditions (Zhao et al., 2019).

Manschadi et al. (2006) estimated that each additional millimetre of water extracted during grain filling generated an additional 55 kg ha^{-1} of wheat grain yield. Root system architecture (RSA) broadly describes the development and growth of root systems targeted to explore and exploit available water and nutrients while anchoring shoots prior to, and during, canopy development (Rebetzke et al., 2022). Wheat develops seminal roots first. These roots remain active throughout the life of the plant and play an important role in early vigour and water uptake from deep soil strata (Rossi et al., 2024). Manschadi et al. (2008) found that RSA is closely linked to the angle of seminal root growth at the seedling stage and that selection for the growth angle and the number of seminal roots may identify drought-tolerant genotypes. Typically, wheats adapted to regions with limited rainfall had a high number of seminal roots descending with a narrow gravitropic angle and deeper root systems, whereas wheats adapted to environments with higher rainfall and/or irrigation tend to have wide seminal root angles, presumably facilitating water and nutrient acquisition from a wider sub-surface area (Hohn and Bektas, 2020).

Progress has been made in screening for drought resistance and evaluating root traits. Selection of wheat genotypes with larger roots and extended seminal and adventitious roots shows promise for improved grain yields, especially in arid and semi-arid regions, indicating substantial genetic heterogeneity in osmotic stress tolerance (Afzal et al., 2017). Several high-throughput phenotyping methods have been used to evaluate roots, including hydroponic systems using growth pouches (or germination paper), aeroponic and agar-plate systems, soil-filled rhizotron, gel-filled chambers with transparent walls, compressed soil columns with X-rays to detect roots, paper-based "cigar roll" system and deep column techniques (Colombo et al., 2022). Current studies on wheat root phenotyping are limited to the very early seedling stage, with only a few phenotyping platforms used for measuring whole root systems in wheat, such as the germination paper technique and clear pots (Chen et al., 2020). The RSA ideotypes used to optimise agronomic performances are different from one environment to another, and the relationships between root traits measured in controlled environments and agronomic performances are inconsistent across locations and/or years (Roselló et al., 2019).

PEG molecules with a molecular weight of more than 3000 cannot penetrate the cell wall, and PEG6000, a commonly used variant, with a molecular weight of approximately around 6,000 g/mol, can simulate drought stress as a non-permeating osmotic agent without causing direct physical damage to the plants (Ahmed et al., 2025). PEG6000 restricts water availability in the growth medium, mimicking drought conditions, and often is used to assess drought-resistance during early growth stages due to its capacity to induce severe water stress (Mustamu et al., 2023). The PEG6000 treatment in the hydroponic experiment has been used in many studies to determine the effects of drought on the root phenotype of wheat seedlings, in order to determine the drought tolerance potential of genotypes and traits contributing to drought tolerance and are valuable for breeding (Tang et al., 2024; Sallam et al., 2024; Li et al., 2024; Kou et al., 2022; Azab et al., 2021).

In the past, there have been four basic approaches to breeding for improved drought tolerance (Dhanda et al., 2004). The first approach was to breed conventionally for high yield and to assume that this will provide a yield advantage under suboptimal conditions. The second approach was to breed for maximum yield in the water-limited environment, but the obstacle was the great temporal variability, which slowed down breeding progress. The third approach encompasses creation of cultivars for water-limited environments by selecting physiological and morphological mechanisms for drought resistance through traditional breeding programmes. Secondary traits have been successfully used to enhance the rate of genetic improvement for wheat under drought stress: early flowering, early vigour, plant height controlled with Rht1, Rht2 and Rht8 dwarfing genes, canopy temperature, osmotic adjustment, water uptake, root system, water use efficiency, and carbon isotope discrimination (Abdolshahi et al., 2015). The fourth approach for breeding under water-limited conditions aims to determine single drought-resistant traits as breeding targets.

The aim of this research was to assess the components of phenotypic variance and broad-sense heritability of early vigour traits of bread wheat genotypes in the hydroponic experiment-polyethylene glycol (PEG) induced osmotic stress vs. the control, in order to choose appropriate traits for breeding for drought resistance.

Material and Methods

Based on previous extensive studies (Blažić et al., 2021) that deeply analysed a total of 101 bread wheat genotypes from Serbia and 16 other countries, 11 genotypes with desirable drought tolerance traits were selected for the current research. A total of 11 genotypes from this collection were chosen as parents with selection criteria aimed at achieving drought tolerance and eight crosses were performed: 1. Euclid (FRA) x CHI 4 (CHN); 2. Dika (SRB) x Ingenio (FRA); 3. Pobeda (SRB) x Donska semi-dwarf (RUS); 4. Phoenix (USA) x NS 40S (SRB); 5. Pobeda (SRB) x Brigant (GBR); 6. Dika (SRB) x Donska semi-dwarf (RUS); 7. Zemunska rosa (SRB) x Ingenio (FRA); 8. WWBMC2 (USA) x Ingenio (FRA). The criteria for choosing parents in the crosses were related traits to increased drought tolerance: longer stem length, longer primary root, large number of seminal roots, large root dry mass and stem dry mass, beginning of root branching at the greatest possible distance from the beginning of the root. Contrasting genotypes that had the widest or narrowest angle between the outermost seminal roots were selected. The crosses were made with plants grown in pots under controlled greenhouse conditions.

The F1 offspring from eight crosses and 11 parental genotypes were grown simultaneously in a hydroponic experiment (induced osmotic stress vs. control) in the laboratory of the Plant GeneBank of the Directorate for the National Reference Laboratories of the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia in 2020. The seedlings were grown in a phytotron (KBW 720, Binder GmbH) under controlled conditions. The seeds of the parental and F1 generation for all crosses were germinated on the filter paper soaked in distilled water at 20˚C for four days. Fifteen uniformly germinated seeds per genotype per treatment and per control were placed on the perforated lid of the plastic box divided in the middle by a plastic partition to create control conditions and conditions for applying stress treatment with two control genotypes (NS 40S, Zemunska Rosa) chosen based on a difference in their reaction to osmotic stress. In the second half of the box, after setting up the experiment, the genotypes were first grown in nutrient culture only for three days, and then polyethylene glycol 6000 (PEG-6000, ACROS OrganicsTM) was added. Osmotic stress represents a surrogate for drought stress in the plant (Li et al., 2019). Wheat seedlings of different genotypes were grown in a completely randomised design with three replicates in both growth conditions. The hydroponic culture consisted of a modified Knop's solution (Blažić et al., 2024). The mode of operation of the phytotron was set as described in Blažić et al. (2021). The relative air humidity was 75%. The cycle was completed after 24 hours and repeated. The mode of operation lasted a further 7 days after PEG treatment, after which the plants were removed from the growth chamber. After 10 days of cultivation in the phytotron, 10 representative seedlings per genotype were selected, washed under running water, scanned, and the photographs were processed using the Image J programme (Rasband, 2020).

The following traits were measured: primary root length (PRL), distance to the first branch on the primary root (DFBR), number of seminal roots (NSR), total seminal root length (TSRL), angle of seminal roots (ASR), and shoot length (SL). After drying the samples for 24 h at a temperature of 80 °C, root dry mass (RDM) and shoot dry mass (SDM) were measured, and RDM/SDM was calculated.

To determine the significance of genotype and environment (treatment) as sources of variation in the examined root and shoot traits, a two-way analysis of variance (ANOVA) was used following a randomised complete block design, where the environment meant growing wheat seedlings without and with osmotic stress by applying treatment with PEG:

$$X_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

X_{ijk} – analysed trait value of the *i*-th genotype $(i = 1, \dots, 19)$, in the *j*-th environment $(j = 1, 2)$ and the *k*-th replication $(k = 1, \dots, 3)$ (μ – mean average; α_i – main effect of the *i*-th genotype; β_j – mean effect of the *j*-th environment; $(\alpha\beta)_{ij}$ – effect of the interaction of the *i*-th genotype and the *j*-th environment; ε_{ijk} – error. The model of a two-way ANOVA is presented in the general table (Table 1).

Table 1. The general model of a two-way ANOVA.

Source of variation	Sum of squares (SS)	DF	Mean squares (MS)	F
Genotype (G)	SS_G	i-1	$MS_G=SS_G/i\text{-}1$	$F_G=MS_G/MS_{Er}$
Environment (E)	SS_E	j-1	$MS_E=SS_E/j\text{-}1$	$F_E=MS_E/MS_{Er}$
G × E	SS_{GE}	(i-1) (j-1)	$MS_{GE}=SS_{GE}/(i\text{-}1)(j\text{-}1)$	$F_{GE}=MS_{GE}/MS_{Er}$
Error	SS_{Er}	ij (k-1)	$MS_{Er}=SS_{Er}/ij(n\text{-}1)$	
Total	SS_T	ik-1		

**i* – number of genotypes, *j* – number of environments, *k* – number of replications.

The variance components were estimated based on the combined two-way ANOVA according to Falconer (1981) as follows:

$$V_g = \frac{MS_g - MS_{ge}}{re}$$

$$V_{ge} = \frac{MS_{ge} - MS_{er}}{r}$$

$$V_{er} = MS_{er}$$

$$V_p = V_g + \frac{V_{ge}}{e} + \frac{V_{er}}{re}$$

where V_g, V_{ge}, V_{er} and V_p are the variances due to genotypes, genotype × environment interaction, experimental error and phenotypes, respectively. MS_g, MS_{ge}, MS_{er} are the mean squares of genotypes, genotype × environment interaction and pooled error with e being the number of environments and r – the number of replications. Broad-sense heritability h^2_{BS} (%) is expressed as:

$$h^2_{BS} = \frac{V_G}{V_P} \times 100$$

The genotypic coefficient of variation CV_G (%) is calculated as follows:

$$CV_G = \frac{\sqrt{V_G}}{\bar{x}} \cdot 100$$

The phenotypic coefficient of variation CV_P (%) is calculated as follows:

$$CV_P = \frac{\sqrt{V_P}}{\bar{x}} \cdot 100$$

where V_G is the genetic variance, V_P is the phenotypic variance, and $\bar{x}$ is the average value of the trait of interest.

All statistical analyses were performed in the MINITAB V. 16 programme (Minitab Inc., 2021).

Results and Discussion

Using a two-way analysis of variance, the significance of the sources of variation for nine investigated root traits and shoot traits of 19 wheat genotypes seedlings grown under PEG-induced osmotic stress and under control conditions is shown (Table 2). Statistically significant ($P < 0.01$) F-test values were determined for both main factors (genotype, environment) for all investigated traits. The GE interaction had a statistically significant ($P < 0.01$) effect on the variation of most investigated root traits and shoot traits, except for TSRL, where the effect was statistically significant ($P < 0.05$) and for NSR, where it had no statistically significant effect. Many authors (Dhanda et al., 2004; Rauf et al., 2007; Bayoumi et al., 2008) determined a significant effect of PEG treatment on the wheat seedling morphology, as well as a significant differential response of the genotypes to the simulated drought stress. For all the traits analysed, genotype had on average the greatest influence (42.51%) on the total variation of the tested traits expressed as percent of the total sum of squares (SS) (Table 2). The environment contributed 17.06% of total sum of squares to the total variation of the studied traits, and the GE interaction contributed 13.79% (Table 2). The genotype had the greatest influence on the ASR (87.12% of total SS) and on the PRL (60.97% of total SS), while it had the least impact on the SDM (17.26% of total SS). The environment had the greatest effect on the SL (43.23% of the total SS) and on the SDM (40.09% of the total SS), and the least influence on the ASR (0.28% of the total SS).

Table 2. Two-way ANOVA for root traits and shoot traits of 19 wheat genotypes under two contrasting water regimes.

Trait		Genotype (G)	Environment (E)	G × E interaction
PRL	F	12438**	167.42**	17.31**
	SS (%)	60.97	9.02	18.54
DFBR	F	97.55**	757.86**	46.39**
	SS (%)	22.95	32.12	36.71
NSR	F	6.75**	13.90**	1,16^{NZ}
	SS (%)	20.04	4.45	1.11
TSRL	F	27.43**	36.91**	1.98*
	SS (%)	49.81	7.18	3.84
ASR	F	287.79**	19.50**	10.95**
	SS (%)	87.12	0.28	6.26
SL	F	53.88**	577.60**	6.97**
	SS (%)	33.77	43.23	8.59
RDM	F	53.01**	128.48**	15.00**
	SS (%)	38.48	12.37	28.86
SDM	F	17.18**	264.39**	5.48**
	SS (%)	17.26	40.09	13.22
RDM/SDM	F	30.93**	28.88**	2.88**
	SS (%)	52.17	4.78	6.96
Mean†	SS (%)	42.51	17.06	13.79

*P < 0.05; **P < 0.01; †residual of explained variance at 100% is error variance; PRL – primary root length, DFBR – distance to the first branch at the primary root, NSR – number of seminal roots, TSRL – total seminal root length, ASR – angle of seminal roots, SL – shoot length, RDM – root dry mass, SDM – shoot dry mass, RDM/SDM – the ratio of root dry mass to shoot dry mass.

Under different environmental conditions, with and without imposed osmotic stress, shoot traits exhibited greater variation in relation to the root traits, thus confirming the above-mentioned results that the examined shoot traits were more sensitive to the effect of the applied treatment. Ahmed et al. (2025) indicated substantial genotype effects (P < 0.01) in osmotic stress tolerance, among the 80 bread wheat genotypes and advanced lines from Iran. PEG treatments (TPEG) displayed statistically (P < 0.01) distinct effects on seedling traits (PRL, SL, RDM, SDM) compared to the control, and the statistically significant (P < 0.01) interaction between G × TPEG, highlighting the differential performance of various genotypes under different osmotic potentials. Jain et al. (2014) analysed 34 genotypes of bread wheat in three different water regimes, and found that the different osmotic potentials had the greatest effect on the SDM. In the same experiment, they showed that the effect of the environment was much stronger than the effect of the genotype and the GE interaction, with the 85.2% contribution to the total variation of the SDM trait. Baloch et al. (2012) also indicated a significant sensitivity of shoot traits under the conditions of PEG-induced osmotic stress. Boudiar et al. (2020) reported in their barley seedling experiment that imposed drought reduced shoot growth (SDM reduction of 43%) almost twice as much as root growth (RDM reduction of 23%).

In general, the effect of the genotype was on average higher on the variability of the tested root traits (46.6%), compared to the tested shoot traits (25.5%). This means that the root traits under investigation can be taken as a more reliable criterion for selection for drought tolerance compared to the investigated shoot traits, because their response to environmental variation will be more stable. Baloch et al. (2012) reported that the development of the root system under water deficit conditions is a very viable selection criterion for resistance to osmotic stress.

Table 3. Components of phenotypic variations, broad-sense heritability, coefficient of phenotypic variation and coefficient of genotypic variation for root traits and shoot traits of 19 wheat genotypes under two contrasting water regimes.

Trait	V_G	V_{GxE}	V_E	V_P	h^2_{BS} (%)	CV_G (%)	CV_P (%)
PRL	2.50	0.76	0.40	2.90	86.1	12.9	13.9
DFBR	0.05	0.08	0.04	0.09	52.4	16.2	22.3
NSR	0.09	0.005	0.02	0.11	82.9	10.5	11.6
TSRL	5.83	0.45	0.45	6.29	92.8	10.6	11.0
ASR	291.41	20.95	11.53	302.94	96.2	18.2	18.6
SL	4.64	1.18	0.69	5.33	87.1	9.5	10.2
RDM	0.28	0.21	0.11	0.39	71.7	8.5	10.1
SDM	1.58	1.21	0.74	2.32	68.1	5.8	7.0
RDM/SDM	0.0012	0.0002	0.0001	0.0013	90.7	11.8	12.4

V_G – genetic variance; V_{GxE} – variance of genotype × environment interaction; V_E – ecological variance; V_P – phenotypic variance; h^2_{BS} – broad-sense heritability; CV_G – coefficient of genotypic variation; CV_P – coefficient of phenotypic variation; PRL – primary root length, DFBR – distance to the first branch on the primary root, NSR – number of seminal roots, TSRL – total seminal root length, ASR – angle of seminal roots, SL – shoot length, RDM – root dry mass, SDM – shoot dry mass, RDM/SDM – the ratio of root dry mass to shoot dry mass.

Manschadi et al. (2008) stated that unlike RSA traits adapted to drought conditions, the use of shoot traits in wheat breeding programmes for drought tolerance was less successful.

GE interaction is defined as variation in the relative performance of genotypes in different environments (Cooper and Byth, 1996). In the absence of GE interaction, a superior genotype in one environment can be considered superior in all other environments, while the presence of GE interaction confirms that certain genotypes are superior in certain environments. Therefore, it is important to know the magnitude of this interaction in order to understand the response of different genotypes to different environments. If a GE interaction is present, breeders strive to identify stable genotypes with relatively consistent performance across a range of environments. Significant GE interaction values reduce the correlation between phenotypic and genotypic values, as well as selection progress (Amare et al., 2020). The term GE interaction is sometimes used as a synonym for plasticity. Phenotypic plasticity is defined as the ability of a genotype to produce different phenotypes under different environmental conditions. Accordingly, as the GE interaction had the greatest influence on the DFBR and the RDM with 36.7% and 28.9%, respectively, these traits were characterised by high plasticity.

Broad-sense heritability was very high (over 90%) for the traits: ASR (96.2%), TSRL (92.8%) and RDM/SDM (90.7%). High h^2_{BS} (80–90%) was observed for: SL (87.1%), PRL (86.1%), and NSR (82.9%). Moderate h^2_{BS} (40–70%) was shown for SDM (68.1%) and for DFBR (52.4%). The assessment of heritability gives us an insight into the degree of genetic control of the expression of a certain trait and phenotypic reliability in predicting the success of selection (Ndukauba et al., 2015). The obtained results indicated that there was a high degree of h^2_{BS} of most of the tested traits and that for most of them consistent genotype performance can be expected in different environmental conditions, and that the selection of wheat genotypes for drought tolerance should focus on these traits. Since the DFBR showed the lowest h^2_{BS} of the examined traits, it should not be taken as a reliable selection criterion due to the greater influence of the external environment on its manifestation. Colombo et al. (2022) showed lower values for h^2_{BS} for NSR (66%), ASR (64%), SDM (67%), RDM/SDM (74%) for 715 bread wheat genotypes tested as seedlings in comparison to our results, by using a high-throughput phenotyping platform. Canè et al. (2014) also documented intermediate to high values for h^2_{BS} for ASR (73%) and for NSR (67%) for 183 durum wheat elite accessions tested as seedlings. Hohn and Bektas (2020) analysed the same traits in three doubled haploid populations of bread wheat and found intermediate values for h^2_{BS} for ASR, in the range 52.3%–70.2%, and for NSR in the range 47%–64%. Higher h^2_{BS} values for RDM (75%) and lower values for ASR (85%)

were found in an experiment with six independent replicates of 201 bread wheat genotypes evaluated as seedlings (Beyer et al., 2019). Abdolshahi et al. (2015) showed moderate h^2_{BS} for PRL and RDW of 60% and 69%, respectively, and low h^2_{BS} for NSR of 39% for 40 genotypes of bread wheat in a two-year glasshouse experiment under drought conditions in Iran. Christopher et al. (2013) estimated h^2_{BS} for ASR with a value of 50% and for NSR with a value of 31% for bread wheat doubled haploid population. Dhanda et al. (2004) determined h^2_{BS} values for RL (86.7% and 84.3%) and SL (87% and 68.5%) for 30 bread wheat genotypes, tested as seedlings under PEG-induced drought stress, under control and drought-induced stress conditions, respectively. Rajamanickam et al. (2024) showed intermediate to high values for h^2_{BS} for TSRL, PRL, RDM, and SDM, of 71%, 72%, 86%, and 85%, respectively for 204 bread wheat genotypes tested as seedlings in the hydroponic experiment. Rebetzke et al. (2022) determined h^2_{BS} values for TSRL, NSR, and SL, of 89%, 81%, and 73%, respectively, for 460 recombinant bread wheat inbred lines, similar to our study.

The genetic stability of a particular genotype for any studied trait is determined by a low coefficient of variation and a high heritability of the corresponding trait (Reddy et al., 2020). Sanguineti et al. (2007) and Canè et al. (2014) stated that the ASR in the seedling phase has two desirable characteristics that make it very suitable for genetic research: first, it does not require a great effort to measure it in a large number of genotypes, and second, it is precisely its high heritability. RSA traits related to root shape and spatial arrangement, such as the NSR and ASR, may provide growth and yield advantages under water deficit conditions (Rogers and Benfey, 2015). In addition to the ASR, among the other examined root traits, the PRL had a significant share of the variance of the genotype (61%) on the variation of this trait, confirming previous studies that selection for increased PRL can be expected to improve the breeding of wheat under drought conditions (Dhanda et al., 2004; Hameed et al., 2010; Shahbazi et al., 2012). The high broad-sense heritability of this trait indicates the possibility of successfully improving the PRL in the breeding efforts by using different germplasm (Ayalew et al., 2018). As the root geometry of adult plants is closely related to RSA (Manschadi et al., 2008), it can be assumed that genotypes that differ in root architecture at early stages of development could also differ in the field at later stages of growth, when water and nutrient uptake becomes critical for yield performance (Canè et al., 2014).

The value of the coefficient of genetic variation (CV_G) was the lowest for the SDM (5.8%), while it was the highest for the DFBR (16.2%). The coefficient of phenotypic variation (CV_P) had the lowest value for the SDM (7.0%), and the highest value for the DFBR (22.3%). The DFBR showed the highest values for both coefficients of variation, which is in accordance with the obtained results that this trait varied the most among all the examined traits, both in conditions without and with osmotic stress. The external environment, i.e. osmotic stress, contributed strongly to the variation of this trait. Abdolshahi et al. (2015) showed high values for CV_P for RDM and NSR, 41.3% and 22.9%, respectively, and medium value for PRL of 16.1%, with CV_G values significantly lower than CV_P, for 40 genotypes of bread wheat tested for two years in glasshouse experiment under drought conditions in Iran. The low values of CV_P and CV_G for SDM have indicated that the existing genetic variability is not sufficient to achieve a significant improvement of this trait under drought stress.

Conclusion

The genetic analysis of the appropriateness of using root system architecture traits and shoot traits of bread wheat seedlings configuring early vigour under simulated drought stress indicated that broad-sense heritability was very high and high (> 82%) for most of the tested traits (primary root length, number of seminal roots, total seminal root length, angle of seminal roots, shoot length, the ratio of root dry mass to shoot dry mass), with low genotype × environment interaction (< 20% of total variation) and that the breeding for drought tolerance should be focused on these traits. Since the distance to the first branch on the primary root showed the lowest value for broad-sense heritability, and the greatest extent of genotype × environment interaction, it should not be considered as a reliable selection criterion due to the greater environmental influence on its manifestation.

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KOMPONENTE FENOTIPSKE VARIJANSE I HERITABILNOST OSOBINA RANOG PORASTA HLEBNE PŠENICE POD KONTRASTNIM SNABDEVANJEM VODOM

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R e z i m e

Istraživanje potencijala za toleranciju na sušu i fenotipske plastičnosti arhitekture korenovog sistema u ranim fazama razvoja moglo bi biti ključno u pogledu oplemenjivanja na otpornost na sušu i za selekciju ideotipova pšenice u uslovima klimatskih promena. Ukupno 11 genotipova iz kolekcije od 101 genotipa hlebne pšenice, poreklom iz Srbije i 16 različitih zemalja sveta, sa poželjnim osobinama u smislu povećane tolerancije na sušu, odabrano je za roditelje i izvršeno je osam ukrštanja. Genotipovi iz P i F1 generacija gajeni su u hidroponskoj kultivaciji u osmotskom stresu izazvanom polietilen glikolom 6000. Cilj ovog istraživanja je bio da se procene komponente fenotipske varijanse i heritabilnosti u širem smislu osobina ranog porasta za devet osobina korena i izdanka genotipova hlebne pšenice u indukovanom vodnom stresu i u kontrolnim uslovima, kako bi se izabrale prikladne osobine za oplemenjivanje na otpornost na sušu. Uticaj genotipa bio je veći na varijabilnost testiranih osobina korena (46,6%), u poređenju sa testiranim osobinama izdanka (25,5%), što znači da se osobine korena mogu uzeti kao pouzdaniji kriterijum za selekciju na toleranciju na sušu u poređenju sa ispitivanim osobinama izdanka. Heritabilnost u širem smislu bila je visoka (> 82%) za većinu ispitivanih osobina (dužina primarnog korena, broj seminalnih korenova, ukupna dužina seminalnih korenova, ugao seminalnih korenova, dužina izdanka, odnos suve mase korena i suve mase izdanka), a malom interakcijom genotip × sredina (< 20% ukupne varijacije) i oplemenjivanje na toleranciju na sušu trebalo bi da bude usmereno na ove osobine.

Ključne reči: *Triticum aestivum* L., sušni stres, arhitektura korenovog sistema, osobine izdanka, heritabilnost u širem smislu, komponente fenotipske varijanse.

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PRINOS, FIZIČKE I HEMIJSKO-TEHNOLOŠKE OSOBINE ZRNA I BRAŠNA NOVE SORTE OZIME PŠENICE U RAZLIČITIM AGROEKOLOŠKIM USLOVIMA

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Sažetak: Potreba za globalnom prehrambenom sigurnošću nameće povećanje proizvodnje pšenice, kao treće najzastupljenije kulture u svetu sa značajnom ulogom u svetskoj i srpskoj ekonomiji. Iako oplemenjivanje i agrotehničke mere doprinose rastu prinosa, poljoprivreda se suočava sa izazovima poput klimatskih promena, degradacije zemljišta i rasta troškova proizvodnje, što zahteva nove pristupe za stabilnost proizvodnje. Cilj istraživanja bio je identifikacija razlika u prinosu i kvalitetu zrna između široko zastupljene sorte pšenice pobeda i nove sorte pšenice ml grigorija, uzimajući u obzir uticaj genotipa i spoljašnjih uslova. Ispitivani su prinos i fizičko-hemijske osobine zrna i brašna sorti ml grigorija i pobeda, na šest lokaliteta u Srbiji tokom 2021/22. i 2022/23. godine. Ogledi su organizovani po planu slučajnog rasporeda u tri ponavljanja, uz standardne agrotehničke mere. Prinos je obračunat i korigovan na standardnu vlagu, dok su fizičko-hemijske i reološke osobine ispitivane u akreditovanim laboratorijama prema standardima SRPS ISO. Dobijeni rezultati su obrađeni statistički, metodom analize varijanse, a za pojedinačna poređenja korišćen je test najmanje značajne razlike (engl. *least significant difference test – lsd test*). Rezultati su pokazali značajne razlike u prinosu, fizičkim i tehnološkim osobinama u zavisnosti od ispitivanih faktora. Sorta ml grigorija je ostvarila visok prinos i dobar tehnološki kvalitet u povoljnim uslovima, dok je pobeda imala stabilnije fizičke osobine i bolju stabilnost testa, što ukazuje da prilagođen izbor genotipa uslovima sredine omogućava postizanje visokih i stabilnih prinosa i kvaliteta.

Ključne reči: pšenica *(Triticum aestivum* L.), kvalitet, prinos zrna, sorta, agroekološki uslovi.

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Uvod

Pšenica predstavlja veoma važnu kulturu u umerenim podnebljima, obezbeđujući prehrambenu sigurnost širom sveta, zahvaljujući visokom prinosu, prilagodljivosti i proteinima koji omogućavaju proizvodnju širokog asortimana proizvoda. Pored energetske vrednosti, pšenica obogaćuje ishranu aminokiselinama, vitaminima, mineralima i dijetetskim vlaknima, posebno u proizvodima od celog zrna. Ipak, povezuje se sa celijakijom, autoimunom bolesti izazvanom imunološkom reakcijom na gluten – protein prisutan u pšenici (Daley et al., 2025). Kvalitet pšenice zavisi od namene, jer različiti proizvodi zahtevaju različite osobine (Iqbal et al., 2022). Analizirajući ulogu pšenice u globalnoj prehrambenoj sigurnosti, Langridge et al. (2022) zaključuju da pšenica obezbeđuje oko 20% ukupnog unosa ugljenih hidrata i proteina.

Brojni su izazovi sa kojima se poljoprivreda suočava u poslednjoj deceniji. Prinos pšenice značajno je povećan zahvaljujući inovacijama u oplemenjivanju i agronomiji, međutim, klimatske promene i ulaganja u proizvodnju predstavljaju izazove, koji zahtevaju nove pristupe za stabilnost proizvodnje. Prema Igrejas i Branlard (2020), savremena istraživanja sve više uključuju genetske resurse i stavljaju akcenat na nutritivnu vrednost. Pored tehnoloških osobina, u fokusu su i nutritivna vrednost i zdravstveni efekti, što zahteva interdisciplinarnu saradnju. Savremeni izazovi u proizvodnji pšenice obuhvataju održivu proizvodnju uz manju primenu pesticida i mineralnih đubriva, kao i razvoj sorti poboljšanog kvaliteta za različite namene (Shewry, 2009).

Kako navode Sharma et al. (2015), procene su da će do 2050. godine svetu biti potrebno oko 840 miliona tona pšenice, što uslovljava povećanje proizvodnje uz očuvanje resursa i zemljišta, što se može postići kroz genetske, fiziološke i agronomske intervencije. Dakle, porast broja stanovnika kao i globalna prehrambena sigurnost zahteva povećanje proizvodnje pšenice. Pšenica kao treća najveća kultura po proizvodnji pored kukuruza i pirinča, značajno doprinosi ekonomiji sveta, kao i Srbije. Svake godine pšenica se u svetu seje na oko 2,20 miliona hektara, a u Srbiji na oko 588.820 hektara (Dončić et al., 2019). Prema Republičkom zavodu za statistiku (https://www.stat.gov.rs/), prosečan prinos ozime pšenice u Srbiji tokom 2022. godine iznosio je oko 5,0 t/ha, a zasejana površina iznosila je oko 639.600 ha. Prema FAO (2018–2022, https://www.fao.org/faostat/), globalni prosečan prinos pšenice kretao se između 2,5 i 2,75 t/ha. Temperaturni uslovi i učesće klimatskih faktora značajno utiču na prinos pšenice (Djekic et al., 2021). Potrebno je pronalaziti nove pogodne lokacije za proširenje proizvodnje pšenice i povećanje globalne proizvodnje hrane za rastuću populaciju (Dadrasi et al., 2023). Takođe, u budućnosti treba pojačati fokus na ulogu agrotehničkih mera, jer pravilna obrada zemljišta, pravilan izbor sortimenta, blagovremena setva, optimalno đubrenje, pravilna primena pesticida, primena održivih praksi kao što je plodored, omogućavaju bolju iskorišćenost resursa i stabilnije prinose.

Genotip pšenice utiče na prinos i kvalitet zrna, određujući kako biljka reaguje na spoljašnje uslove i kako koristi raspoložive resurse. Prinos zavisi od genetskog potencijala sorte za broj klasića, zrna po klasu i masu zrna, dok kvalitet zrna, uključujući sadržaj proteina, gluten i tehnološke osobine, u velikoj meri zavise od naslednih osobina. Pravilnim izborom genotipa, koji je prilagođen lokalnim agroekološkim uslovima, moguće je ostvariti visoke i stabilne prinose uz očuvanje ili unapređenje tehnološkog kvaliteta pšenice. Posmatrano sa aspekta stvaranja novih sorti pšenice (Jocković et al., 2023), ističe se da je važno pored prinosa i kvaliteta, uključiti i otpornost na poleganje, niske temperature i sposobnost prilagođavanja različitim uslovima. Brz napredak u genetičkim istraživanjima otvara mogućnosti za obezbeđivanje globalne prehrambene sigurnosti (Sharma et al., 2015).

Oplemenjivanje pšenice igra važnu ulogu u poboljšanju prinosa, otpornosti na stresne uslove i kvalitet pečenja (Ljubičić et al., 2022). Nagarajan et al. (2007) su ispitivali uticaj genotipa (G), okruženja (E) i njihove interakcije (G × E) na pet osobina kvaliteta i prinos zrna 20 sorti pšenice u sedam različitih okruženja tokom dve godine. Ustanovili su značajne razlike između sorti i okruženja za ispitivane osobine, kao i značajnu interakciju G × E, što ukazuje na potrebu procene kvaliteta u različitim sredinama. Takođe, interakciju godine na prinos i tehnološki kvalitet pšenice ispitivali su i Živančev et al. (2022). Kaya i Akcura (2014) navode da ciljeve selekcije treba prilagoditi konkretnim uslovima proizvodnje na osnovu ispitivanja uticaja genotipa (G), sredine (E) i njihove interakcije (GEI) na prinos i kvalitet zrna pšenice jer su utvrdili veliku varijabilnost kvalitativnih osobina, s većim rasponima uticaja sredine nego genotipa.

Uzimajući u obzir značaj genotipa i spoljašnje sredine, cilj rada je da se ustanove razlike u prinosu i kvalitetu zrna pšenice gajene u različitim agroekološkim uslovima, jedne veoma zastupljene sorte pšenice na proizvodnim površinama Srbije i jedne nove sorte pšenice.

Materijal i metode

Ispitivanje prinosa, kao i fizičko-hemijskih osobina zrna i brašna dveju sorti ozime pšenice priznatih u Srbiji, ml grigorija, osnovne sorte u tipu B2 čiji je tvorac Institut PKB Agroekonomik i pobeda kao hlebne sorte u tipu B1 čiji je tvorac Institut za ratarstvo i povrtarstvo, Novi Sad, sprovedeno je na šest različitih lokaliteta (Novi Sad, Pančevo, Kikinda, Sombor, Kruševac i Požarevac). Ispitivanja su obavljena u dvogodišnjem periodu 2021/22–2022/23. Ogledi su postavljeni po planu slučajnog rasporeda u tri ponavljanja. Veličina elementarne parcele iznosila je 6 m^2. Osnovna obrada zemljišta obavljena je plugom na svim lokalitetima u jesen, kada je uneto osnovno đubrivo u količini od 600 kg/ha u obliku NPK (15:15:15). Predsetvena priprema je obavljena početkom oktobra tanjiračom za usitnjavanje i mešanje žetvenih ostataka i drljačom – za dodatno usitnjavanje i nivelaciju zemljišta. Time je dobijen fini površinski sloj za ujednačeno sejanje i obezbeđena je dobra zbijenost ispod semenskog sloja za kapilarni dotok vode.

Setva je u obe godine obavljena u optimalnim rokovima, sredinom oktobra meseca, a od mera nege primenjene su standardne mere u tehnologiji gajenja ozime pšenice: kontrola nicanja i sklop, valjanje, jesenja prihrana, praćenje prezimljavanja i prolećna prihrana. Suzbijanje korova vršeno je u jesenjem tretmanu – protiv širokolisnih i uskolisnih korova, kao i u prolećnom tretmanu – pojava korova posle zime (do početka vlatanja). Zaštita od bolesti i štetočina vršena je fungicidima – protiv bolesti lista i klasa, insekticidima – protiv lisnih vaši, tripsa i žitnih pijavica. Uzorci za određivanje morfoloških i produktivnih osobina su uzimani pri kraju bokorenja (mart/april), na početku klasanja (maj), pri cvetanju i mlečnoj zrelosti (kraj maja/jun) i fiziološkoj zrelosti (kraj juna/početak jula). Prinos zrna je obračunat po sledećoj formuli: prinos (kg/ha) = masa zrna (kg)/površina uzorka (m^2) x 10.000.

Zatim je po sledećoj formuli urađena korekcija na standardnu vlagu zrna 14%:

Korigovani prinos = izmereni prinos x ((100 – vlaga)/(100 – 14)).

Ispitivanja fizičkih i hemijskih osobina zrna, reoloških osobina testa i pecivosti, izvedena su u Institutu za prehrambene tehnologije u Novom Sadu, dok je izvođač ispitivanja DUS bila Poljoprivredna stručna služba Sombor, iz Sombora.

Izbrašnjavanje I_1 kao ključan pokazatelj mlevne vrednosti pšenice, koji govori koliko brašna se može dobiti iz određene količine zrna, određeno je u laboratorijskim uslovima na mlevnom automatu Bühler MLU-202 (Kaluđerski i Filipović, 1998).

Ispitivanje farinoloških osobina testa, uključujući vreme razvoja testa, stabilnost testa, stepen omekšanja (Fj) i određivanje farinogramske kvalitetne grupe, izvršeno je metodom SRPS EN ISO 5530-1, u skladu sa Pravilnikom o kvalitetu žita, mlinskih i pekarskih proizvoda i testenina („Sl. glasnik RS”, br. 68/2016 i 56/2018).

Metode korišćene za određivanje tehnoloških pokazatelja, su sledeće: određivanje sadržaja vlage; određivanje zapreminske mase žita/hektolitarske mase; određivanje mase 1000 zrna; određivanje količine sirovih proteina; određivanje sedimentacione vrednosti pšenice za preradu (Pravilnik o metodama fizičkih i hemijskih analiza za kontrolu kvaliteta žita, mlinskih i pekarskih proizvoda, testenina i brzo smrznutih testa, Sl. List SFRJ br 74/1988). Za određivanje staklavosti zrna, sadržaja vlažnog glutena pšenice i sadržaja suvog glutena primenjuju se metode prema Kaluđerskom i Filipoviću (1998).

Brabenderov farinograf i ekstenzograf proizvedeni su u Duizburgu u Nemačkoj i namenjeni su merenju kako se pšenično testo ponaša kada se razvlači u jednom pravcu (uniaksijalno), odnosno da meri njegov otpor, elastičnost i rastegljivost tokom tog procesa.

Dobijeni rezultati su obrađeni statistički metodom analize varijanse, a za pojedinačna poređenja korišćen je test najmanje značajne razlike (engl. *least significant difference test – lsd test*). Obrada statističkih parametara izvršena je u programu STATISTICA 12 (StatSoft, Inc. 1984-2014, Tulsa, OK, USA).

Agroekološki uslovi u toku izvođenja ogleda

Prema podacima u tabelama 1 i 2 uočavamo da su prosečne temperature vazduha u vegetacionom periodu bile nepovoljnije u drugoj ispitivanoj godini, posebno za lokalitete Novi Sad, Pančevo, Sombor i Kikinda. Tu se pre svega misli na veće vrednosti temperature vazduha na početku i na kraju vegetacionog perioda, koje su istovremeno praćene malom količinom padavina. S druge strane, ukupna količina padavina u svim lokalitetima bila je veća u drugoj ispitivanoj godini. Što se tiče rasporeda padavina, nepovoljan je u obe ispitivane godine, posebno u zimskim i ranim prolećnim mesecima, što je generalno trend koji će u budućnosti biti sve češći zbog intenziviranja globalnih klimatskih promena (Tošić et al., 2025). Navedena činjenica je razlog dobijenih nižih prinosa zrna obe ispitivane sorte u drugoj u odnosu na prvu godinu ispitivanja.

Tabela 1. Meteorološki podaci za vegetacioni period ozime pšenice u ispitivanim lokalitetima 2021/22. godine.
Table 1. Meteorological data for the winter wheat growing season at the studied localities in 2021/22.

Lokalitet/ meseci Location/ months	Kikinda		Kruševac		Pančevo		Sombor		Novi Sad		Požarevac	
	t	Pad./preci pitation	t	Pad./preci pitation	t	Pad./preci pitation	t	Pad./preci pitation	t	Pad./preci pitation	t	Pad./preci pitation
X	10,6	41,2	9,9	62,7	11,7	73,4	9,7	81,6	10,6	88	10,5	43,6
XI	7	72,9	8,4	29,6	8,9	122,8	6,2	57,7	7,2	114,6	8,3	62
XII	2,9	70,4	3,8	92,4	4,1	157,8	2,6	73,5	3	88,9	3,8	124,4
I	0,9	13,7	0,5	55,6	2,4	45,7	1,7	8,5	1,5	11,6	0,7	55,1
II	5,3	12,5	4,8	27,6	6,9	22,2	5,2	19,1	5,7	23,7	5,5	33,1
III	5,6	5,2	5	22,2	7,1	10,5	5,4	3,9	5,6	1,1	5,1	13,9
IV	10,9	37,7	11,6	40,1	12,3	80,1	10,6	35,5	10,9	54,5	11,2	75,4
V	19,1	53,3	18,1	28,9	20,3	32,2	18,9	56,4	19,2	17,9	18,5	63,5
VI	23,8	61,5	22,2	93,3	24,8	43,3	23,3	36,1	24	43,6	22,3	120,9
Prosek/ Average Suma/ Sum	9,57	368,4	9,36	452,4	10,9	588	9,29	372,3	9,74	443,9	9,54	591,9

Tabela 2. Meteorološki podaci za vegetacioni period ozime pšenice u ispitivanim lokalitetima 2022/23. godine.
Table 2. Meteorological data for the vegetation period of winter wheat at the studied localities in 2022/23.

Lokalitet /Meseci Location/ months	Kikinda		Kruševac		Pančevo		Sombor		Novi Sad		Požarevac	
	t	Pad./precipitation	t	Pad./precipitation	t	Pad./precipitation	t	Pad./precipitation	t	Pad./precipitation	t	Pad./precipitation
X	13,7	19,3	12,7	3,6	15,5	13,1	13,2	20,1	13,7	31	13,2	9
XI	8	44,3	8,9	95,6	9,3	64,9	7,6	56,8	23,7	58,7	8,7	61,2
XII	4,8	74,3	5,2	50,8	7	76,1	4,4	62,6	1,1	44,7	5,9	89,5
I	5,1	57,6	4,6	82,1	5,7	79,3	4,3	64,1	4,9	66,4	5,1	78,9
II	3,5	30,9	3,4	12	4,9	62,9	3,6	38,1	3,6	57,2	3,3	33
III	8,6	18,6	8,7	50	10,1	37,6	8,5	17,8	9	25,3	8,5	46,2
IV	10,3	79,3	10,1	89,6	11,3	79	10,5	75,8	10,5	63,9	10,1	76,9
V	17,2	89,5	16,1	67,9	17,5	92,8	16,9	98,1	17,2	124,8	16,2	43,9
VI	21	81,8	20,1	172,8	21,7	75,6	20,8	79,5	21,4	35,4	20,2	92,1
Prosek/ Average Suma/ Sum	10,24	495,6	9,98	624,4	11,4	581,3	9,98	512,9	11,7	507,4	10,13	530,7

Tipovi zemljišta na kojima su gajene ispitivane sorte ozime pšenice su povoljnih fizičkih, hemijskih i fizičko-hemijskih osobina, izuzev Kruševca i Požarevca (tabela 3). U lokalitetima gde je predusev bio kukuruz (Kruševac, Sombor i Požarevac) u pitanju je dvopoljni plodored, kao najčešće zastupljen sistem gajenja u našoj zemlji kao posledica dominacije ova dva useva u setvenoj strukturi. Tropoljni plodored, gde je uključena soja kao predusev ozimoj pšenici (Kikinda, Pančevo i Novi Sad) veliki je pomak u izmenama i modifikaciji tehnologije gajenja, a u skladu sa osobinama zemljišta i klimatskim promenama.

Tabela 3. Tip zemljišta i predusevi ozimoj pšenici u ispitivanim lokalitetima.
Table 3. Soil type and preceding crops of winter wheat at the studied localities.

Lokalitet/Locality	Tip zemljita/Soil type	Predusev/pre-crop
Kikinda	černozem/chernozem	soja/soybean
Kruševac	smeđe šumsko zemljište/brown forest soil	kukuruz/corn
Pančevo	černozem/chernozem	soja/soybean
Sombor	černozem/chernozem	kukuruz/corn
Novi Sad	černozem/chernozem	soja/soybean
Požarevac	smonica (vertisol)/vertisol	kukuruz/corn

Rezultati i diskusija

Podaci o prinosu zrna ispitivanih sorti pšenice (kg/ha) prikazani su u tabeli 4.

Na osnovu prikazanih podataka zaključujemo da je, prosečno posmatrano, najveći prinos zrna dobijen na lokaciji Novi Sad (9.417 kg/ha), dok je najniži prinos ostvaren na lokaciji Sombor (5.859 kg/ha). Razlika između prosečno najnižeg i najvišeg prinosa statistički je vrlo značajna. Posmatrano po ispitivanim sortama, najviši dobijeni prinos dala je sorta ml grigorija na lokalitetu Novi Sad (9.894 kg/ha), a najniži sorta pobeda na lokalitetu Sombor (5.520 kg/ha). Razlika između ovih prinosa takođe je statistički vrlo značajna. Posmatrano po godinama ispitivanja, možemo primetiti da je prosečno viši prinos ostvaren 2021/22. godine (11.153 kg/ha), i najviši ostvaren prinos nove ispitivane sorte ml grigorija je u ovoj godini na lokalitetu Novi Sad (11.973kg/ha). Najniži prinos u 2021/22. godini ostvaren je gajenjem sorte pobeda (4.277 kg/ha) na lokalitetu Kikinda.

Sorta pšenice ml grigorija svrstava se u osnovne sorte tipa B2, što ukazuje na srednji kvalitet zrna i brašna, koji se u tehnologiji prerade češće koristi u kombinaciji sa kvalitetnijim sortama. Sorta pobeda pripada hlebnim sortama tipa B1 i odlikuje se višim sadržajem proteina i glutena, kao i boljim reološkim osobinama brašna, zbog čega se ubraja u sorte pogodnije za proizvodnju hleba bez dodatka poboljšivača. Indeks prinosa nove sorte ukazuje da je sorta ml grigorija imala za 84,97% veći prinos od pobede. U 2022/23. godini situacija je izmenjena, te je nova sorta, u većini ispitivanih lokaliteta dala manje prinose zrna od sorte sa kojom se poredi. U interakcijama između sva tri ispitivana faktora dolazimo do zaključka da su razlike u prinosu između različitih sorti u različitim godinama i na različitim lokalitetima statistički vrlo značajne.

Iz podataka u tabeli 4 uočavamo da je 2022/23. godina bila značajno lošija godina za proizvodnju pšenice, jer su prinosi opali u poređenju sa prethodnom godinom. Takođe, u ovoj godini, nova sorta imala je dosta niže prinose i u više slučajeva ostvarivala prinose niže od prinosa sorte sa kojom se poredi, što se vidi iz indeksa prinosa. Indeks prinosa ukazuje da je u 2021/22. godini, prosečno posmatrano, nova sorta dostigla prinose veće za 31,64%, posmatrano sa svih lokaliteta, dok je u 2022/23. godini njen prinos bio manji za 6,79%.

Prema Kristensen et al. (2011), veće varijacije u prinosu uglavnom su posledica ekstremnih zimskih temperatura, uz zaključak da visoke letnje temperature smanjuju prinos ozime pšenice, dok tokom proleća i leta imaju pozitivan uticaj. Najveći prinos ostvaruje se pri zimskoj temperaturi od 4,4° C. Carew et al. (2009), analizirajući faktore koji utiču na prinos pšenice, zaključuju da su potrebna dodatna istraživanja o uzajamnim uticajima agrotehničkih i genetskih faktora na prinos.

Tabela 4. Prinos zrna ispitivanih sorti pšenice (kg/ha).
Table 4. Grain yield of the tested wheat varieties (kg/ha).

Lokalitet/ Locality	Godina/ Year	Prinos zrna/Grain yield		Prosek/Average	Indeks prinosa nove sorte/ Yield index of new varieties
		ml grigorija	pobeda		
	21/22	7911	4277	6094	184,97
Kikinda	22/23	5778	7523	6651	76,80
	Prosek/Average	6844	5900	6372	116,00
	21/22	6781	5733	6257	118,28
Kruševac	22/23	5556	5575	5566	99,66
	Prosek/Average	6169	5654	5912	109,11
	21/22	11973	10332	11153	115,88
Novi Sad	22/23	7815	7546	7681	103,56
	Prosek/Average	9894	8939	9417	110,68
	21/22	11285	10400	10843	108,51
Pančevo	22/23	4725	6043	5384	78,19
	Prosek/Average	8005	8221	8113	97,37
	21/22	7991	7544	7768	105,93
Požarevac	22/23	7200	7237	7219	99,49
	Prosek/Average	7595	7390	7493	102,77
	21/22	7628	6514	7071	117,10
Sombor	22/23	4765	4526	4646	105,28
	Prosek/Average	6197	5520	5859	112,26
Prosek/	21/22	9828	7466	8647	131,64
Average	22/23	5973	6408	6191	93,21
(2021/22–2022/23)		7900	6937	7418	113,88

LSD prinos zrna/ LSD grain yield		A	B	C	AB	AC	BC	ABC
	0,05	0,081	0,047	0,047	0,114	0,114	0,66	0,162
	0,01	0,014	0,079	0,079	0,196	0,196	0,113	0,277
		**	**	**	**	**	**	**

LSD – pragovi značajnosti; * – statistički značajna razlika; ** – statistički vrlo značajna razlika; Faktor A – godina; Faktor B – sorta; Faktor C – lokalitet.
*LSD – significance thresholds; * – statistically significant difference; ** – statistically very significant difference; Factor A – year; Factor B – variety; Factor C – locality.*

Što se tiče prinosa, Đurić et al (2013) u istom radu navode prinose od 8,00 do 9,60 t/ha Prinos zrna u postavljenom ogledu kreće se od 4,27 do11,97 t/ha, što su značajna variranja u zavisnosti od lokaliteta, godine i sorte.

Đurić et al (2020) ističu prinose zrna pšenice na lokalitetu Požarevac u 2017 godini, koji se kreću od 5,05 do 6,40 t/ha, dok se u ovom ogledu vrednosti prinosa kreću od 7,2 do 7,9 t/ha Najviši prinosi zabeleženi su prema Đuriću et al (2020) na lokalitetu Pančevo, dok je najveći prinos u ovom ogledu iznosio 11,97 t/ha na lokalitetu Novi Sad Na lokalitetu Kruševac 2018 godine prosečan prinos, kako navode Đurić et al (2020), iznosio je 7,71 t/ha, dok je u ovom radu prinos zrna na pomenutom lokalitetu prosečno bio 5,9 t/ha.

Podaci o fizičkim i hemijsko-tehnološkim osobinama zrna i brašna prikazani su u tabeli 5 Od fizičkih osobina ispitivani su: sadržaj vlage zrna kod prispeća, hektolitarska masa očišćene pšenice, masa 1000 zrna i staklavost zrna Sadržaj vlage zrna kod prispeća prosečno je isti kod obe sorte Hektolitarska masa prosečno je bila veća kod sorte pobeda i iznosila je 83,15 kg/hl Najveća masa zabeležena je kod pobede na lokalitetu Novi Sad (83,9 kg/hl), a najniža na lokalitetu Sombor kod sorte ml grigorija (80,4 kg/hl) Sorta pobeda dala je prosečno najveću masu 1000 zrna (33 g) Posmatrano po lokalitetima, najveća masa bila je od uzoraka iz Sombora kod pobede (36,8 g), a najniža kod iste sorte na lokalitetu Novi Sad (29,2 g) Najveća staklavost zrna izmerena je kod sorte ml grigorija (82,5%) na lokalitetu Sombor, a najniža kod iste sorte na lokalitetu Novi Sad (69%).

Na osnovu ispitivanja povezanosti ispitivane težine pšenice sa različitim fizičkim i hemijskim svojstvima zrna, Lakić-Karalić et al (2021), analizom šest uzoraka utvrdili su značajne korelacije između zapreminskih masa i parametara kao što su: težina 1000 zrna (TKW), staklavost, sadržaj vlage, masti, proteina, vlažnog glutena i zapremina sedimentacione vrednosti (engl *Zeleny test*) Živančev et al (2022) konstatovali su da je sposobnost razvlačenja testa u pozitivnoj korelaciji sa sadržajem proteina, vlažnim glutenom i apsorpcijom vode, dok je u negativnoj korelaciji sa odnosom otpornosti i rastegljivosti.

Istraživanja koja su sproveli Zečević et al (2024) obuhvatila su ocenu tehnološkog kvaliteta zrna i brašna pet sorti ozime pšenice u uslovima održive proizvodnje tokom dve sezone Utvrdili su značajne razlike između sorti, godina i njihove interakcije za sedimentacionu vrednost, sadržaj glutena i moć upijanja vode Sorte ana, morava i aleksandra pokazale su bolje rezultate od sorte pobeda Prema rezultatima koje su dobili Živančev et al (2022), tokom trogodišnjeg ispitivanja utvrđeno je da moderne srpske sorte pšenice, iako daju viši prinos, poseduju slabiji tehnološki kvalitet u odnosu na rasprostranjene sorte, pri čemu su klimatski uslovi najviše uticali na zapreminu hleba i ekstenzografske parametre, dok su razlike među sortama i njihove interakcije bile značajne za farinografske osobine i stepen omekšavanja.

Sorta ml grigorija je imala najveći sadržaj sirovih proteina na lokalitetu Sombor (12,5% na sm), a najniži na drugom ispitivanom lokalitetu (9,4% na sm), što je u skladu sa vrednostima koje su naveli Anjum i Walker (2000) za različite sorte pšenice (11,99–13,80%) U ovom radu drugi najniži je sadržaj sorte pobeda na istom lokalitetu (9,5% na sm).

Najveća sedimentaciona vrednost pšenice iznosila je 58 kod ml grigorije, na lokalitetu Sombor, a najmanja 29 u Novom Sadu kod sorte pobeda Pobeda je dala prosečno manju sedimentacionu vrednost, dok su niže vrednosti prikazane za lokalitet Novi Sad kod obe sorte U skladu sa navedenim su i istraživanja koja su sproveli Banjac et al (2022), koji ukazuju i na to da uticaj mikroklime na kvalitet zrna omogućava bolje planiranje gajenja u različitim uslovima.

Prosečno veći sadržaj vlažnog glutena izmeren je kod sorte ml grigorija (25%), ali je vrednost ove osobine kod pobede približno isti (24%) Najveća vrednost je kod sorte ml grigorija u Somboru, ali za njom ne zaostaje druga ispitivana sorta na istom tom lokalitetu.

Studija koju su sproveli Mastilović et al (2018) pokazuje da klimatske promene smanjuju sadržaj vlažnog glutena u pšenici, što negativno utiče na ukupnu snagu i sposobnost upijanja testa, dok gluten sa višim indeksom može delimično održati elastičnost i otpornost testa, pa je veći sadržaj vlažnog glutena i dalje poželjan za bolje tehnološke osobine.

Rezultati dobijeni u aktuelnom radu, gde sorta ml grigorija u Somboru daje veće izbrašnjavanje (74%) u odnosu na sortu pobeda u Novom Sadu (69%), mogu biti objašnjeni razlikama u zapreminskoj masi i masi 1000 zrna (engl *thousand-kernel weight – TKW*), što je u skladu sa nalazima Dziki i Laskowski (2004), koji ističu da veličina i oblik zrna značajno utiču na proces mlevenja i prinos brašna Najveću moć upijanja vode pokazala je sorta pobeda na lokalitetu Sombor (62,4% na 13% vlage brašna), dok je ova veličina najmanja bila kod sorte ml grigorija na lokalitetu Novi Sad (58,6%) Najduže vreme razdvajanja testa pokazala je ml grigorija, na lokalitetu Sombor (2,4 min), a najkraće ista sorta na lokalitetu Novi Sad (1,4 min) Najvišu vrednost za stabilitet pokazala je pobeda u Somboru, a najnižu ista sorta za lokalitet Novi Sad.

Stepen omekšanja testa kod posmatranih sorti pokazuje značajne razlike između lokaliteta i sorti Najviša vrednost je zabeležena kod sorte ml grigorija na lokalitetu Novi Sad (116 Fj), dok sorta pobeda na istom lokalitetu pokazuje malo niži stepen omekšanja (115 Fj) Suprotno tome, najniža vrednost je zabeležena kod sorte pobeda na lokalitetu Sombor (18 Fj) S obzirom na to da niži stepen omekšanja ukazuje na bolji tehnološki kvalitet testa, sorta pobeda na lokalitetu Sombor pokazuje povoljnije osobine u tom smislu, dok visoke vrednosti kod ml grigorije i pobede u Novom Sadu sugerišu na testo slabije strukture i elastičnosti, što može negativno uticati na kvalitet hleba i drugih pekarskih proizvoda U aktuelnom istraživanju sve vrednosti farinografskog kvalitetnog broja bile su ispod 100, što ukazuje na nizak kvalitet brašna, posebno na lokalitetu Novi Sad, uz pretpostavku mikroklimatskih uticaja Nasuprot tome, na lokalitetu Sombor kvalitet brašna bio je znatno bolji, što se ogleda i kroz kvalitetnu grupu sorte pobeda (A-2), ukazujući na dobar tehnološki kvalitet brašna Najviša vrednost izmerena je kod sorte pobeda na lokalitetu Sombor i iznosi 81,3, a najniža kod sorte ml grigorija na lokalitetetu Novi Sad i iznosi 40,5 Nizak sadržaj glutena, i mali farinografski broj u Somboru odgovaraju za keks, ali vrednosti energije su visoke 70 cm^2, odnosno otpora za ovu namenu.

Na lokalitetu Novi Sad obe sorte (ml grigorija i pobeda) spadaju u farinogramsku kvalitetnu grupu C-1, što ukazuje na nizak kvalitet brašna u tim uslovima, verovatno zbog nepovoljnih mikroklimatskih faktora Nasuprot tome, na lokalitetu Sombor kvalitet brašna je značajno bolji: ml grigorija pripada B-1, a pobeda grupi A-2, što ukazuje na dobar do vrlo dobar kvalitet brašna, naročito kod sorte pobeda Kada se posmatraju prosečne vrednosti, ml grigorija spada u B-2, a pobeda u grupu B-1, što znači da, ukupno gledano, prosečan kvalitet brašna obe sorte može se oceniti kao dobar, s tim što pobeda ima nešto povoljniji tehnološki potencijal.

Što se tiče ekstenzograma, najvišu energiju ima ml grigorija na lokalitetu Sombor, a najnižu ista sorta na lokalitetu Novi Sad Prosečno posmatrano, veću energiju prikazuje sorta pobeda Najveći otpor rastezanja u aktuelnom istraživanju sorta pobeda pokazuje na lokalitetu Novi Sad (566 Ej), što ukazuje na relativno tvrdo testo, dok ml grigorija na istom lokalitetu ima najmanji otpor Najveću rastegljivost postiže ml grigorija u Somboru, dok je na lokalitetu Novi Sad rastegljivost iste sorte znatno niža Prosečno, ml grigorija pokazuje veću rastegljivost u odnosu na pobedu, što ukazuje na to da je ova sorta pogodnija za dobijanje elastičnog i dobro formabilnog testa.

Na osnovu istraživanja koje su obavili Jocković et al (2023), nova sorta ns lenija čije su osobine ispitivane na različitim lokalitetima dala je sledeće rezultate: veći prinos zrna od ml grigorije na svim lokalitetima osim Požarevca, hektolitarska masa iznosila je 85,3 kg/hl, što je više od obe naše ispitivane sorte Ns lenija pokazala je veće vrednosti i za: masu 1000 zrna (33,7g na sm), sadržaj proteina (12% na sm), sadržaj vlažnog glutena (28,3%), sadržaj suvog glutena (9,3%), izbrašnjavanje (74,5%), moć upijanja vode (61,8%), farinogramski kvalitetni broj (74,6), energiju na ekstenzogramu (115 cm^2) Ns lenija imala je niži sedimentacioni broj u odnosu na dve ispitivane sorte u ovom radu Interesantno je da je ns lenija pokazala veće vrednosti tehnoloških pokazatelja od sorte pobeda.

Na osnovu odnosa otpora rastezanju (Ej) i rastegljivosti testa (mm) može se uočiti (tabela 5) razlika u reološkim svojstvima između sorti i lokaliteta Kod sorte pobeda sa lokaliteta Novi Sad dobijen je najveći odnos (5,34), što ukazuje na najveću čvrstoću testa uz najmanju elastičnost Najniži odnos je kod sorte ml gligorija sa lokaliteta Sombor (2,44), što pokazuje mekše i elastičnije testo Ostali uzorci imaju srednje vrednosti (3,19–4,64), što ukazuje na uravnotežen odnos otpornosti i elastičnosti Dobijeni rezultati potvrđuju da različiti genotipovi i lokaliteti gajenja značajno utiču na fizičko-hemijska i reološka svojstva brašna, a time i na njegovu tehnološku vrednost.

Tabela 5. Fizičke i hemijsko-tehnološke osobine zrna i brašna.
Table 5. Physical and chemical-technological characteristics of grain and flour.

Obeležje/Parameter	ml gligorija			pobeda		
	Novi Sad	Sombor	Prosek/ Average	Novi Sad	Sombor	Prosek/ Average
Fizičke osobine/Physical characteristics						
Sadržaj vlage zrna kod prispeća (%)/ Moisture content of grain upon arrival (%)	11,3	12,3	11,8	11,4	12,1	11,8
Hektolitarska masa očišćene pšenice (kg/hl)/Hectoliter weight of cleaned wheat (kg/hl)	82,8	80,4	81,6	83,9	82,4	83,15
Masa 1000 zrna (g na sm)/ 1000-grain Mass (g, on dry matter basis)	32,1	33,8	32,9	29,2	36,8	33
Staklavost zrna (%)/Grain vitreousness (%)	69	82,5	75,75	75	80	77,5
Hemijsko-tehnološke osobine/Chemical-technological characteristics						
Sadržaj sirovih proteina pšenice (% na m)/Crude protein content of wheat (% on dry matter basis)	9,4	12,5	10,9	9,5	12,2	10,8
Sedimentaciona vrednost pšenice (engl Zeleny test)/Sedimentation value of wheat (Zeleny test)	31	58	45	29	55	42
Sadržaj vlažnog glutena(%)/Wet gluten content(%)	20	30	25	19	29	24
Sadržaj suvog glutena (%)/Dry gluten content (%)	7	10	9	7	10	8
Izbrašnjavanje I1 (%)/Flouring I1 (%)	73,9	74	74	69	70,7	69,9
Farinogram/Farinogram						
Moć upijanja vode (% na 13% vlage brašna)/ Water absorption (% at 13% flour moisture)	58,6	61,8	60,2	59,4	62,4	60,9
Vreme razvoja testa (min)/Dough development time (min)	1,4	2,4	1,9	1,5	2,2	1,9
Stabilitet testa (min)/Dough stability (min)	0,2	0,8	0,5	0,1	2,1	1,1
Stepen omekšanja testa (Fj)/Dough softening degree (Fj)	116	66	91	115	18	67
Farinogramski kvalitetni broj/Farinogram quality number	40,5	62,6	51,6	44,5	81,3	62,9
Farinogramska kvalitetna grupa/Farinogram quality group	C-1	B-1	B-2	C-1	A-2	B-1
Ekstenzogram/Extensogram						
Energija (cm2)/Energy (cm2)	69	100	85	84	94	89
Otpor rastezanja (Ej)/ Dough resistance to extension (Ej)	478	376	427	566	424	495
Rastegljivost (mm)/ Dough extensibility (mm)	103	154	129	106	133	120
Odnos između otpora rastezanja i rastegljivosti testa/The relationship between dough resistance to extension and dough extensibility	4,64	2,44	3,31	5,34	3,19	4,13
Energija (cm2)/Energy (cm2)	69	100	85	84	94	89

Tabela 6. Statistička analiza ispitivanih parametara.
Table 6. Statistical analysis of the investigated parameters.

	A	B	AB
Fizičke osobine/Physical characteristics			
Sadržaj vlage zrna kod prispeća (%)/Moisture content of grain upon arrival (%)	nsz	nsz	nsz
Hektolitarska masa očišćene pšenice (kg/hl)/Hectoliter weight of cleaned wheat (kg/hl)	*	**	nsz
Masa 1000 zrna (g na sm)/Mass of 1000-grain (g, on dry matter basis)	nsz	**	**
Staklavost zrna (%)/Grain vitreousness (%)	*	**	**
Hemijsko-tehnološke osobine/Chemical-technological characteristics			
Sadržaj sirovih proteina pšenice (% na sm)/Crude protein content of wheat (% on dry matter basis)	*	**	**
Sedimentaciona vrednost pšenice (engl *Zeleny test*)/Sedimentation value of wheat (*Zeleny test*)	**	**	nsz
Sadržaj vlažnog glutena (%)/Wet gluten content (%)	nsz	**	nsz
Sadržaj suvog glutena (%)/Dry gluten content (%)	nsz	**	nsz
Izbrašnjavanje I_1 (%)/Extraction I_1 (%)	**	nsz	nsz
Farinogram/Farinogram			
Moć upijanja vode (% na 13% vlage brašna)/Water absorption (% at 13% flour moisture)	nsz	**	nsz
Vreme razvoja testa (min)/Dough development time (min)	nsz	nsz	nsz
Stabilitet testa (min)/Dough stability (min)	nsz	**	*
Stepen omekšanja testa (Fj)/Dough softening degree (Fj)	**	**	**
Farinogramski kvalitetni broj/Farinogram quality number	**	**	**
Farinogramska kvalitetna grupa/Farinogram quality group			
Ekstenzogram/Extensogram			
Energija (cm^2)/Energy (cm2)	**	**	**
Otpor rastezanja (Ej)/Dough resistance to extension (Ej)	**	**	**
Rastegljivost (mm)/Dough extensibility (mm)	**	**	**
Odnos između otpora rastezanja i rastegljivosti testa/ The relationship between dough resistance to extension and dough extensibility	nsz	**	nsz

* – statistički značajna razlika; ** – statistički vrlo značajna razlika; nsz – nema statistički značajne razlike; Faktor A – sorta; Faktor B – lokalitet.

** – statistically significant difference; ** – statistically very significant difference; nsz –* not statistically significant*; Factor A – variety; Factor B – localityn.*

Zaključak

Na osnovu dobijenih rezultata može se zaključiti da su prinosi pšenice značajno varirali u zavisnosti od sorte, godine i lokaliteta Sorta ml grigorija pokazala je izuzetan potencijal u povoljnim uslovima, ostvarivši najveće prinose u sezoni 2021/22, dok su u nepovoljnijoj 2022/23 godini ti prinosi značajno opali, što ukazuje na visoku osetljivost prinosa na meteorološke uslove i potrebu za višegodišnjim ispitivanjima radi pouzdane ocene sortnog potencijala.

Na osnovu ispitivanih fizičkih osobina zrna može se zaključiti da su sorte pokazale različite rezultate u zavisnosti od lokaliteta Sadržaj vlage zrna bio je sličan kod obe sorte Sorta pobeda imala je veće vrednosti hektolitarske mase i mase 1000 zrna, što ukazuje na bolju zrelost i krupnoću zrna S druge strane, sorta ml grigorija pokazala je veću staklavost zrna, naročito na lokalitetu Sombor, što može ukazivati na bolji kvalitet zrna u pogledu tehnološke obrade.

Na osnovu prikazanih rezultata može se zaključiti da je sorta ml grigorija, u povoljnijim uslovima (lokalitet Sombor), pokazala bolje osobine u pogledu sadržaja proteina, vlažnog glutena, izbrašnjavanja i rastegljivosti testa, dok je sorta pobeda imala veće vrednosti hektolitarske mase, mase 1000 zrna, moći upijanja vode, stabilnosti testa, farinogramskog kvalitetnog broja, kao i niži stepen omekšanja Farinogramski kvalitet brašna kod obe sorte bio je nizak, naročito kod ml grigorije na lokalitetu Novi Sad Ekstenzografska analiza ukazuje na veću prosečnu energiju testa kod sorte pobeda, dok ml grigorija ima veću rastegljivost.

Vremenski uslovi u drugoj godini ispitivanja bili su nepovoljniji na većini lokaliteta, zbog viših temperatura i smanjenih padavina na početku i kraju vegetacije Iako je ukupna količina padavina bila veća, njihov loš raspored, naročito zimi i u rano proleće, negativno je uticao na useve.

Zahvalnica

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YIELD, PHYSICAL AND CHEMICAL - TECHNOLOGICAL CHARACTERISTICS OF GRAIN AND FLOUR OF NEW WINTER WHEAT VARIETIES IN DIFFERENT AGROECOLOGICAL CONDITIONS

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R e z i m e

The need for global food security requires an increase in wheat production, as the third most common crop in the world with a significant role in the global and Serbian economy Although breeding and agrotechnical measures contribute to yield growth, agriculture faces challenges such as climate change, land degradation and rising input costs that require new approaches to production stability The aim of the research was to identify differences in grain yield and quality between the widely used wheat variety Pobeda and the new wheat variety ML Grigorija, taking into account the influence of the genotype and external conditions The yield and physicochemical properties of grain and flour of ML Grigorija and Pobeda were examined at six locations in Serbia during 2021/22 and 2022/23 The trials were organized according to a random distribution plan in three repetitions, with standard agrotechnical measures The yield was calculated and corrected for the standard moisture content, while the physico-chemical and rheological properties were tested in accredited laboratories according to SRPS ISO standards The obtained results were processed statistically, using the variance analysis method, and the least significant difference test (lsd test) was used for individual comparisons The results showed significant differences in yield, physical and technological properties depending on the factors examined The ML Grigorija variety achieved high yields and good technological quality in favorable conditions, while Pobeda had more stable physical properties and better test stability, which indicates that the selection of the genotype adapted to environmental conditions enables the achievement of high and stable yields and quality.

Key words: wheat (*Triticum aestivum* L), quality, grain yield, variety, agroecological conditions.

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COMPARATIVE EFFECTS OF GROWING MEDIA AND STARTER NITROGEN FERTILIZATION ON THE GROWTH AND NUTRIENT UPTAKE OF LETTUCE (*LACTUCA SATIVA* L.) SEEDLINGS

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Abstract: The growth and nutrient uptake of lettuce (*Lactuca sativa* L.) are strongly influenced by the choice of growing medium and nitrogen fertilization strategy. This study investigated the comparative performance of soil, coco peat, and hydroponics under different starter nitrogen regimes using a completely randomized design (CRD) with six treatments and four replications. Data on seedling emergence, chlorophyll content, plant height, root number, dry weight, and nutrient concentrations (N, P, K) were collected. The results revealed significant differences ($p < 0.001$) across the treatments. Hydroponics supported the fastest seedling emergence (2 days), the highest chlorophyll content (0.632 at week 2), the tallest plants (5.53 cm at week 3), and the greatest biomass (1.31 g), while soil with NPK recorded the highest nitrogen concentration (7.17%). Hydroponic full-strength and half-strength nutrient solutions achieved the greatest phosphorus uptake (7.69 and 7.67 g kg^{-1}, respectively). Root number did not differ significantly among treatments. Overall, hydroponics demonstrated superior performance, with the half-strength nutrient solution offering a more economical approach without compromising growth. These findings highlight the potential of hydroponics and coco peat as sustainable alternatives to soil-based systems for lettuce production in resource-limited environments.

Key words: lettuce, growing medium, hydroponics, coco peat, soil, nitrogen fertilization, seedling emergence, chlorophyll content, nutrient uptake.

Introduction

Lettuce (*Lactuca sativa L.*) is a globally significant leafy vegetable valued for its nutritional content, low caloric value, and economic importance. As a rich source of vitamins A, C, and K, folate, and dietary fiber, lettuce contributes significantly to human health, especially in urban diets. Its cultivation supports food security and income generation, making it a preferred crop for smallholder

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farmers and urban agriculture in many parts of the world (Gonzaga et al., 2018; Nyam et al., 2021).

In Nigeria, lettuce is increasingly grown in urban and peri-urban settings, supported by the rise in demand for fresh vegetables. However, the diverse soil conditions pose challenges to its cultivation. Nigerian soils, particularly those in the southeastern and savanna regions, are often characterized by low fertility, acidity, and nutrient imbalances. These issues include deficiencies in essential nutrients such as nitrogen, phosphorus, and potassium, and poor organic matter content, which limits their suitability for optimal lettuce production (Mustapha et al., 2022a; Mustapha and Muhammad, 2023). Additionally, soil erosion and soil degradation further reduce productivity, emphasizing the need for alternative solutions to improve crop yields (Mustapha and Idris, 2023).

Alternative growing media, such as coco peat and hydroponics, have gained traction as sustainable methods to address soil-related limitations of lettuce cultivation. Coco peat, derived from coconut husks, is known for its excellent water retention capacity, aeration, and pH stability. Its use has proven to be particularly beneficial in seedling production, offering uniform growth conditions and improved root establishment (Fathidarehnijeh et al., 2023). In Nigeria, coco peat is increasingly being integrated into urban farming practices due to its affordability and ease of use, particularly for high-value vegetables such as lettuce and tomatoes, due to its affordability and ease of use (Mensah et al., 2020; Ogunwole et al., 2001). Hydroponics, a soilless cultivation system that uses nutrient-enriched solutions, offers another viable alternative. It allows for precise control of nutrient delivery, leading to faster growth rates, higher yields, and reduced water usage compared to traditional soil-based systems. Hydroponics is especially effective in urban settings where space and soil quality are constraints, making it an attractive option for lettuce production in Nigerian cities (Alam et al., 2024; Raj et al., 2023; Sharma et al., 2018).

Nitrogen is a key macronutrient that plays a critical role in lettuce growth and yield. It is essential for chlorophyll synthesis, leaf area development, and overall plant biomass. However, excessive nitrogen application can result in nitrate accumulation in the leaves, posing health risks to consumers and contributing to environmental pollution through leaching (Hong et al., 2022; Mensah et al., 2020). Starter nitrogen fertilization, which involves applying nitrogen during the early growth stages, has been shown to enhance seedling establishment and improve nitrogen use efficiency. This approach is particularly relevant in resource-constrained environments, where efficient nutrient management is essential (Al-Taey et al., 2018; Alam et al., 2024).

Despite the growing interest in soilless systems and advanced fertilization strategies, there is limited research on the comparative effects of soil, coco peat, and hydroponics under varying nitrogen fertilization regimes on lettuce growth in

Nigeria. This study seeks to evaluate the influence of these growing media on lettuce seedling growth, focusing on germination time, chlorophyll content, plant height, root development, and nutrient uptake. The findings will contribute to identifying sustainable and efficient practices for lettuce cultivation in diverse agro-ecological zones, particularly in regions with degraded soils or limited resources.

Material and Methods

Study location

The greenhouse experiment was conducted at the Center for Dryland Agriculture, Bayero University Kano. Laboratory analyses were carried out at the Research and Experimental Laboratory of the Department of Soil Science, Faculty of Agriculture, Bayero University Kano.

Growing media

Three different growing media were used in this study: soil, coco peat, and hydroponics. The soil was collected from the research farm of the Center for Dryland Agriculture. Coco peat and hydroponic materials were sourced from certified commercial suppliers.

Experimental design and treatments

The experiment followed a completely randomized design (CRD) with six treatments. The treatments included three growing media (soil, coco peat, and hydroponics) combined with two fertilizer doses: zero fertilizer dose (no fertilizer applied), and complete fertilizer dose (for hydroponics, half the recommended fertilizer rate was used in the zero-dose treatment).

Urea, single super phosphate (SSP), and muriate of potash (KCl) were used as fertilizers. The fertilizer was applied based on the standard recommendations for lettuce: 30 kg N ha^{-1}, 15 kg P_2O_5 ha^{-1}, and 10 kg K_2O ha^{-1}.

Collection of agronomic data

The agronomic data collected included chlorophyll content, plant height, and root characteristics.

1. Chlorophyll content and plant height: Measurements were recorded at 9, 18, and 27 days after sowing (DAS). Chlorophyll content was assessed using a

GreenSeeker™ handheld optical sensor (Model 505, NTech Industries, Ukiah, CA, USA).

2. Root samples: Root samples were collected from each treatment at the end of 27 DAS for further analysis of root growth parameters.

3. Dry weight: Plants were harvested at 27 DAS, dried at room temperature for three days, and weighed using a precision balance.

This approach ensured a comprehensive evaluation of the effects of the growing media on the growth and development of the lettuce seedlings.

Laboratory analysis

The soil and the coco peat were analyzed for their basic properties as follows:

- Soil texture was determined using the Boyoucous hydrometer method (Bouyoucous, 1962);
- The electrical conductivity and pH were measured using a pH meter with a glass electrode based on the method outlined by Mclean (1982);
- Organic matter was determined using the Walkley-Black wet oxidation method as described by Mustapha et al. (2022a);
- Total nitrogen was determined by the micro-Kjedhal digestion method (Bremmer, 1996);
- Available phosphorus was determined using the Bray method as outlined by Mustapha et al. (2022b), while total P was determined using the procedure of Malá and Lagová (2014);
- Exchangeable bases were determined as described by Silva et al. (2010).

Statistical analysis

The results of soil physical and chemical properties were analyzed using descriptive statistics, while analysis of variance was used to evaluate the response of lettuce to different growing media and fertilizers. Means were separated using the Fisher's protected least significant difference. All analyses were carried out using the JMP version 16 statistical package.

Results and Discussion

Soil and coco peat characterization

The results of the physical and chemical properties of the soil and the coco peat are shown in Table 1. The soil used was classified as sandy loam, a common soil type in the Nigerian savannah, which is often classified as nutrient-deficient and requires fertilization for optimal vegetable production (Mustapha et al., 2022a; Nyam et al., 2021). These characteristics make coco peat particularly effective in supporting seedling growth in environments with inconsistent water availability.

Cahyo et al. (2019) and Abad et al. (2002) highlighted the ability of coco peat to provide a stable water supply, promoting uniform germination and root development. Both media displayed slightly acidic pH values within the optimal range for lettuce cultivation (5.5–6.8), with coco peat having a slightly higher pH (6.70 and 5.90) than soil (6.50 and 5.60) in both water and CaCl2, respectively. These pH levels enhance nutrient solubility and availability to plants (Amran et al., 2024).

Table 1. Characterization of the soil and the coco peat.

Property	Soil	Coco peat
Texture (%)	Clay loam	
Sand	40.24	
Silt	31.28	-------
Clay	28.48	
pH		
Water	6.50	6.70
$CaCl_2$	5.60	5.90
Electrical conductivity (dsm-^{1})	0.14	0.17
Exchangeable bases ($cmol_{(+)}kg^{-1}$)		
Na	0.14	14.13
Mg	1.11	14.37
Ca	3.16	2.61
K	0.42	0.41
Exchange acidity	0.67	----
Organic carbon (%)	1.06	2.37
Total nitrogen (%)	0.28	4.37
Available P (mg kg^{-1})	4.09	8.82

Electrical conductivity values for both media were low (soil: 0.14 dS m-1, coco peat: 0.17 dS m-1), indicating minimal salinity stress, which is critical for optimal lettuce growth (Sublett et al., 2018). Organic carbon plays a crucial role in improving soil structure, water-holding capacity, and nutrient availability. Coco peat had a higher organic carbon content (2.37%) compared to soil (1.06%). The higher organic carbon content of coco peat aligns with its use as an effective growing medium for crops that require consistent moisture and nutrient availability during germination and early growth stages (Cahyo et al., 2019). In contrast, the lower organic carbon content in the soil may limit its ability to retain nutrients and water, potentially impacting seedling development. Coco peat exhibited significantly higher levels of exchangeable bases, particularly magnesium (14.37 cmol kg-1) and sodium (14.13 cmol kg-1), compared to soil. These findings support the use of coco peat as a medium for maintaining a balanced nutrient supply as reported by Amran et al. (2024). However, the soil had a higher calcium concentration (3.16 cmol kg-1), which is beneficial for plant structural development. Despite this, the overall nutrient retention capacity of coco peat, credited to the high amounts of cations observed (Na (14.13 cmol(+)kg-1); Mg (14.37 cmol(+)kg-1); Ca (2.61 cmol(+)kg-1) and K (0.41cmol(+)kg-1)), makes it a more reliable medium for nutrient delivery. Coco peat contained significantly higher nitrogen (4.37%) and phosphorus (8.82 g kg-1) levels than soil (0.28% nitrogen and 4.09 g kg-1 phosphorus). Nitrogen is essential for chlorophyll synthesis and leaf growth, while phosphorus supports root development and energy transfer. The higher nutrient content in coco peat highlights its suitability for promoting rapid seedling growth (Asiah et al., 2004). Conversely, the lower nutrient levels in the soil underscore the need for supplemental fertilization to support healthy lettuce growth.

Effect of different growing media and fertilizer rates on the chlorophyll content of lettuce

The chlorophyll content of lettuce as affected by different growing media and fertilizer rates are as shown in Table 2. The chlorophyll concentration represents the production of energy nutrition in plants and an indication of the sufficiency of required nutrients, especially N (Sandadevani et al., 2025).

Table 2. Effect of different growing media and fertilizer rates on the chlorophyll content of lettuce.

Growing medium	Chlorophyll w1	Chlorophyll w2	Chlorophyll w3
Hydroponics full	0.540a	0.632a	0.560a
Hydroponics half	0.520a	0.535a	0.565a
Coco peat	0.283b	0.310b	0.407a
Coco peat with NPK	0.253b	0.312b	0.362a
Soil	0.288b	0.335b	0.487a
Soil with NPK	0.355b	0.387b	0.502a
S.e.d	0.0575	0.570	0.0660
F probability	<0.001	<0.001	0.043

Means followed by different letters are significantly different at $p<0.001$, W1: week 1, W2: week 2, W3: week 3, NPK: nitrogen, phosphorus and potassium fertilizers.

Significant differences ($p < 0.05$) in the chlorophyll content were observed between the treatments, indicating that the different growing media and fertilizer regimes had an impact on the chlorophyll content of lettuce. The highest chlorophyll content (0.520 – 0.632) was observed with the use of the hydroponics which was attributed to optimal nutrient supply, excellent aeration essential for root respiration and nutrient supply as well as reduced or low competition with weeds and soil-borne pathogens (Çekin et al., 2025). The soil-based systems, both with and without NPK fertilizer, showed lower chlorophyll content (0.288–0.502) compared to hydroponics, with the lowest chlorophyll content of 0.288–0.407 recorded across the coco peat media.

The addition of fertilizer to soil-based systems was observed to significantly increase chlorophyll content by 3.08–23.03%, thus indicating the positive effect of NPK fertilizer and its ability to affect nutrient availability which may have an influence on the synthesis of chlorophyll (Rathnayaka et al., 2024). In contrast, the addition of NPK to coco peat-based systems did not significantly increase chlorophyll content. This suggests that the high nutrient-holding capacity of coco peat may already meet the crop requirements without additional fertilizer (Abad et al., 2002; Amran et al., 2024).

Generally, chlorophyll content increased over time in all treatments except for the full-strength hydroponics. The rate of increase was highest in the half-strength hydroponic systems followed by the soil-based systems with NPK.

Effect of different growing media and fertilizer rates on days to emergence, number of roots and dry weight of lettuce

The effect of different growing media and fertilizer rates on days to emergence, number of roots and dry weight of lettuce is shown in Table 3. The result showed highly significant differences (<0.001) in the days to emergence across the different media. The hydroponic systems, both with full and half-strength nutrient solutions, showed the fastest emergence time of 2 days, followed by the coco peat-based media (3 days) and finally the soil-based system of 5 days. Significant differences ($p<0.05$) in the number of roots among the different media were observed in the hydroponic systems, particularly the half-strength nutrient solution had significantly higher number of roots (20) compared to the full-strength solution (18). The soil-based and coco peat-based systems, both with and without NPK fertilizer, had a similar number of roots. The rapid emergence and higher root number in hydroponic systems can be attributed to the optimal nutrient and oxygen availability in the nutrient solution (Ahmed et al., 2021; Liu et al., 2014; Raj et al., 2023). The slower emergence and lower root number in soil-based and coco peat-based systems may be due to factors such as soil compaction, waterlogging, or nutrient deficiencies (Cahyo et al., 2019; Ogunwale et al., 2021; Olasupo et al., 2018).

Highly significant differences (<0.001) in the dry weight of lettuce were observed among the different media. Hydroponic systems, both with full and half-strength nutrient solutions, produced a significantly higher dry weight (1.308 and 1.258, respectively). The superior biomass production in hydroponics is a cumulative effect of early emergence, higher chlorophyll content, and increased root number. This reflects the efficient conversion of photosynthates into biomass under optimized nutrient and water conditions (Boroujerdnia and Ansari, 2007; Sandadevani et al., 2025). On the other hand, lower dry weights were observed in the soil-based and coco peat-based systems, with no significant weight increase with additions of NPK fertilizer. The addition of NPK fertilizer did not significantly improve weight, suggesting that other factors, such as soil structure and microbial activity, may limit plant growth (Nyam et al., 2021; Ogunwale et al., 2021; Olasupo et al., 2018).

Table 3. Effects of different growing media and fertilizer rates on days to emergence, number of roots and dry weight of lettuce.

Growing medium	Days to emergence	Number of roots	Dry weight
Hydroponics full	2b	18b	1.308a
Hydroponics half	2b	220a	1.258a
Coco peat	3b	16b	0.785b
Coco peat with NPK	3b	17b	0.808b
Soil	5a	15b	0.713b
Soil with NPK	5a	16b	0.758b
S.e.d	0.357	1.567	0.0591
F probability	<0.001	0.013	<0.001

Means followed by different letters are significantly different at $p<0.001$, NPK: nitrogen, phosphorus and potassium fertilizers.

Effect of different growing media and fertilizer rates on plant height

Table 4 presents the plant height of lettuce seedlings grown in various media and under different fertilizer rates over 3 weeks. Statistical differences ($p < 0.05$) in the plant height were observed among all the growing media. The tallest seedlings of 5.525 cm were observed in both the full-strength and the half-strength nutrient solutions of the hydroponic system. The plant height was observed to increase significantly over the time period. The excellent performance of the hydroponic system may be attributed to optimal nutrient supply, reduced competition as well as excellent aeration (Raj et al., 2023; Rathnayaka et al., 2024).

Table 4. Effect of plant height on lettuce seedlings grown on different media.

Growing medium	Plant height w1	Plant height w2	Plant height w3
Hydroponics full	1.800ab	3.450a	5.525a
Hydroponics half	2.250a	3.825a	5.525a
Coco peat	1.150c	2.150b	4.125b
Coco peat with NPK	1.150bc	2.200b	4.250b
Soil	0.850c	2.000a	3.950b
Soil with NPK	0.825c	1.950b	4.000b
S.e.d	0.3031	0.3432	0.2296
F probability	0.001	<0.001	<0.001

Means followed by different letters are significantly different at $p<0.001$, NPK: nitrogen, phosphorus and potassium fertilizers, W1: week 1, W2: week 2, W3: week 3.

On the other hand, the plant soil-based and coco peat-based systems were significantly shorter compared to hydroponic systems (3.95 cm and 4.125 cm, respectively). The low plant heights observed might be due to factors such as soil compaction, waterlogging, or nutrient deficiencies (Olasupo et al., 2018).

Effect of different growing media and fertilizer rates on the content of nitrogen, phosphorus and potassium

The nitrogen, phosphorus, and potassium content of lettuce grown under the various media is presented in Table 5. There were no significant differences in the content of nitrogen across the different growing media (p = 0.963) which is probably due to nitrification and other chemical changes within the environment of the growing media (Vought et al., 2024). Similar results were obtained for potassium. Significant differences ($p<0.001$) were found for phosphorous content across the different growing media. Hydroponic systems, both with full-strength (7.691 g kg^{-1}) and half-strength nutrient solutions (7.669 g kg^{-1}), had a significantly higher phosphorus content compared to soil-based (5.674 g kg^{-1}) and coco peat-based systems (5.660 g kg^{-1}). The enhanced phosphorus uptake in hydroponics underscores the efficiency of direct nutrient supply in soilless systems (Çekin et al., 2025). This aligns with Karimaei et al. (2004), who have highlighted that phosphorus availability is often constrained in soil-based systems but optimized in hydroponics systems.

Table 5. Effect of nitrogen, phosphorus, and potassium content on lettuce grown on different media.

Growing medium	Nitrogen %	Phosphorus (g/kg)	Potassium (mg/kg)
Hydroponics full	6.30a	7.691b	0.580a
Hydroponics half	6.30a	7.669b	0.667a
Coco peat	6.30a	5.660a	0.384a
Coco peat with NPK	6.45a	5.843a	0.627a
Soil	7.00a	5.674a	0.661a
Soil with NPK	7.17a	5.07a	0.510a
S.e.d	1.291	0.1974	0.1449
F probability	0.963	<0.001	0.387

Means followed by different letters are significantly different at $p<0.001$, NPK: nitrogen, phosphorus and potassium fertilizers.

Conclusion

This study demonstrated that growing media and starter nitrogen fertilization significantly affected lettuce growth, development, and nutrient uptake. Hydroponics consistently outperformed soil and coco peat in terms of seedling emergence, chlorophyll content, plant height, and dry matter accumulation, confirming its efficiency as a high-performance cultivation system. Notably, half-strength hydroponic solutions produced results comparable to full-strength treatments, suggesting that input costs can be reduced without compromising productivity.

Coco peat proved to be a promising sustainable alternative to soil, with higher organic carbon and nutrient content, though its nutrient release dynamics require supplementation—particularly of calcium—to support structural development. Soil-based systems, while widely available, exhibited lower performance and highlighted the need for improved management practices such as fertilization or organic amendments to optimize productivity.

The findings emphasize the potential of hydroponics and coco peat to strengthen urban and peri-urban lettuce production in Nigeria and similar regions facing soil degradation. Future studies should focus on integrating coco peat with soil or other organic amendments, evaluating organic nutrient solutions, and assessing the environmental and economic sustainability of hydroponic adoption in resource-constrained settings.

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UPOREDNI EFEKTI SUPSTRATA I OSNOVNOG ĐUBRENJA AZOTOM NA RAST I USVAJANJE HRANLJIVIH MATERIJA KOD RASADA ZELENE SALATE (*LACTUCA SATIVA* L.)

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R e z i m e

Rast i usvajanje hranljivih materija kod zelene salate (*Lactuca sativa* L.) u velikoj meri zavise od izbora supstrata i načina đubrenja azotom. Ovim istraživanjem se ispitivao uporedni efekat zemljišta, kokosovog treseta i hidroponike u različitim režimima osnovnog đubrenja azotom, korišćenjem potpuno slučajnog rasporeda (engl. *completely randomized design – CRD*) sa šest tretmana u četiri ponavljanja. Prikupljeni su podaci o nicanju, sadržaju hlorofila, visini biljaka, broju korenova, suvoj masi i koncentracijama hranljivih materija (N, P, K). Rezultati su pokazali značajne razlike ($p < 0,001$) među tretmanima. Hidroponika je omogućila najbrže nicanje (2 dana), najveći sadržaj hlorofila (0,632 u 2. nedelji), najviše biljke (5,53 cm u 3. nedelji) i najveću biomasu (1,31 g), dok je kod zemljišta u kome je dodato đubrivo NPK zabeležena najviša koncentracija azota (7,17%). Hidroponski hranljivi rastvori sa punom i sa pola doze postigli su najveće usvajanje fosfora (7,69 odnosno 7,67 g kg^{-1}). Broj korenova se nije značajno razlikovao među tretmanima. U celini, hidroponika je pokazala najbolje efekte, pri čemu je hranljivi rastvor sa pola doze predstavljao ekonomičniji pristup bez ugrožavanja rasta. Ovi nalazi ističu potencijal hidroponike i kokosovog treseta kao održivih alternativa sistemima zasnovanim na zemljištu za proizvodnju zelene salate u okruženjima sa ograničenim resursima.

Ključne reči: zelena salata, supstrat, hidroponika, kokosov treset, zemljište, đubrenje azotom, nicanje, sadržaj hlorofila, usvajanje hranljivih materija.

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PHENOLOGY AND BIOCHEMICAL ANALYSIS OF SPONTANEOUS *PRUNUS SPINOSA* L. POPULATION IN THE VICTINITY OF BELGRADE

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Abstract: The aims of the research were to determine the variability of the phenological patterns of natural populations of *Prunus spinosa* L. in the ecotones of the southeastern part of the Balkan Peninsula and to create an informational database regarding the impact of air temperature and precipitation on the phenology of this species, based on research conducted during the period from 2007 to 2024. The obtained data were analysed using descriptive and multivariate statistical methods. The results showed that the onset of spring phenological phases has been significantly accelerated by the increase in air temperature, especially in 2024, when flowering started 31 days earlier, the flowering phase lasted 13 days longer, and fruit ripening occurred 65 days earlier compared to the 2007–2023 period. The content of phenols, flavonoids, anthocyanins, and antioxidant activity in fruits was determined in distilled water and 70% ethanol. The analysed extracts contained a high percentage of phenols and demonstrated significant antioxidant activity. The obtained results are the starting point for studying changes in the phenological patterns of blackthorn in response to climate change under the agro-ecological conditions of the southwestern suburb of Belgrade. The study confirmed the adaptability of blackthorn, except for the vulnerability of its flowering phenophase to late spring frosts, as observed in 2023. The resilience of the species to climatic challenges was further confirmed by its drought tolerance in 2024 and a significant correlation between fruit ripening and high air temperatures during the growing season over 18 consecutive years of research.

Key words: blackthorn, adaptability, functionality, antioxidant capacity of fruits, ecosystem services, heat waves, ecotones.

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Introduction

Climate change, with increasingly intense extreme events, is a limiting factor for the use of woody plants in the green infrastructure of urban and suburban areas, especially near roads (Rahman et al., 2017). Research confirms that taxa reach the upper limit of plasticity in the conditions of the temperate continental climate in Central Europe (LWG, 2018). Plasticity, or changes in morphology, phenological patterns, and bioactive components in response to environmental conditions (Price et al., 2003), can be assessed through the benefits of ecosystem services. Plant attributes that influence condition, growth, reproduction, survival, and ecosystem services are functional plant traits classified as morphoanatomical, bioactive, and phenological, and have been confirmed to be influenced by climate (Floret et al., 1990; Ocokoljić et al., 2023a). For future challenging environmental conditions, according to Klein (2014), plants that grow in conditions of seasonal drought have a greater tolerance to rainfall deficits compared to taxa from wetter habitats. In addition, there are specific morphological traits and phenological patterns that have evolved to enable survival (Roloff et al., 2013; Ocokoljić et al., 2023b, Petrov et al., 2024), as well as theoretical estimates aimed at identifying the sensitivity of woody plants based on knowledge of environmental conditions in natural and semi-natural habitats (Sjöman et al., 2012).

Blackthorn is an ornamental species commonly used in the design of hedges as an element of green infrastructure. However, it is also important for ecosystem services as it provides nectar for early pollinators in spring. Its thorny branches (in young plants) offer safe nesting sites for birds, as well as shelter and food for small mammals. Particularly in semi-natural ecosystems, its dense and thorny canopy in oak forest zones creates an impenetrable barrier through which large herbivores find it difficult to pass and thus serves to protect young, palatable seedlings such as oak saplings (Ružičkova et al., 1996; Ocokoljić and Petrov, 2022). Like most species of this genus, blackthorn exhibits a gametophytic self-incompatibility system (GSI), and a high degree of intra and interspecies hybridisation. Alongside with cherry plum – *Prunus cerasifera* – it is considered as one of the progenitors of the European plum (*Prunus domestica*) (Crane and Lawrence, 1952). Fully ripe fruits are a valuable source of natural antioxidants due to their high content of vitamin C and phenolic acids (Ruiz-Rodriguez et al., 2014).

Therefore, the paper analyses the responses of the flowering and fruiting phenophases of blackthorn (*Prunus spinosa* L.) to climatic factors during the 2007–2024 period in agroforestry ecotones near roads. *Prunus spinosa* L. belongs to the *Prunus* genus of the *Rosaceae* family, with its range extending across much of southern and central Europe, reaching northwards to the southern part of the Scandinavian peninsula (Ocokoljić and Petrov, 2022). The paper analyses: (1) the phenological responses of blackthorn to climatic variables at the population level over 18 consecutive years, and (2) the fruits as a source of bioactive compounds, with the aim of promoting the species as a source of safe natural antioxidants.

Material and Methods

Study site

The experimental area is located in Ostružnica, on the right bank of the Sava River, in a suburban zone within the Belgrade municipality of Čukarica, in the settlement of Ostružnica, as part of the local green road infrastructure on flat, unexposed terrain. By identifying the ecotone *in situ*, the conventional landscape pattern of the experimental area was completed, as the remote sensing bypasses transitional zones (Arnot et al., 2004). Seven linear agroforestry ecotones were selected, dominated by blackthorn and characterised by a uniform topography, with the soil classified as eutric brown (eutric cambisol) according to Tanasijević et al. (1963). The structure, composition, and diversity of the ecotones have changed significantly over the 18 years of research, and the presence of woody species (with the clustering of individual trees) revealed an inequality in tree distribution, although the blackthorn remained stable and occupied a larger area within the ecotones.

During 2024, which has been recorded as the warmest year since measurements started, the highest yielding blackthorn population was selected, covering an area of 124 m², and centred at coordinates 44°43'36.80"N and 20°19'35.91"E, at an altitude of 112 m.

Phenology, yield and meteorological data

The phenological patterns during the 2007–2024 period were classified according to the identification keys of BBCH phenological stages for stone fruit (plum, genus *Prunus* L.) as a mean value at the population level (Meier, 1997). Observations were made every other day, at the same time in all ecotones, recording: the onset of flowering (BF) – the day when more than 10% of the flowers on the bush were open, full flowering (FF) – the day when more than 50% of the flowers were open, the end of flowering (EF) – when no more flowers were open, and fruit ripening (RP) – the day when the fruits acquired the characteristic colour of ripe fruit.

The fruit abundance of blackthorn in the ecotones was assessed at the population level by quantifying phenological observations according to Stilinović (1985) on a five-point scale, where 0 stands for no yield (0% of branches with fruits); 1 – very small yield (<20%); 2 – small yield (20–40%); 3 – moderate yield (40–60%); 4 – abundant yield (60–90%); and 5 – maximum yield (>90%).

The sum of degree days, or accumulated heat (GDD), was determined for the key phenological stages of flowering and fruiting according to Lalić et al. (2021). Dates are presented as DOY, i.e., day of the year, with January 1st as DOY 1. Climatic variables were analysed based on hourly and daily values obtained from the main meteorological station Surčin (φ44º47'54.44"N; λ20º27'53.35"E, Figure 1). Bearing in mind that the fruits were under the influence of climatic parameters before sampling, the air temperature and precipitation after their ripening were analysed in relation to the reference period 1991–2020 (RHMZ, 2024; OGI, 2024).

Chemical analysis

In August 2024, physiologically mature fruits from the southern part of the bush of the selected genome (rating 5) were collected for mesocarp analysis to determine the impact of high air temperatures and drought on the total content of polyphenols and the antioxidant activity of blackthorn fruits in a laboratory oven at 80°C.

Five grammes of the fruit mesocarp were weighed and dried to constant weight and extracted with 70% ethanol and distilled water (10 ml) in a ratio of 1:50. The samples were then kept in a dark place for 24 hours, and centrifuged at 10,000rpm for 15 minutes. The content of total phenols and total tannins was determined using a Folin-Ciocalteu reagent (Nagavani and Raghava Rao, 2010). The absorbance at λ=730 nm was read using a Thermo Scientific Evolution 220 spectrophotometer. The total phenol and tannins were expressed as mg of quercetin equivalents/g of fresh weight. The method for determining the amount of total flavonoids was based on the properties of flavonoids to build appropriate metal complexes with metal ions (Markham, 1989). The absorbance at λ=430 nm was read on a spectrophotometer. The content of total flavonoids in the tested extracts was calculated using the calibration curve of the quercetin standard and expressed as mg of quercetin equivalents/g of fresh weight. The qualitative determination of total anthocyanins was based on the property of anthocyanins to reversibly change their structure when the pH of the medium changes, whereby the absorption spectrum changes. The content of monomeric anthocyanins was determined by the pH differential method, based on the properties of the monomeric anthocyanins at pH 1.0 in the form of oxonium ions (red coloured), while at pH 4.5 anthocyanins were in the semi-metallic form (colourless). The concentration of total anthocyanins was expressed as mg of cyaniding-3-glucoside/g of fresh weight.

The antioxidant activity of the blackthorn fruit extracts was evaluated using the FRAP test (reducing power) and DPPH and ABTS tests (radical scavenging).

The FRAP method was determined as previously reported by Benzie and Strain (1996) with adaptations by Kolarov et al. (2021). The calibration curve was constructed using a series of dilutions of FRAP in water. After 30 minutes, the absorbance was read at λ=593 nm. The total FRAP reducing capability was expressed in mg of trolox equivalents/g of fresh weight. The ability of the extracts of the tested plant species to neutralise the radical cation 2.2′-azino-bis- (3-ethylbenzothiazoline-6-sulfonic acid), $ABTS^{\cdot +}$, was measured according to the method of Re et al. (1999), with slight modifications. The absorbance at λ=734 nm was measured on a spectrophotometer. The calibration curve was constructed using a series of dilutions of trolox in water. The $ABTS^{\cdot +}$ radical scavenging activity was expressed in mg of trolox equivalents/g of fresh weight. The spectrophotometric determination of the "skewing" activity of the extracts studied was based on monitoring the transformation of $DPPH^{\cdot}$ radicals (2,2-diphenyl-1-picrylhydrazyl radicals) into reduced form (DPPH-H) (Przybylski et al., 1998). The $DPPH^{\cdot}$ radical itself was purple in colour and had an absorption maximum at 517 nm. By reacting with antioxidants, it bound the hydrogen atom to its unpaired electron and thus turned into the yellow DPPH-H. The absorbance at λ=517 nm was read on a spectrophotometer. The calibration curve was constructed using a series of dilutions of trolox in water. After 30 minutes, the absorbance was read at λ=517 nm. The $DPPH^{\cdot}$ radical scavenging activity was expressed in mg of trolox equivalents/g of fresh weight.

Statistical analysis

To assess statistical significance, the non-parametric Mann-Kendall test (Kendall's tau) was applied, which, according to the WMO, is suitable for trend analysis in environmental time series data (Rustum et al., 2017). The Spearman rank correlation coefficient (ρ) was also used, as it indicates whether there is a consistently increasing or decreasing relationship between variables, and because it does not require any assumptions about the frequency distribution of the variables (Quade, 1974). The range of ρ is from -1 to 1, with the sign determining the strength and direction of the relationship. For data interpretation, the scale of Horvat and Mijoč (2012) was used to interpret the data: 0 (no correlation), 0–0.24 (very weak), 0.25–0.49 (weak), 0.50–0.74 (moderate), 0.75–0.99 (strong to very strong), and 1 (perfect). Coefficients with statistically significant correlations were interpreted with a probability of $p < 0.05$. The data processing and figure preparation were performed using the software packages XLSTAT 2020, Past 4.11, ArcGIS 10.8/ArcMap 10.8, and Google Earth Pro.

Results and Discussion

Relationship between phenophases and climate variables

The average monthly and annual air temperatures and average monthly, annual and total precipitation were calculated for the reference and research periods (Table 1).

Table 1. Climate variables for the reference period (1991–2020), the research period (2010–2024) and 2024 and deviations in 2024 compared to the reference period and the 2007–2023 period, according to the MMS Surčin data.

Mean air temperature (°C)													
Months Period	1	2	3	4	5	6	7	8	9	10	11	12	$\bar{x}$
$\bar{x}$ 2024	2.9	10.1	11.6	15.7	18.6	24.7	26.8	27.4	19.9	14.4	5.7	3.2	15.1
$\bar{x}$ 2007/2023	2.0	4.2	8.1	13.3	17.8	22.1	24.2	24.0	18.8	13.2	8.3	3.4	13.3
$\bar{x}$ 1991/2020	1.0	3.0	7.5	12.9	17.6	21.4	23.2	23.2	18.0	12.8	7.4	2.2	12.5
Deviation 2024(%) from normal value 1991–2020	1.8	7.1	4.2	2.8	1.0	3.3	3.6	4.2	1.9	1.6	-1.7	1.1	2.6
Deviation 2024(%) from normal value 2007–2023	0.9	5.9	3.6	2.4	0.9	2.6	2.6	3.4	1.1	1.2	-2.5	-0.2	1.8
Total and mean precipitation amounts (mm)													
Months Period	1	2	3	4	5	6	7	8	9	10	11	12	∑
$\bar{x}$ 2024	37.1	4.8	27.7	23.3	99.8	92.5	69.4	6.5	86.8	44.0	45.6	63.2	600.7
2007/2023	46.5	39.1	47.3	41.5	82.1	75.4	50.6	46.2	52.0	48.1	51.1	48.9	628.9
$\bar{x}$ 1991/2020	42.4	34.0	41.7	47.4	68.1	80.1	58.2	54.0	56.0	50.7	45.5	48.3	626.4
Deviation 2024(%) from normal value 1991–2020	-5.3	-29.2	-14.0	-24.1	31.7	12.4	11.2	-47.5	30.8	-6.7	0.1	14.9	-25.7
Deviation 2024(%) from normal value 2007–2023	-9.4	-34.3	-19.6	-18.2	17.7	17.1	18.8	-39.7	34.8	-4.1	-5.5	14.3	-28.2

Based on the analysis of the parameters, it can be seen that the mean annual air temperature for the reference period (1991–2020) was 12.5°C, 13.3°C for the research period (2007–2023), and 15.1°C for 2024. The mean annual rainfall for the reference period (1991–2020) was 626.4mm, 628.9mm for the research period (2007–2023), and 600.7mm for 2024. The deviations are shown in Table 1. The mean air temperatures and precipitation amounts by month and on an annual basis for the research period (2007–2024) are shown in Figure 1.

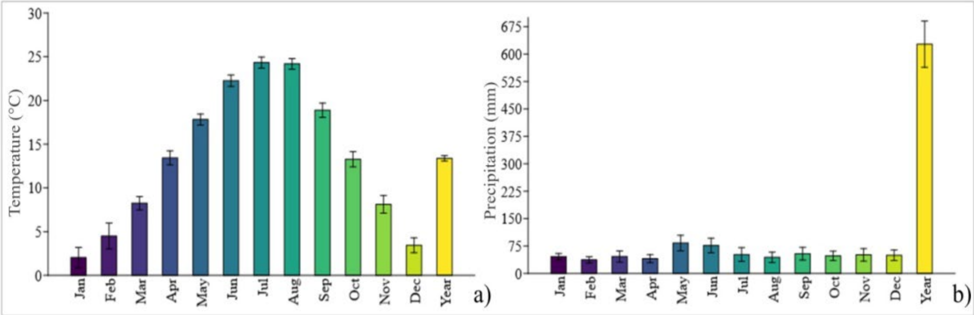

Figure 1. Mean air temperatures (a) and precipitation amounts (b) by month and on an annual basis for the research period (2007–2024), according to the MMS Surčin data. Data are expressed as the mean ± SD (n=18).

Descriptive statistics were used to assess the variability of the climate variables (Tables 2 and 3). The standard deviation and other deviation parameters indicate the variation of mean air temperatures and precipitation during the 18 years of research.

Table 2. Descriptive statistics for mean monthly and annual air temperatures for the 2007–2024 period according to MMS Surčin.

Parameters	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Min	-4.40	-4.00	6.00	10.00	15.00	20.40	22.40	21.90	15.70	9.90	3.30	0.00	12.30
Max	6.30	10.10	11.60	17.40	20.80	24.70	26.80	27.40	21.70	17.00	12.10	6.40	15.10
Sum	36.60	80.90	148.5	241.7	320.8	400.7	438.0	435.4	339.9	239.0	146.2	61.90	240.9
Mean	2.03	4.49	8.25	13.43	17.82	22.26	24.33	24.19	18.88	13.28	8.12	3.44	13.38
Std. error	0.59	0.75	0.39	0.41	0.32	0.33	0.32	0.31	0.42	0.44	0.52	0.43	0.15
Variance	6.34	10.25	2.73	2.99	1.89	2.01	1.84	1.73	3.13	3.55	4.80	3.33	0.42
Stand. dev	2.52	3.20	1.65	1.73	1.38	1.42	1.36	1.32	1.77	1.88	2.19	1.83	0.65
Median	2.10	4.75	8.30	13.50	17.60	21.95	23.60	24.20	19.20	13.65	8.40	3.80	13.30
25 percentiles	0.20	2.88	6.80	12.60	16.80	20.88	23.35	23.40	17.25	11.38	6.95	2.03	13.10
75 percentiles	3.88	6.53	9.53	14.25	18.83	23.68	25.45	25.13	20.10	14.53	9.25	4.75	13.80
Skewness	-0.69	-0.88	0.35	0.16	0.19	0.23	0.44	0.38	-0.10	-0.02	-0.65	-0.33	0.78
Kurtosis	1.27	1.98	-0.74	1.05	0.34	-1.49	-1.13	1.08	-0.87	-0.53	0.76	-0.65	2.22
Geom. mean	0.00	0.00	8.10	13.32	17.77	22.22	24.30	24.16	18.80	13.15	7.77	0.00	13.37
Coeff. var	123.8	71.24	20.02	12.88	7.72	6.37	5.58	5.44	9.37	14.19	26.98	53.10	4.82

Based on the analysed data, the trends of increasing air temperature and decreasing precipitation can be observed. However, the Mann-Kendall trend tests only confirmed a statistically significant increase (significance level: $p<0.05$) in mean annual air temperatures ($S=71$, $Z=2.6733$, $p=0.00751$). Statistical significance was not confirmed for monthly air temperatures and total precipitation, which indicates extreme climatic events (WMO, 2021; RHMZ, 2024).

Table 3. Descriptive statistics for average monthly and annual rainfall for the 2007–2024 period according to MMS Surčin.

Parameters	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Year
Min	18.8	4.8	2.5	5.9	32.1	15.8	6.5	3.6	10.1	9.7	1.6	2	378.3
Max	89.6	68.9	110.3	86.9	233.4	156	181	126.6	161	97.4	152.7	125.5	937.3
Sum	827.6	669.9	832.4	728.1	1496	1375	929.5	791.5	971	861.3	914.8	895.1	11292
Mean	45.98	37.22	46.24	40.45	83.1	76.36	51.64	43.97	53.94	47.85	50.82	49.73	627.3
Std. error	4.395	4.359	7.739	5.696	10.94	10.23	9.575	7.218	8.797	6.507	8.703	7.418	32.31
Variance	347.7	342	1078	584	2154	1882	1650	937.8	1393	762.1	1363	990.5	18785
Stand. dev	18.65	18.49	32.84	24.17	46.41	43.38	40.62	30.62	37.32	27.61	36.92	31.47	137.1
Median	43.2	41.35	37.4	39.35	73.65	80.3	42.45	43.25	52.05	46.3	45.7	50.5	620.6
25 percentiles	33.55	20.2	24.6	21	58.2	33.93	21.25	20.5	22.65	17.03	27.18	30.63	519.3
75 percentiles	59.95	52.08	68.7	58.13	95.75	103.4	70.13	61.83	78.9	69.25	65.15	65.2	734.6
Skewness	0.571	-0.34	0.729	0.233	2.17	0.274	1.979	0.917	1.298	0.226	1.341	0.503	0.295
Kurtosis	0.269	-0.99	-0.52	-0.71	6.22	-0.99	5.419	1.727	2.726	-0.99	2.38	0.829	0.312
Geom. mean	42.34	30.74	33.33	31.45	74.02	62.9	39.39	31.36	42.05	38.83	36.12	33.74	613
Coeff. var	40.55	49.69	71	59.74	55.85	56.81	78.67	69.64	69.18	57.69	72.65	63.29	21.85

Descriptive statistics were used to assess the impact of climatic variables on the adaptability of blackthorn (Table 4). The standard deviation and other deviation parameters indicate a shift in the key phenophases of blackthorn.

Table 4. Descriptive statistics for the studied phenological parameters of blackthorn for the 2007–2024 period.

Parameters	BF DOY	FF DOY	EF DOY	RP DOY	BF GDD	FF GDD	EF GDD	RP GDD
Min	56.0	64.0	83.0	227.0	141.3	167.3	287.7	2697.6
Max	98.0	101.0	112.0	319.0	270.0	303.6	434.1	3333.2
Sum	1344.0	1408.0	1662.0	4893.0	3412.7	3890.7	5711.6	52275.0
Mean	79.06	82.82	97.76	287.82	200.75	228.86	335.98	3075.00
Std. error	2.74	2.68	1.87	5.34	9.06	8.16	9.01	34.71
Variance	127.68	122.03	59.57	484.90	1394.21	1133.26	1380.26	20476.74
Stand. dev	11.30	11.05	7.72	22.02	37.34	33.66	37.15	143.10
Median	82.00	87.00	98.00	289.00	203.40	233.30	343.00	3086.40
25 percentiles	68.50	71.50	92.00	273.50	173.90	202.50	303.55	3021.85
75 percentiles	87.50	91.50	103.50	305.50	225.35	243.20	355.10	3172.30
Skewness	-0.51	-0.36	-0.18	-1.14	0.22	0.55	0.91	-0.95
Kurtosis	-0.42	-0.97	-0.31	2.51	-0.28	0.82	1.64	2.26
Geom. mean	78.25	82.10	97.47	286.98	197.46	226.58	334.13	3071.77
Coeff. var	14.29	13.34	7.89	7.65	18.60	14.71	11.06	4.65

Considering the pronounced variability of the elements of the phenological patterns during the research period, the mean daily air temperatures were determined for the corresponding periods. To assess the impact of air temperature on the elements of the phenological patterns, statistical parameters were used to indicate the variation in the number of days within the phenological phases and the mean values of air temperatures during the relevant periods (Table 5).

Table 5. Descriptive statistics for the investigated phenological parameters and Tmean in the relevant elements of the phenological pattern of blackthorn (2007–2024).

Parameters	№ days BF-FF	Tmean BF-FF	№ days FF-EF	Tmean FF-EF	№ days BF-EF	Tmean BF-EF	№ days EF-RP	Tmean EF-RP
Min	2	6.8	9	6.5	12	7.3	142	17.9
Max	8	13.3	24	16.1	30	14.4	218	21.8
Sum	64	176.5	254	191.2	334	188.4	3248	337.1
Mean	3.76	10.38	14.94	11.25	19.65	11.08	191.06	19.83
Std. error	0.41	0.42	1.19	0.67	1.23	0.52	4.76	0.27
Variance	2.82	3.01	24.06	7.57	25.74	4.63	385.81	1.26
Stand. dev	1.68	1.73	4.90	2.75	5.07	2.15	19.64	1.12
Median	3.00	10.60	14.00	11.10	18.00	11.10	195.00	19.70
25 percentiles	3.00	9.20	11.50	9.05	16.50	9.50	177.00	18.90
75 percentiles	4.50	11.50	18.00	13.55	22.50	13.00	205.50	20.90
Skewness	1.41	-0.39	0.84	0.07	0.93	-0.20	-0.77	-0.05
Kurtosis	1.71	0.13	-0.39	-0.67	-0.02	-0.86	0.95	-0.86
Geom. mean	3.47	10.24	14.25	10.92	19.08	10.88	190.05	19.80
Coeff. var	44.58	16.71	32.83	24.47	25.82	19.41	10.28	5.67

Mann-Kendall trend tests confirmed a statistically significant earlier fruit ripening with a decrease in accumulated heat, as well as a reduction in the number of days from the end of flowering to fruit ripening, and an increase in the average daily air temperature during the EF-RP period (Table 6).

Table 6. Results of the Mann-Kendall trend test for statistically significant elements of the phenological patterns of blackthorn (RP DOY, RP GDD, number of days from EF to RP, and Tmean during EF-RP), at the population level, for the study period 2007–2024.

Phenological observation	RP DOY	RP *GDD*
S*	-61	-64
Z	-2.4737	-2.5951
p	0.013374	0.0094554
Phenological observation	№ days EF-RP	Tmean EF-RP
S*	-68	50
Z	-2.5799	2.0253
p	0.0057818	0.042834

*It has a negative sign (–) when the trend is decreasing, a value of (0) if there is no trend, and a positive sign (+) for an increasing trend.

The values of the Spearman correlation coefficient (ρ) for GDD and DOY, for the relevant flowering phenophase periods, were statistically significant for DOY, confirming a very strong and consistently increasing relationship between BF DOY and FF DOY, BF DOY and EF DOY, and FF DOY and EF DOY. This indicates that changes in the DOY of the onset and full flowering influenced the onset and end of other key stages in the flowering phenological pattern. Strong and consistently increasing positive correlations were observed between BF GDD and FF GDD, as well as between RP DOY and RP GDD, which suggests that an increase in accumulated heat for the onset of flowering affected the accumulated heat for full flowering, and that an increase in RP DOY influenced a higher accumulation of heat for fruit ripening.

Spearman's correlation coefficient indicates a very strong positive correlation between the number of days from full flowering to the end of flowering and the total duration of the flowering phenophase. It also shows that the average air temperature in this interval also rose with the number of days from full flowering to the end of flowering (Figure 2b). The moderate negative correlation between Tmean FF-EF and Tmean BF-EF confirms that if the average temperature from full flowering to the end of flowering is lower, the average temperature from the beginning to the end of flowering will also be lower (Figure 2b).

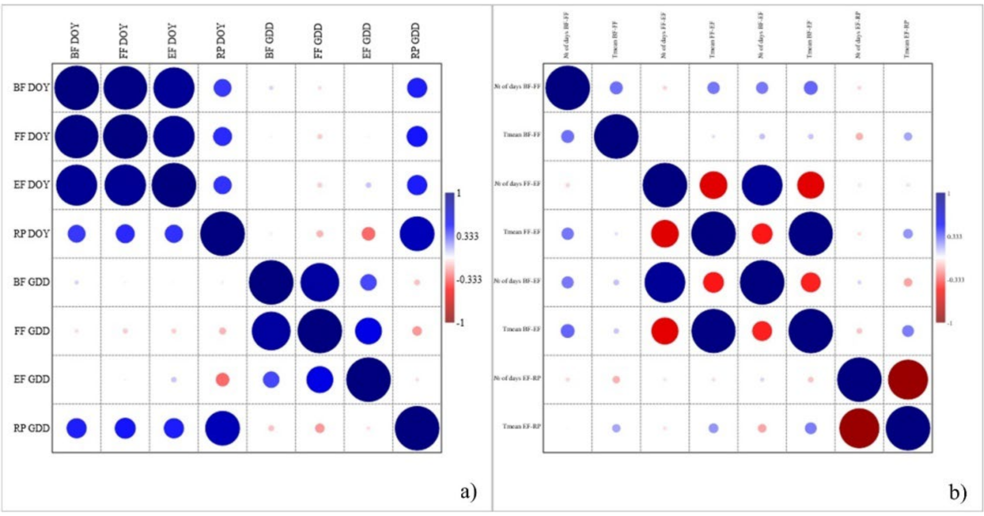

Figure 2. Graphical representation of Spearman's correlation coefficients: a) for the investigated phenological parameters of blackthorn (BF DOY, FF DOY, EF DOY, RP DOY, BF GDD, FF GDD, EF GDD, RP GDD), and b) for the investigated phenological parameters (number of days BF-FF, number of days FF-EF, number of days BF-EF, number of days EF-RP) and Tmean in the relevant elements of the phenological pattern of blackthorn (Tmean BF-FF, Tmean FF-EF, Tmean BF-EF, Tmean EF-RP) during the study period (2007–2024).

Strong and constantly increasing positive correlations were recorded between BF GDD and FF GDD and RP DOY and RP GDD, suggesting that an increase in accumulated heat for the beginning of flowering affected an increase in accumulated heat for full flowering, as well as that an increase in RP DOY affected a greater heat accumulation for fruit ripening (Figure 2a). Other correlations were not statistically significant.

The key phenological stages of blackthorn flowering during 2024 were recorded after the heat accumulation (Figure 3) at 183.3°C (BF), 249.3°C (FF), and 370.6°C (EF), which is approximately in line with the average values for the 2007–2023 period: 204.8°C (BF), 231.5°C (FF), and 334.9°C (EF).

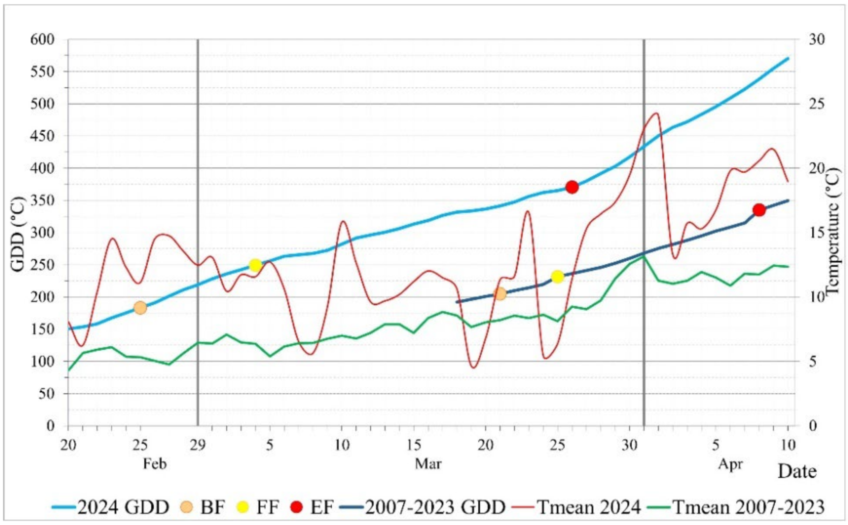

Figure 3. Graphical presentation of the temperature sums (GDD, °C) for the onset of flowering (BF), full flowering (FF), and the end of flowering (EF) of blackthorn (own observations), and the mean daily temperatures, in the study area, for 2024 and the average values, or normal values, for the 2007–2023 period (MMS Surčin). Legend: 2024 GDD (cumulative totals of GDD, with BF, FF and EF markers for 2024); 2007–2023 GDD (average values of cumulative sums of GDD, with BF, FF and EF markers for the 2007–2023 period); Tmean 2024 (mean daily air temperatures during the flowering period during 2024) and Tmean 2007–2023 (mean daily air temperatures during the flowering period during the 2007–2023 period).

The data obtained through descriptive statistics confirm that the temperature sums triggered flowering (Tables 1, 2, 3, 4 and 5). To compare the GDD and the mean daily air temperatures shown in Figure 3, both values during the flowering phenophase of *P. spinosa* L. in 2024 are presented graphically. The statistical analysis of the average daily air temperatures and the phenological observations revealed that: 1) the average value of the daily air temperatures for BF-FF during the 2007–2023 period was 11.1°C, 2) the average value of the daily air temperatures for BF-FF for 2024 was 10.8°C, 3) the average value of the daily air temperatures for the period from DOY 56 to DOY 86 for 2007–2023 was 7.2°C, and 4) the average value of the daily air temperatures for the period from DOY 80 to DOY 96 for 2024 was 15.0°C. It is evident that the average daily air temperatures during 2024 were higher by 3.6°C and 3.9°C, respectively, compared to the previous seventeen-year period. Additionally, an average annual air temperature of 13.3°C was determined for the research period , while it was 12.5°C for the reference period (1991–2020), indicating an increase in air temperature of 0.8°C. These findings are directly related to climate change (WMO, 2021).

Figure 4 shows the phenogram of blackthorn flowering for 2024 and the 2007–2023 period. The year of 2024 stands out clearly, with the flowering phase lasting 31 days, which is 13 days longer than in the 17-year period (2007–2023). Compared to the previous 17-year period, flowering began 24 days earlier.

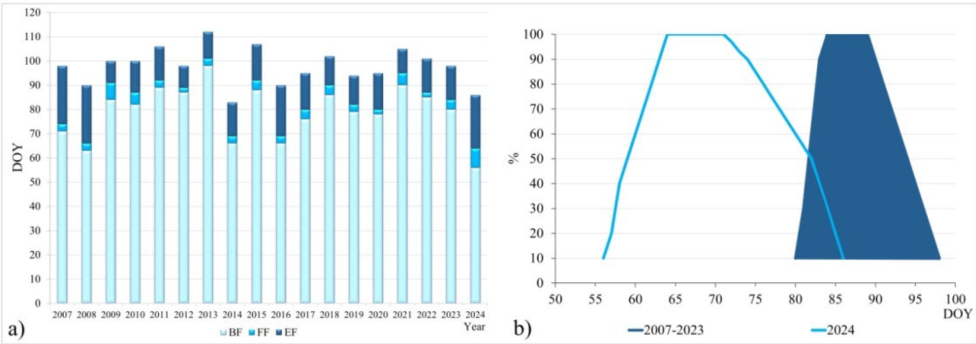

Figure 4. Phenological observations (a) DOY for the onset of flowering (BF), full flowering (FF), and end of flowering (EF) of blackthorn during the 2007–2024 period and (b) in 2024 and the average values for the 2007–2023 period.

The phenological patterns of fruit ripening in blackthorn are influenced by numerous ecological factors at the population level over 18 consecutive years. The most important factors are air temperature and precipitation, which affect ecosystem services and plant phenology (Cosmulescu and Ionescu, 2018; Ocokoljić et al., 2023b). The main response of blackthorn to changes in climatic variables is an earlier fruit ripening, which occurred 65 days earlier in 2024 compared to the average DOY for the 2007–2024 period (Figure 5). During this period, the earliest fruit ripening was recorded on DOY 227 (2024), and the latest on DOY 319 (2011). The average accumulated heat sum (GDD) for fruit ripening is 3075°C, with the lowest value recorded in 2024 (2697.6°C), and the highest in 2012 (3333.2°C).

Statistical analysis of the mean daily air temperatures and phenological observations revealed the following: 1) the average mean daily air temperature for EF-RP during the 2007–2023 period was 19.7°C, 2) the average mean daily air temperature for EF-RP in 2024 was 21.8°C, 3) the average mean daily air temperature for the period 56 to 227 DOY for 2007–2023 was 19.6°C, and 4) the average mean daily air temperature for the period 99 to 292 DOY in 2024 was 21.7°C. It is evident that the mean daily air temperatures from the end of flowering to fruit ripening in 2024 were higher by 2.2°C and 2.0°C compared to the previous seventeen-year period. A comparative analysis showed almost identical average air temperatures from the end of flowering to fruit ripening for the period 2007–2023 and 2024, regardless of DOY, confirming the thesis that phenophases occur within the corresponding accumulated heat sums.

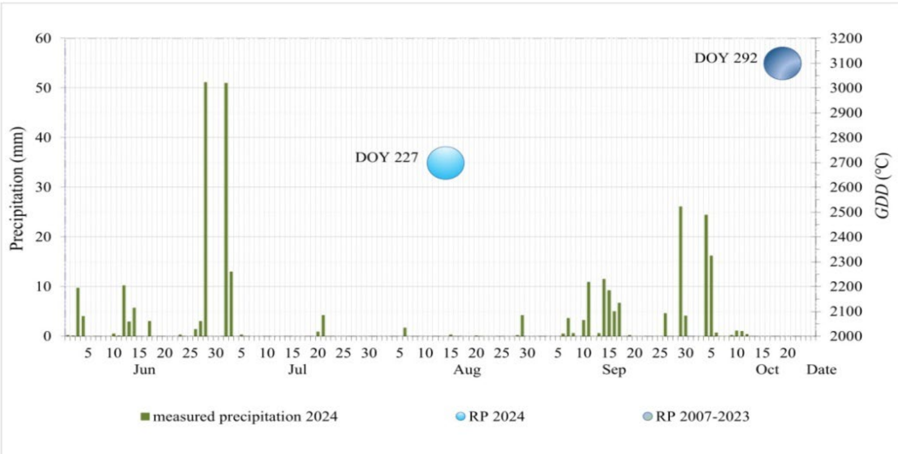

Figure 5. Phenomonitoring of blackthorn fruit ripening (RP) at the population level, DOY and GDD in 2024, and the mean values for the period 2007–2023, along with a graphical representation of the daily precipitation amounts during the summer of 2024 at the Surčin meteorological station.

The lowest and highest GDD values were recorded in the warmest years during the study period, which is correlated with the DOY being lower in 2024, following the warm 2023 and the hottest year of 2024. This is confirmed by the daily mean, maximum, and minimum air temperatures for Surčin, according to the corresponding percentiles. It can be observed that the mean, maximum, and minimum temperatures were above the multi-year average for most of the summer, apart from mid-June, early and late July, and early August, when temperatures were below normal. Heat waves were evident, with five consecutive days above the normal temperature threshold, and the most intense heat wave occurred over 8 days in August, during which fruit ripening was recorded on 227 DOY (14 August) in 2024. Combined with the air temperatures, the rainfall totals indicate specific patterns, such as the rainfall sum for July 2024, which, according to the percentile method, falls within the ‘normal’ category. A closer look at the distribution of daily rainfall amounts reveals that both July and August, as well as the entire summer of 2024, were dry in the study area (Figure 5). The monthly rainfall totals resulted from heavy showers and intense rainfall events, where the amount of rainfall in a single day was equivalent to the amount expected for a whole month in the reference period.

To the best of our knowledge, research on the phenology of *Prunus spinosa* L. is limited. The results of our 18-year study on phenological patterns are consistent with the findings of Réaumur (1735) and Ocokoljić et al. (2023), who stated that plant growth and development are proportional to accumulated thermal units (GDD), which measure the accumulation of heat above a threshold temperature over 24 hours, rather than to daily air temperatures during phenophases. Our results are also in line with the World Meteorological Organisation (WMO, 2021), which asserts that each taxon has an optimal period for its phenophases. Our findings are similar to those of studies in Romania, where a two-year study confirmed a correlation between flowering and spring air temperature (Cosmulescu and Gavrila Calusaru, 2020), and to those of Vander et al. (2016a), who highlighted that flowering occurred earlier in southern Europe compared to populations in Belgium. Additionally, they have noted that the timing of flowering is correlated with the local climate, suggesting that *Prunus spinosa* L. displays plasticity in response to rising air temperatures (Vander et al., 2016b). As in the study by Flynn and Wolkovich (2018), our research shows that in a moderately continental climate, phenological patterns were influenced by both air temperature and photoperiod, which interact to make plant responses complex and nonlinear. The start of flowering and subsequent phenophases in *Prunus spinosa* L. were correlated with temperature and precipitation from January to November. Increased heat accumulation has led to a longer flowering phase (particularly in 2024) and earlier fruit ripening, which aligns with the findings of Teskey et al. (2015). Given that our research has confirmed the adaptability of *Prunus spinosa* L. and its increased coverage in the studied ecotones, these findings are important for agriculture (beekeeping), landscape contexts aimed at creating a sense of place by predicting future changes in biodiversity, as well as for the conservation of habitats for wildlife such as the harvest mouse, great tit, blackbird, starling, tawny owl, and little owl, which exist in the study area (Batrićević and Batanjski, 2014).

The quality of the studied fruits

The comparative analysis of the phytochemical extraction efficacy between distilled water and 70% ethanol reveals significant differences in the content of total phenolics, tannins, flavonoids, anthocyanins, and antioxidant activities, highlighting the importance of solvent choice in phytochemical studies (Table 7).

Table 7. Total polyphenolic content and antioxidant activity of physiologically mature fruits of blackthorn in 2024.

	Total phenolic[1]	Total tannins[1]	Total flavonoids[1]	Total anthocyanins[2]	FRAP[3]	ABTS[3]	DPPH[3]
Distilled water	4.50 ± 0.21	2.30 ± 0.11	0.28 ± 0.04	0.13 ± 0.02	15.25 ± 0.73	34.09 ± 1.19	10.54 ± 0.42
Ethanol	9.99 ± 0.48	4.94 ± 0.50	2.44 ± 0.24	2.76 ± 0.51	31.18 ± 0.52	72.47 ± 1.38	33.86 ± 0.47

The data are mean values ± standard error; [1] Expressed as mg of quercetin equivalents/g of fresh weight; [2] Expressed as mg of cyaniding-3-glucoside/g of fresh weight; [3] Expressed as mg of trolox equivalents/g of fresh weight.

The antioxidant capacity of blackthorn fruit (*Prunus spinosa* L.) extracts is notable, particularly when comparing the efficacy of distilled water and ethanol as solvents. Ethanol extracts exhibited a significantly higher total phenolic content (9.99 ± 0.48 mg/g) compared to distilled water (4.50 ± 0.21 mg/g), enhancing the release of bioactive compounds that combat oxidative stress. The elevated tannin further demonstrates the superior extraction capabilities of ethanol, as tannins are known for their antioxidant properties through metal chelation and radical scavenging. The total flavonoid content was also markedly higher in the ethanol extracts (2.44 ± 0.24 mg/g) than in water (0.28 ± 0.04 mg/g), contributing to cellular protection against oxidative damage. Additionally, total anthocyanin levels were significantly higher in ethanol (2.76 ± 0.51 mg/g) compared to water (0.13 ± 0.02 mg/g), underscoring the role of these pigments as powerful antioxidants. The antioxidant activities assessed through FRAP, ABTS, and DPPH assays confirmed the effectiveness of the ethanol extracts, with FRAP values of 31.18 ± 0.52 mg/g for ethanol versus 15.25 ± 0.73 mg/g for water.

The fruits of *Prunus spinosa* L. analysed in this study had a high content of phenolic compounds and a strong antioxidant capacity, which, according to Jiménez et al. (2017), is important for both the food chain and the pharmaceutical industry. The differences in the content of the analysed compounds, depending on the extraction medium used, are a result of the varying polarity of the organic solvents and their mixtures, which selectively extract individual compounds. The examined extracts showed a strong DPPH radical scavenging activity. Phenols, flavonoids, tannins, and anthocyanins in the ethanol extracts exhibited significantly higher values, similar to the findings of Veličković et al. (2014), who analysed sloe fruits collected in Serbia in October. The sloe fruits (*Prunus spinosa* L.) from the selected ecotone in Southeastern Europe, grown under climate challenges as part of the local roadside green infrastructure, which ripened in August, confirmed their high antioxidant capacity. This finding aligns with studies on sloe fruits in the Romanian flora from 2015 (Skrovankova et al., 2015) and in September 2019 (Andronie et al., 2019). These findings highlight blackthorn as a valuable source of natural antioxidants with potential health benefits (Pinacho et al., 2015), and provide researchers with more detailed information on blackthorn identification in

Southeastern Europe for multipurpose uses (cosmetics, phytotherapy, pharmaceuticals, and the food industry, for the development of new functional foods or the enhancement of existing products). The results support the further evaluation of the functional potential of sloe fruits, as studies from different parts of the world have demonstrated a high concentration of antioxidant compounds (Opriş et al., 2021). Previous studies on other species have revealed that antioxidant capacity is influenced by factors such as cultivar, genotype, geographical region, and stage of ripeness (Memete et al., 2023). However, genotype has proven to be the most important factor influencing the anthocyanin content and antioxidant capacity of sloe fruits in northeastern Turkey (Ilhan, 2023).

Conclusion

The findings provide a basis for studying changes in the phenological patterns of *Prunus spinosa* L. in response to climate change in the ecotones of the road green infrastructure. Furthermore, these results are significant for research in the fields of fruit growing, horticulture, landscape architecture, and for defining guidelines for landscape design aimed at enhancing the physiognomic composition of landscapes and promoting *Prunus spinosa* L. as a species for the future. It has been confirmed that blackthorn (*Prunus spinosa* L.) exhibits plasticity in key functional traits associated with drought tolerance. Fruit ripening shows a significant correlation with high air temperatures during the growing season and rainfall, as well as with extreme climatic events such as late frosts during flowering, making this phenophase useful for assessing the tolerance of the species to climate adaptation and plasticity in response to climate challenges. Understanding the functional traits of blackthorn is essential for directed selection to improve resilience to future climate changes and for the sustainable use of wild fruits. Based on the results, it is recommended to study the genetic variability of blackthorn and consider its introduction into cultivation, as it has been confirmed that, as a wild fruit, it is an exceptionally healthy food suitable for directed use and the creation of sustainable, climate-smart landscapes.

Acknowledgements

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FENOLOGIJA I BIOHEMIJSKA JEDINJENJA PLODOVA *PRUNUS SPINOSA* L. POD UTICAJEM TEMPERATURE VAZDUHA I PADAVINA

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Rezime

Fenologija je proučavanje periodičnih bioloških promena kroz koje biljke prolaze, pod uticajem geografskih i ekoloških uslova sredine, posebno klimatskih varijabli. Ciljevi istraživanja bili su utvrđivanje varijabilnosti fenoloških obrazaca *Prunus spinosa* L. prirodnih populacija u ekotonima jugoistočnog dela Balkanskog poluostrva i formiranje informacione baza podataka o uticaju temperature vazduha i padavina na fenologiju vrste na osnovu istraživanja u periodu 2007–2024. Dobijeni podaci analizirani su deskriptivnim i multivarijantnim statističkim metodama. Rezultati pokazuju da je početak prolećnih fenoloških faza značajno ubrzan porastom temperature vazduha posebno u 2024. godini, kada je fenofaza cvetanja počela 31 dan ranije, 13 dana duže trajala, a dozrevanje plodova bilo 65 dana ranije u odnosu na period 2007–2023. S obzirom na to da trnjina značajno doprinosi ekosistemskim uslugama kao medonosna i vrsta sa primenom u prehrambenoj industriji i fitoterapiji određen je sadržaj fenola, flavonoida, antocijana i antioksidativna aktivnost plodova u destilovanoj vodi i 70% etanolu. Rezultati sugerišu da etanol ne samo da efikasnije ekstrahuje bioaktivne komponente, već i pojačava njihov potencijal za neutralizaciju slobodnih radikala. Istraženi ekstrakti sadrže visok procenat fenola i pokazuju značajnu antioksidativnu aktivnost. Dobijeni rezultati su polazna osnova za proučavanje promena fenoloških obrazaca trnjine kao odgovora na klimatske promene u agroekološkim uslovima jugozapadne prigradske zone Beograda. Potvrđeno je da je trnjina adaptivna sa izuzetkom ranjivosti fenofaze cvetanja na pozne prolećne mrazeve, što je evidentirano 2023. godine. Plastičnost vrste na klimatske izazove potvrdjena je tolerancijom na sušu, posebno tokom 2024, ali i značajnom korelacijom dozrevanja plodova sa visokim temperaturama vazduha tokom vegetacionog perioda tokom 18 uzastopnih godina istraživanja.

Ključne reči: trnjina, adaptivnost, funkcionalnost, antioksidativni kapacitet plodova, usluge ekosistema, toplotni talasi, ekotoni.

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ENHANCING THE SHELF LIFE AND QUALITY OF MEXICAN LIME THROUGH γ-AMINOBUTYRIC ACID TREATMENT

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Abstract: The key factors limiting the postharvest shelf life of limes are weight loss, rapid peel discoloration, and the degradation of their green color, which significantly reduces their market value. The Mexican lime, a citrus fruit of considerable economic and nutritional importance, was evaluated for the effects of γ-aminobutyric acid (GABA) immersion on postharvest quality and storage life. Fruits were treated with GABA solutions (0, 4, 8, or 12 mM) and stored at 7°C with 80 ± 5% relative humidity for 30 days. Measurements of fruit weight loss, total soluble solids (TSS), titratable acidity (TA), taste index (TSS/TA ratio), ascorbic acid content in juice, and rind pigments (chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids) were conducted every 10 days. GABA treatment, particularly at higher concentrations, significantly reduced weight loss and slowed TSS increases while enhancing TA and ascorbic acid levels. By the end of storage, the treated fruits exhibited higher organic acid levels and a more desirable taste index, as indicated by a reduced TSS/TA ratio. Additionally, GABA treatments preserved higher chlorophyll levels for up to 20 days and delayed carotenoid accumulation, effectively slowing peel yellowing. The 8 mM concentration was the most effective in maintaining postharvest quality. These findings demonstrate that GABA can enhance the storage life and quality of Mexican lime, providing a promising strategy to minimize postharvest losses and boost economic value.

Key words: *Citrus aurantifolia*, γ-aminobutyric acid, peel discoloration, taste index, weight loss.

Introduction

The global lemon and lime market, which accounts for a substantial share of global citrus production, yielded 21.53 million tons from 1.33 million hectares (FAO, 2022c). A diverse range of producers and exporters supports this market. The top ten producers—India, Mexico, China, Argentina, Brazil, Spain, the United

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States, Turkey, Italy, and Iran—highlight the global significance of these citrus fruits (FAO, 2022c).

Spain leads the world in lemon and lime exports, contributing approximately 60% of its production to the global market (FAO, 2022a). Mexico, the second-largest exporter, allocates 23% of its production to exports, while Turkey, ranked eighth in production, stands third in exports, exporting nearly half of its harvest (FAO, 2022a; 2022c). Argentina, ranked fourth in both production and exports, significantly contributes to the global market by exporting 18% of its production (FAO, 2022a; 2022c). Interestingly, despite not being among the top ten producers, South Africa ranks fifth in global exports, underscoring its strong international market presence (FAO, 2022a; 2022c). Iran, while ranking tenth in production, has relatively low export volumes, highlighting a production-export disparity (FAO, 2022a; 2022c).

The United States, despite ranking seventh in production, imports as much as 50% of its production, making it the world's largest importer of lemons and limes (FAO, 2022b). Other leading importers are Russia, the Netherlands, Germany, and France (FAO, 2022b).

This diverse landscape illustrates the critical role of various countries in shaping the global lemon and lime market, where distinct production and export strategies influence the global supply chain.

The Mexican lime (*Citrus aurantifolia* Swingle cv.), an acidic citrus fruit, is extensively cultivated in tropical and subtropical regions worldwide because of its economic importance. It is the third most widely grown citrus fruit, and it is valued for its delightful taste and the presence of beneficial natural compounds (Singh et al., 2021). This fruit contains abundant phytochemicals, including phenols, flavonoids, steroids, terpenoids, and alkaloids, which contribute to its antioxidant and anti-inflammatory properties (Chriscensia et al., 2020; Izah et al., 2024). Additionally, its essential oils demonstrate antimicrobial activity, suggesting their potential as natural food preservatives (Izah et al., 2024). The health benefits of Mexican lime extend to its antidiabetic effects, making it a valuable dietary addition (Izah et al., 2024). Furthermore, combining Mexican lime extracts with compounds such as doxorubicin (a chemotherapy medication used to treat cancer) has shown promise in enhancing therapeutic effects, such as inducing apoptosis in cancer cells (Adina et al., 2014).

Although the Mexican lime is a non-climacteric fruit with a low respiration rate, it still undergoes some softening and compositional changes after harvest, leading to a short postharvest shelf life and a loss of approximately 18–25% (CABI, 2022; Lerslerwong et al., 2023; Mohammadi et al., 2024a). The primary obstacles limiting the postharvest longevity of limes are weight loss and rapid peel discoloration and degradation of the green color, reducing their market value (Mohammadi et al., 2024a; 2024b).

γ-aminobutyric acid (GABA) is a non-protein amino acid that plays a significant role in plant physiology, including stress response, growth, and development. It is mainly metabolized via a short pathway called the GABA shunt, which bypasses two steps of the TCA cycle (Sheng et al., 2017). Exogenous pre- or postharvest application of GABA enhances citrus fruit quality by modulating biochemical pathways, including the elevation of phytohormone levels critical for development and ripening (Badiche-El Hilali et al., 2023) and the improvement of citrate and amino acid accumulation, contributing to better quality and storage performance (Sheng et al., 2017). Additionally, GABA boosts the antioxidant system in fruits, maintaining quality during storage by reducing oxidative stress and prolonging shelf life, as shown in studies on mangoes (Rastegar et al., 2020). Research by Nehela and Killiny (2023) sheds light on the crucial role of GABA accumulation in the response of *Citrus sinensis* to huanglongbing (HLB), a devastating bacterial disease lacking a sustainable cure. Their findings demonstrate that GABA significantly influences the metabolic and defence mechanisms of plants, boosting the biosynthesis of essential compounds such as amino acids, organic acids, fatty acids, and phytohormones. This, in turn, activates antioxidant defences, reducing oxidative stress and bolstering plant resilience against HLB. The implications of the study extend beyond HLB, suggesting that GABA may play a critical role in mitigating the effects of various biotic stresses.

Building on this understanding and addressing the existing knowledge gap regarding the effects of GABA on the postharvest quality of Mexican lime, this study aimed to evaluate the influence of this generally recognized as safe (GRAS) compound (Sheng et al., 2017) on key quality parameters during 30 days of storage. Specifically, it investigated the effects of GABA on fruit weight loss, total soluble solids (TSS), titratable acidity (TA), taste index (TSS/TA ratio), ascorbic acid content in fruit juice, and rind pigments, including chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoids.

Material and Methods

Plant material, experimental design, and treatments

Mexican lime fruits at the mature green stage were harvested from a commercial orchard located in Maniyan, a village in the Jolgah Rural District, Central District of Jahrom County, Fars Province, Iran, with geographic coordinates of 53°12′57″E and 28°34′38″N. The fruits were immediately transferred to the laboratory, where they were selected and sorted based on uniformity in color, shape, and size, as well as freedom from physical damage, pests, and diseases. Before applying the treatments, the fruits were disinfected with a 2% sodium hypochlorite solution for 2 minutes, followed by rinsing with distilled water and drying at room temperature.

The experimental design was factorial within a completely randomized design framework, including two experimental factors: GABA immersion (four levels: 0, 4, 8, and 12 mM) and sampling time (three levels: days 10, 20, and 30 of the storage period). Each treatment was replicated four times, with 15 fruits per replicate. Fruits were immersed in GABA (Sigma-Aldrich, St. Louis, MO, USA) solutions for 5 minutes, with distilled water serving as a control, and then dried at room temperature for one hour. They were then placed in perforated zip-top plastic bags with a 3%-hole ratio and stored at 7°C and 80 ± 5% relative humidity for one month. Additionally, for informational purposes and not for statistical comparison, the parameters were assessed on the initial day of the experiment, before the start of the storage period.

Fruit weight loss

The mass variation method was used (Taghipour and Assar, 2021). The weight of the fruits was measured at different time points during storage using a digital scale with an accuracy of 0.1 g. The weight loss was calculated as the difference between the initial mass and the mass at each time point, expressed as a percentage of the initial mass using Equation (1):

$$\textit{Weight loss}\ (\%) = \frac{W_0 - W_1}{W_0} \times 100 \qquad [1]$$

where W_0 represents the initial mass and W_1 represents the final mass.

Juice total soluble solids (TSS)

The TSS of the fruit juice was measured using a digital refractometer (Milwaukee MA871, Hungary) at room temperature and expressed as a percentage (%) (Taghipour and Assar, 2021).

Juice titratable acidity (TA)

The TA of the fruit juice was determined by titration with a 0.1 N NaOH solution (Taghipour and Assar, 2021). The endpoint of the titration was reached when the pH of the solution reached 8.2. The results were subsequently expressed as a percentage of citric acid.

Juice taste index

The taste index was calculated by dividing the TSS value by the TA value (Taghipour and Assar, 2021).

Juice ascorbic acid content

The ascorbic acid content of the fruit juice was measured using a titration method with a 2,6-dichlorophenolindophenol (DCPIP) solution (Taghipour and Assar, 2021). The results were expressed as milligrams per 100 grams (mg 100 g^{-1}) of the fruit juice.

Chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid content of peels

The chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents in the citrus fruit peel were measured using the method described by Wellburn (1994). Fresh citrus fruit peel was thoroughly cleaned and cut into small pieces (about 1–2 cm^2). Approximately 0.5 g of the peel was weighed and ground into a fine paste using a mortar and pestle with a small amount of 80% acetone (v/v). The homogenized paste was transferred to a centrifuge tube, and mixed with 10 mL of 80% acetone. The mixture was allowed to stand for 10 minutes in the dark to prevent pigment degradation. The sample was then centrifuged at 4000 rpm for 10 minutes, and the supernatant was carefully decanted into a clean tube, avoiding any pellet at the bottom. The supernatant was filtered through a filter paper to remove any remaining particles. The absorbance of the filtrate was measured at 663, 645, and 470 nm using a spectrophotometer. The chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid contents were calculated using Equations (2), (3), (4), and (5), respectively.

$$\text{Chlorophyll } a \text{ (mg g}^{-1}\text{ FW)} = 12.25 \times A_{663} - 2.79 \times A_{645} \quad [2]$$

$$\text{Chlorophyll } b \text{ (mg g}^{-1}\text{ FW)} = 21.50 \times A_{645} - 5.10 \times A_{663} \quad [3]$$

$$\text{Total chlorophyll (mg g}^{-1}\text{ FW)} = \text{Chlorophyll } a + \text{Chlorophyll } b \quad [4]$$

$$\text{Carotenoid (mg g}^{-1}\text{ FW)} = (1000 \times A_{470} - 1.82 \times \text{Chlorophyll } a - 85.02 \times \text{Chlorophyll } b)/198 \quad [5]$$

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) at a significance level of $P < 0.05$, and mean comparisons were performed using the least significant difference (LSD) test. All statistical analyses were performed using SAS software version 9.4.

Results and Discussion

The Mexican lime (*Citrus aurantifolia* Swingle cv.) is an economically important acidic citrus fruit, that is widely cultivated in tropical and subtropical regions worldwide. Renowned for its significant health benefits, it is characterized by a short postharvest life and high global postharvest losses (CABI, 2022; Lerslerwong et al., 2023; Mohammadi et al., 2024a; Singh et al., 2021). Key indicators such as fruit weight loss, juice attributes including total soluble solids (TSS), titratable acidity (TA), taste index (TSS/TA ratio), and ascorbic acid content, as well as peel color, are critical for assessing citrus fruit quality and consumer acceptance (Sheng et al., 2017). In this context, the authors investigated the effect of exogenous GABA (a GRAS compound) applied via the dip method on these parameters during a one-month storage period.

The analysis of variance (Table 1) revealed a significant effect of all experimental factors and their interactions on the evaluated traits, except for the lack of interaction effects on juice TSS and peel carotenoid content.

Weight loss in the control group increased steadily throughout the storage period and was significantly higher than that in the treated fruits. In the treated fruits, weight loss rose significantly until day 20, after which no further increase was observed. Additionally, no differences in weight loss were detected among fruits treated with different GABA concentrations on day 20. By the end of the storage period, the fruits treated with 12 mM GABA exhibited the lowest weight loss among all treated groups (Figure 1).

GABA immersion was associated with a reduction in juice total soluble solids (TSS), although no significant differences were observed among the different GABA concentrations (Figure 2a). The juice TSS increased significantly during storage up to 20 days, with no significant changes detected between the final two sampling times (Figure 2b).

The juice titratable acidity (TA) of the control fruits increased with storage time, showing a statistically significant rise up to day 20. Beyond this point, the increase in TA was no longer significant. On day 10, the fruits treated with 8 and 12 mM GABA exhibited a significantly higher TA compared to the control fruits. On day 20, no differences were observed between the treated and control fruits. However, by the final sampling time, TA peaked in the treated fruits. Although no significant differences were found among the different GABA concentrations, the TA in the treated fruits remained significantly higher than in the control fruits. Notably, in the control fruits, TA increased significantly between days 10 and 20, while in the treated fruits, the significant increase occurred during the final 10 days of storage (Figure 3).

The juice taste index (TSS/TA ratio) of the control fruits decreased over the storage period, with a significant reduction observed up to day 20. Beyond this point, the decrease in the ratio was no longer significant. On day 10, the fruits treated with all GABA concentrations exhibited significantly lower TSS/TA ratios compared to the control fruits. On day 20, no differences were observed between the treated and control fruits. However, at the final sampling, the TSS/TA ratio of the treated fruits reached its lowest value. Although no significant differences were found among the various GABA concentrations, the TSS/TA ratio was significantly lower in the treated fruits than in the control. Notably, the control fruits showed a significant decrease in the TSS/TA ratio between days 10 and 20, whereas the treated fruits experienced this significant decrease during the final 10 days of storage (Figure 4).

In the control fruits, the ascorbic acid content decreased by approximately 17.48% after 10 days of storage, followed by a slight and gradual increase until the end of the storage period. Despite this increase, the final ascorbic acid content in the control fruits remained 11.14% lower than at the start of the experiment. In contrast, the fruits treated with GABA, especially at 8 mM, consistently exhibited the highest ascorbic acid levels. After 10 days, these fruits had a significantly higher ascorbic acid content compared to the control fruits and those treated with 4 mM GABA. On day 20, the 8 mM GABA-treated fruits maintained a significantly higher ascorbic acid content than the control group and the other GABA-treated groups. By the end of the 30-day storage period, fruits treated with 4, 8, or 12 mM GABA had 0.43%, 4.95%, and 2.32% more ascorbic acid, respectively, compared to the beginning of the experiment. Throughout the storage period, the 8 mM GABA-treated fruits consistently had the highest ascorbic acid content, surpassing the initial levels and showing the most significant difference compared to the control fruits (Figure 5).

The chlorophyll *a* content in the peels of both the control and the treated fruits decreased significantly over time. Up to day 20 of storage, the fruits treated with higher concentrations of GABA had a significantly higher chlorophyll *a* content compared to the control fruits and those treated with the lowest GABA concentration. However, by the end of the 30-day storage period, no significant difference was observed in chlorophyll *a* content between the treated and control fruits (Figure 6a).

As storage progressed, the chlorophyll *b* content in the peels of both the control and the treated fruits decreased significantly. After 10 days, the fruits treated with higher GABA concentrations exhibited higher chlorophyll *b* content compared to the control group and those treated with the lowest GABA concentration, with no significant difference between the control group and the group with the lowest GABA concentration. On day 20, the chlorophyll *b* content was similar across the treated fruits and significantly higher than in the control fruits. By the end of the storage period, only the fruits treated with 8 mM GABA exhibited a significantly higher chlorophyll *b* content compared to the control group, with no significant differences observed among the treated groups. Furthermore, the chlorophyll *b* content in the peels of the fruits treated with the lowest and highest GABA concentrations was similar to that of the control fruits (Figure 6b).

The changes in total chlorophyll content followed a pattern similar to that of the chlorophyll *a* content (Figure 6c).

GABA treatment at higher concentrations was associated with a significantly lower carotenoid content in the peel compared to the control (Figure 6d). Nevertheless, carotenoid levels increased significantly in the fruit peel over the storage period (Figure 6e).

Table 1. Analysis of variance for the effect of γ-aminobutyric acid (GABA) treatment on the evaluated characteristics of Mexican lime fruit during storage.

		Mean square									
Source of variation	Degrees of freedom	Weight loss	Juice total soluble solids (TSS)	Juice titratable acidity (TA)	Juice taste index	Juice ascorbic acid	Peel chlorophyll *a*	Peel chlorophyll *b*	Peel total chlorophyll	Peel carotenoid
Dip in GABA	3	23.05**	0.244**	0.946**	0.034**	1062.6**	0.069**	0.0006**	0.083**	0.042**
Storage time (day)	2	62.48**	0.479**	9.51**	0.096**	63.63*	1.44**	0.0049**	1.609**	0.231**
Dip in GABA × Storage time (day)	6	3.74**	0.040^{ns}	0.476**	0.0093**	50.74*	0.013**	0.0002**	0.0142**	0.002^{ns}
Error	36	0.116	0.042	0.086	0.0024	16.07	0.003	0.00002	0.0028	0.0049
Coefficient variation (%)		4.2	2.3	3.6	4.5	3.8	10.6	13.2	9.9	10.9

**, *, and ^{ns} denote significant differences at P ≤ 0.01, P ≤ 0.05, and no significant difference, respectively, as determined by the LSD test.

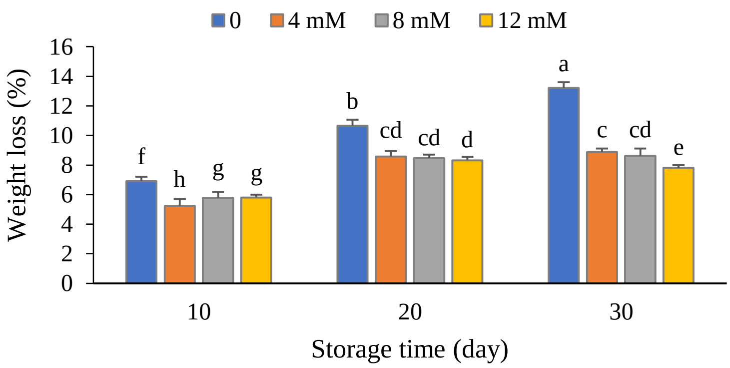

Figure 1. Changes in the weight loss of Mexican lime fruits during 30 days of storage at 7°C and 80 ± 5% RH. Fruits were dipped in γ-aminobutyric acid (GABA) solutions at 0, 4, 8, or 12 mM for 5 minutes prior to storage. Measurements were taken on days 10, 20, and 30. Data are presented as means of 4 replicates ± SD. Different letters indicate significant differences according to the LSD test (P ≤ 0.05).

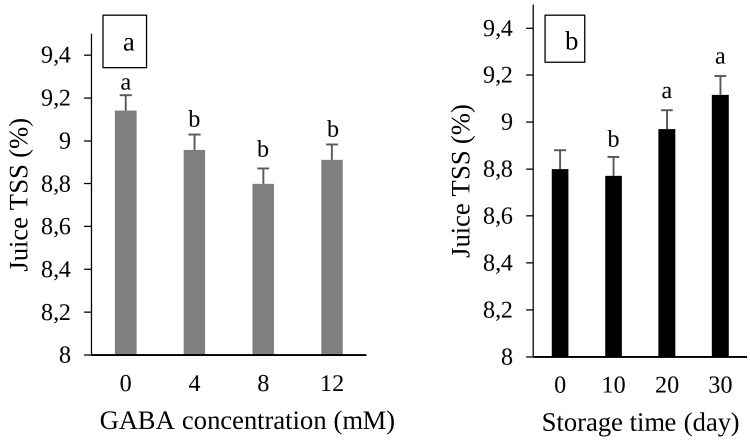

Figure 2. Changes in the juice total soluble solids (TSS) of Mexican lime fruits under the main effects of γ-aminobutyric acid (GABA) treatment (a) and storage time (b) during 30 days of storage at 7°C and 80 ± 5% RH. Fruits were dipped in GABA solutions at 0, 4, 8, or 12 mM for 5 minutes prior to storage. Measurements were taken on days 10, 20, and 30. Data are presented as means of 12 replicates for (a) and 16 replicates for (b) ± SD. Different letters indicate significant differences according to the LSD test (P ≤ 0.05).

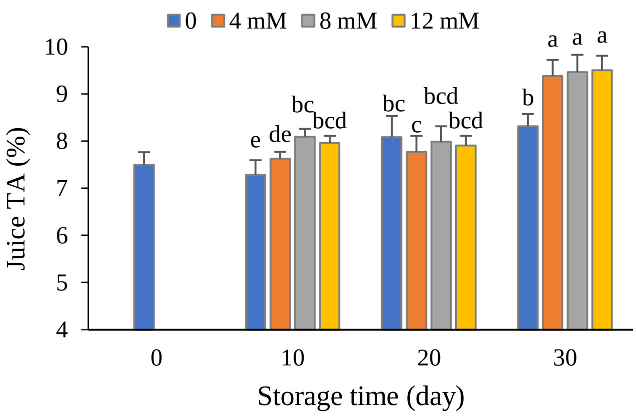

Figure 3. Changes in the juice titratable acidity (TA) of Mexican lime fruits during 30 days of storage at 7 °C and 80 ± 5% RH. Fruits were dipped in γ-aminobutyric acid (GABA) solutions at 0, 4, 8, or 12 mM for 5 minutes prior to storage. Measurements were taken on days 10, 20, and 30. Data are presented as means of 4 replicates ± SD. Different letters indicate significant differences according to the LSD test (P ≤ 0.05).

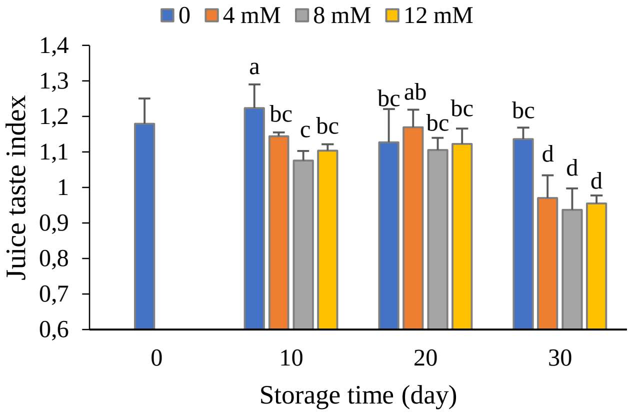

Figure 4. Changes in the juice taste index (TSS/TA ratio) of Mexican lime fruits during 30 days of storage at 7°C and 80 ± 5% RH. Fruits were dipped in γ-aminobutyric acid (GABA) solutions at 0, 4, 8, or 12 mM for 5 minutes prior to storage. Measurements were taken on days 10, 20, and 30. Data are presented as means of 4 replicates ± SD. Different letters indicate significant differences according to the LSD test ($P \leq 0.05$).

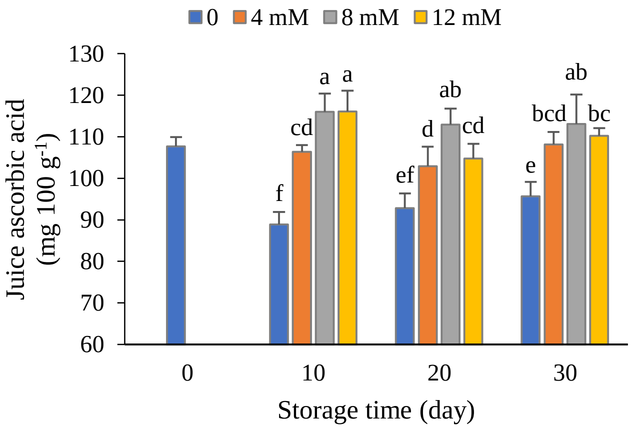

Figure 5. Changes in the juice ascorbic acid content of Mexican lime fruits during 30 days of storage at 7°C and 80 ± 5% RH. Fruits were dipped in γ-aminobutyric acid (GABA) solutions at 0, 4, 8, or 12 mM for 5 minutes prior to storage. Measurements were taken on days 10, 20, and 30. Data are presented as means of 4 replicates ± SD. Different letters indicate significant differences according to the LSD test ($P \leq 0.05$).

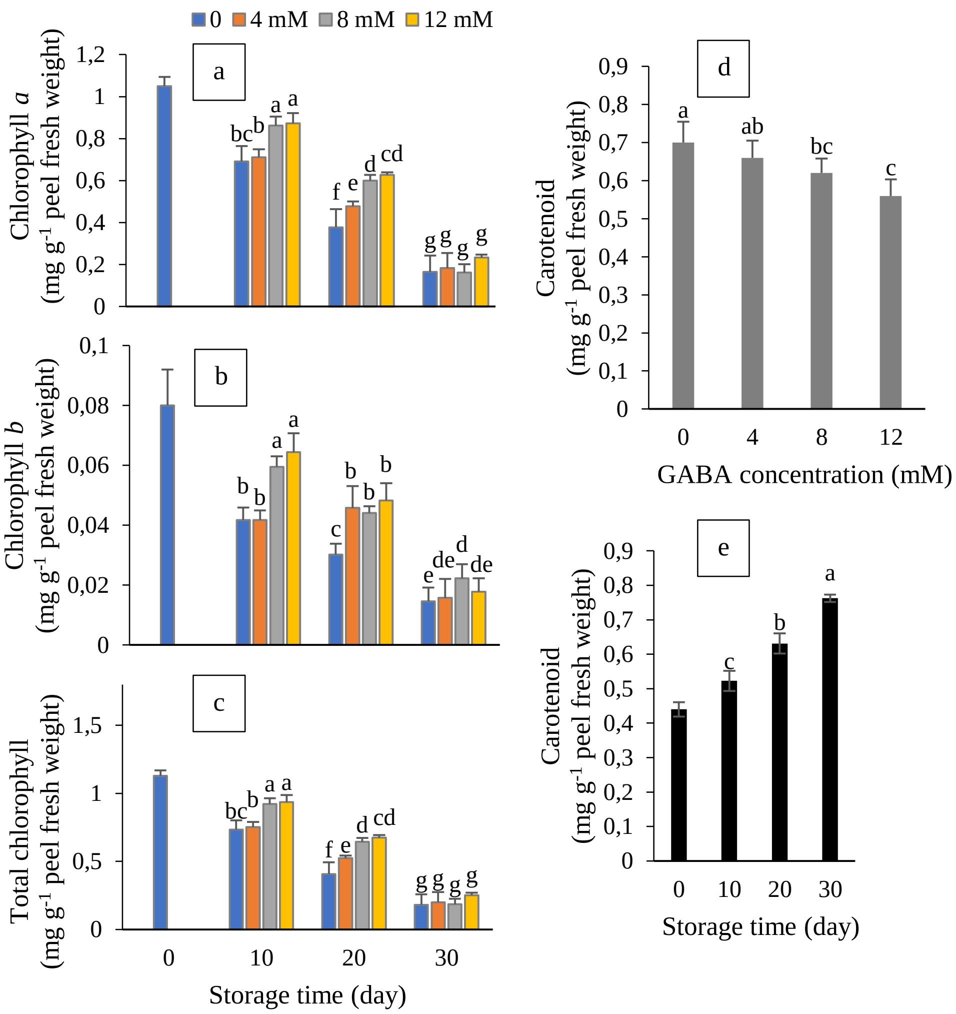

Figure 6. Changes in peel chlorophyll *a* (a), chlorophyll *b* (b), and total chlorophyll (c) of Mexican lime fruits during 30 days of storage. Changes in peel carotenoid content under the main effects of γ-aminobutyric acid (GABA) treatment (d) and storage time (e). Fruits were dipped in GABA solutions at 0, 4, 8, or 12 mM for 5 minutes prior to storage at 7°C and 80 ± 5% RH. Measurements were taken on days 10, 20, and 30. Data are presented as means of 4 replicates for (a), (b), and (c); 12 replicates for (d); and 16 replicates for (e) ± SD. Different letters indicate significant differences according to the LSD test ($P \leq 0.05$).

The substantial water loss in postharvest limes due to transpiration and respiration, particularly in hot and humid climates, limits their storage life to 6–9 days (Mohammadi et al., 2024a; 2024b). The reduced internal water content leads to increased juice TSS levels, whereas cell wall-degrading enzymes further elevate TSS levels (Zhang et al., 2024). For the Mexican lime, which is valued for its sour taste, an increase in TSS is undesirable. According to previous studies, GABA treatment enhances the postharvest quality of various horticultural crops by influencing physiological mechanisms. GABA application upregulates antioxidant enzymes, including superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT), which play a key role in scavenging reactive oxygen species (ROS) and alleviating postharvest oxidative stress. Consequently, by stabilizing cell membranes, GABA reduces electrolyte leakage and prevents cell wall degradation (Badiche-El Hilali et al., 2023; Ge et al., 2018; Sheng et al., 2017). Additionally, GABA affects crop preservation by decreasing ethylene biosynthesis, thereby reducing respiration rates and delaying senescence (Han et al., 2018; Li et al., 2021). Collectively, these effects can lead to improved water retention in fruit tissues, reducing dehydration and limiting subsequent increases in juice TSS (Badiche-El Hilali et al., 2023; Rastegar et al., 2020).

Organic acids and amino acids are crucial for citrus fruit quality, with organic acids being particularly significant (Sheng et al., 2017). Citric acid is the main organic acid in juice, whereas ascorbic acid is present in very low amounts (Sheng et al., 2017). Our results revealed an accelerated increase in titratable acidity (TA) and a higher ascorbic acid content in GABA-treated fruits compared with the control fruits during the storage period. Sheng et al. (2017) reported that exogenous GABA improves the postharvest quality and storage performance of citrus fruits by increasing citrate content through inhibiting glutamate decarboxylase and enhancing amino acid accumulation. They also found that higher antioxidant enzyme activities and ATP content in GABA-treated fruits reduced organic acid consumption during storage.

Our results indicated that the titratable acidity (TA) in the juice increased during storage, with the treated fruits exhibiting a more pronounced increase than the controls. Therefore, the increase in the TSS of the juice is likely due to weight loss rather than to the conversion of organic acids into sugars (Mohammadi et al., 2024a; 2024b).

The TSS/TA ratio reflects the changes in the juice TSS and TA. After one month of storage, fruits treated with various GABA concentrations showed a significantly lower TSS/TA ratio than controls, indicating superior quality and a better taste index.

Postharvest peel discoloration results from chlorophyll degradation and carotenoid accumulation related to fruit senescence (Mohammadi et al., 2024b). Harvest maturity affects storage life and quality, with climate influencing preharvest rind color. Warm temperatures hinder chlorophyll loss and carotenoid accumulation, while cool temperatures enhance fruit color development. Therefore, lime fruits in tropical regions remain greener compared to those in subtropical regions. Extended storage in subtropical regions generally leads to peel yellowing, reducing postharvest shelf life (CABI, 2022). Market preferences also impact peel color acceptability, namely, dark green or transitioning green is preferred for exports, while light-yellow is suitable for domestic markets. Managing fruits picked at the mature green stage is crucial for maintaining their green color, as storage conditions and treatments influence peel color. Treatments that alleviate postharvest stress and delay senescence can help preserve the green color of the peel.

Statistical results showed that GABA treatment, especially at higher concentrations, effectively preserved chlorophyll *a*, chlorophyll *b*, and total chlorophyll up to the 20th day. After one month, there was no significant difference between the treated fruits and the control fruits, except for chlorophyll *b*, where the fruits treated with 8 mM GABA had higher levels. Although carotenoid content increased over time, treatment with higher concentrations of GABA attenuated this increase. These effects may be attributed to the modulation of oxidative stress and delayed senescence by GABA (Badiche-El Hilali et al., 2023; Ge et al., 2018; Sheng et al., 2017).

A study by Sun et al. (2013) demonstrated that organic acids in citrus fruits activate the antioxidant enzymatic system under storage stress. Therefore, the positive effects of GABA on increasing TA and ascorbic acid content could enhance antioxidant activity and maintain peel color and health better in treated fruits than in controls.

Although the Mexican lime is a non-climacteric fruit, its internal ethylene concentration is related to peel color, as ethylene promotes chlorophyll degradation and color change (CABI, 2022). Thus, the ability of GABA to inhibit ethylene biosynthesis could positively influence the postharvest quality and lifespan of Mexican lime (Han et al., 2018; Li et al., 2021).

Conclusion

This study has demonstrated that GABA treatment significantly enhanced the postharvest quality of Mexican lime by reducing weight loss, improving juice quality, and delaying peel discoloration. Among the tested concentrations, 8 mM GABA was the most effective in preserving and improving postharvest quality. GABA treatment effectively minimized fruit weight loss and mitigated the increase in juice TSS, while enhancing TA and ascorbic acid content in the juice. These changes contributed to a more desirable taste index, as indicated by a lower TSS/TA ratio. Furthermore, GABA treatment delayed the degradation of chlorophyll pigments and the accumulation of carotenoids in the peel, slowing the transition to a yellow color. These findings underscore the potential of GABA as a safe treatment for extending shelf life and enhancing the quality of citrus fruits during postharvest storage. Future studies are recommended to investigate the molecular mechanisms underlying the effects of GABA, assess its efficacy in other citrus and horticultural fruits, and optimize application methods to maximize its benefits.

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POBOLJŠANJE VEKA SKLADIŠTENJA I KVALITETA MEKSIČKIH LIMETA TRETMANOM γ –AMINOBUTERNOM KISELINOM

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R e z i m e

Ključni faktori koji ograničavaju postharvest rok trajanja limete su gubitak težine, brzo žutenje kore i degradacija njene zelene boje, što značajno smanjuje tržišnu vrednost. Meksička limeta, citrusno voće od značajnog ekonomskog i nutritivnog potencijala, ocenjivana je efektima potapanja u γ-aminobuternu kiselinu (GABA) na postharvest kvalitet i vek skladištenja. Plodovi su tretirani GABA rastvorima (0, 4, 8 ili 12 mM) i čuvani na 7°C sa relativnom vlažnošću od 80 ± 5% tokom 30 dana. Merenja gubitka mase plodova, rastvorne suve materije (TSS), titracione kiselosti (TA), indeksa ukusa (TSS/TA odnos), sadržaja askorbinske kiseline u soku i pigmenata kore (hlorofil a, hlorofil b, ukupni hlorofil i karotenoidi) vršena su svakih 10 dana. GABA tretman, posebno na višim koncentracijama, značajno je smanjio gubitak mase i usporio porast TSS, dok je poboljšao nivo TA i askorbinske kiseline. Na kraju skladištenja, tretirani plodovi su pokazali viši nivo organskih kiselina i poželjniiji indeks ukusa, što je naznačeno smanjenjem TSS/TA odnosa. Pored toga, GABA tretmani su očuvali viši nivo hlorofila do 20 dana i odložili akumulaciju karotenoida, efektivno usporavajući žutenje kore. Koncentracija od 8 mM bila je najučinkovitija u očuvanju postharvest kvaliteta. Ovi rezultati ukazuju da GABA može poboljšati vek skladištenja i kvalitet meksičke limete, pružajući obećavajuću strategiju za smanjenje postharvest gubitaka i povećanje ekonomske vrednosti.

Ključne reči: *Citrus aurantifolia*, požutelost kore, indeks ukusa, vitamin C, gubitak težine.

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Acknowledgements

Acknowledgements should contain the title and the number of the project that is the title of the program within which the paper was written, as well as the name of the institution which financed the project or program. It should be placed between the conclusion and references.

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Periodicals

Gvozdenović, S., Saftić Panković, D., Jocić, S., & Radić, V. (2009). Correlation between heterosis and genetic distance based on SSR markers in sunflower (Helianthus annuus L.). *Journal of Agricultural Sciences*, *54*, 1-10.

Books

Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Book chapter

Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R. M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Proceedings

Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon, France.

Thesis

Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Report

Ballard, J. (1998). *Some significant apple breeding stations around the world*. Selah, Washington.

Web site

Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

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The summary in Serbian is given at the end of the paper and should comprise 200 to 250 words. Before the main text of the summary, as well as in English, the title of the paper, first name, middle initial(s) and last (family) name of all authors and the names and addresses of affiliations should be given. The title of the summary is centred and written separately. Below the title, the text of the summary should follow, without any indentation, and immediately after the text of the summary, the key words are given with the full stop at the end. The e-mail address of the corresponding author should be given at the bottom of the page.

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Tables numbered with Arabic numerals (1, 2, etc.), followed by the title should be placed in the text using 9 font size and a maximum width of 13 cm. They should be clear, simple and unambiguous. The vertical sections should be avoided, and the number of columns should be limited so that the table is not too wide. Also, an unnecessary usage of horizontal sections should be avoided. The title of the table, single spaced above the table, justified, and with the full stop at the end should be given. The detailed explanation of abbreviations, symbols and signs used in the table should be provided below the table. Each table must be mentioned in the text.

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All graphs, diagrams and photographs should be titled "Figure" (1, 2, etc.). They should be placed in the text. Graphs and diagrams should be computer drawn, using 9 font size and a maximum width of 13 cm, so that they can be legible and distinct after the size reduction. The overuse of colours and hues should be avoided for aesthetic reasons. The detailed legend without abbreviations for each graph and diagram should be given. The photographs must be of high quality so that they can technically be well reproduced. They should be submitted in "TIF" or "JPG" format, and they will be printed in black and white. The title of the illustration should be justified, with a full stop at the end, single spaced from the illustration and given below it. Each illustration should be mentioned in the text.

Abbreviations and units
Only standardised abbreviations should be used in the paper. Measure units should be expressed using International System of Units (SI). The abbreviations can be used for other expressions provided these expressions are stated in the full form when appear for the first time with the abbreviated form in the brackets. Values from 1 to 9 can be written in letters, but others numerically.

Nomenclature
The complete nomenclature (chemical and biochemical, taxonomical, genetic etc.) must be adjusted to international codes and commissions, such as *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* etc.

Formulae
All formulae and equations in the paper should be worked out by means of the programme "WORD Equation". An ample space should be left around the formulae for the sake of visibility. Subscripts and superscripts should be clear. Greek letters and other non-Latin symbols should be explained when they are first used. The meaning of all symbols should be given immediately after the equation where these symbols are first used. Equations should be numbered by Arabic numerals, serially in brackets, at the right-hand side. Each equation must be mentioned in the text as Eq. (1), Eq. (2), etc.

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Ključne reči
Ključne reči su termini ili fraze koje najbolje opisuju sadržaj članka za potrebe indeksiranja i pretraživanja. Broj ključnih reči može biti od 3 do 10. Navode se ispod sažetka. Naslov „Ključne reči“ piše se boldovano i uvlači jednim

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Uvod

Uvod treba da sadrži informacije o dosadašnjim istraživanjima po navedenom pitanju i šta se datim istraživanjem želi postići. Prilikom osvrta na literaturu, navesti autora i godinu, a autora citirati u spisku literature. Naslov „Uvod" piše se sa prvim velikim slovom, centrirano i boldovano, nakon čega sa jednim razmakom ispod naslova sledi tekst uvoda poravnat po levoj i desnoj margini. Svaki novi pasus uvlači se jednim tabulatorom. Ova pravila važe i za sva ostala poglavlja.

Materijal i metode

Materijal i metode treba izložiti jasno uz objašnjenje svih primenjenih postupaka u radu. Opšte poznate metode izložiti kratko, a detaljnije ih objasniti ukoliko se odstupa od ranije objavljenih postupaka. Za radove eksperimentalnog karaktera obavezno navesti način statističke obrade podataka. U ovom poglavlju, kao i u poglavlju „Rezultati i diskusija", po potrebi se mogu dati i određena podpoglavlja.

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U poglavlju „Rezultati i diskusija" interpretiraju se podaci dobijeni na osnovu zapažanja i izvršenih eksperimenata. U komentaru rezultata treba se pozivati na literaturu koja se navodi na kraju rada, čime se obezbeđuje poređenje dobijenih rezultata sa dosadašnjim saznanjima u toj oblasti.

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U zaključku treba ukratko navesti najznačajnije rezultate dobijene u radu. Izbegavati nabrajanje svih rezultata istraživanja sa ponavljanjem brojčanih vrednosti koje su prethodno već navedene u poglavlju „Rezultati i diskusija". Zaključak ne sme da sadrži reference.

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Zahvalnica treba da sadrži naziv i broj projekta, odnosno naziv programa u okviru koga je rad nastao, kao i naziv institucije koja je finansirala projekat ili program.

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Primeri navođenja referenci su sledeći:

Periodičan časopis
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Knjiga
Steel, R.G.D., & Torrie, J.H. (1980). *Principles and procedures of statistics*. New York: McGraw-Hill Book Company.

Poglavlje u knjizi
Bell, R.L., Quamme, H.A., Layne, R.E.C., & Skirvin, R.M. (1996). Pears. In J. Janick & J.N. Moore (Eds.), *Fruit breeding, Volume I: Tree and tropical fruits.* (pp. 441-514). New York: John Wiley and Sons, Inc.

Zbornik
Behera, T.K., Staub, J.E., Behera, S., Rao, A.R., & Mason, S. (2008). One cycle of phenotypic selection combined with marker assisted selection for improving yield and quality in cucumber. In M. Pitrat (Ed.), *Proceedings of the IXth EUCARPIA meeting on genetics and breeding of Cucurbitaceae* (pp. 115-121). Avignon.

Teza
Singh, N.K. (1985). *The structure and genetic control of endosperm proteins in wheat and rye*. University of Adelaide.

Izveštaj

Ballard, J. (1998). *Some significant apple breeding stations around the world.* Selah, Washington.

Veb sajt

Platnick, N.I. (2010). The world spider catalog, version 10.5. *American Museum of Natural History*. Retrieved February 12, 2016, from http://research.amnh.org/entomology/spiders/catalog/index.html

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Tabele

Tabele obeležene arapskim brojevima (1, 2, itd.) praćene naslovom treba da se nalaze na odgovarajućem mestu u tekstu, u fontu 9. Maksimalna širina tabela treba da bude 13 cm. One treba da budu jasne, što jednostavnije i pregledne. Treba izbegavati vertikalne crte, a broj kolona ograničiti tako da tabela ne bi bila preširoka. Takođe, treba izbegavati nepotrebnu upotrebu horizontalnih crta. Naslov tabele, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom iznad tabele. Ispod tabele treba dati detaljno objašnjenje skraćenica, simbola i znakova korišćenih u samoj tabeli. Svaka tabela mora biti pomenuta u tekstu.

Ilustracije

Svi grafikoni, dijagrami i fotografije treba da se nazovu „Slika" (1, 2, itd.). Prilažu se na odgovarajućem mestu u tekstu. Grafikone i dijagrame treba uraditi fontom 9, u crno-beloj tehnici i sa maksimalnom širinom od 13 cm. Voditi računa da oni budu čitki i jasni i nakon redukcije veličine. Za svaki grafikon i dijagram treba obezbediti detaljnu legendu bez skraćenica. Fotografije moraju biti visokog kvaliteta da bi se tehnički mogle dobro reprodukovati. Prilažu se u „TIF" ili „JPG" formatu, u crno-beloj tehnici. Naslov ilustracije, poravnat po levoj i desnoj margini, sa tačkom na kraju, navodi se sa jednim razmakom ispod ilustracije. Svaka ilustracija mora biti pomenuta u tekstu.

Skraćenice i jedinice

U radu treba koristiti samo standardne skraćenice. Merne jedinice treba izražavati u internacionalnom sistemu jedinica (SI). Kod navođenja jedinica posle broja treba da stoji razmak (osim za % i °C). Skraćenice se mogu koristiti i za druge izraze pod uslovom da se ti izrazi navedu u punom obliku prilikom prvog pominjanja, sa skraćenim oblikom u zagradi. Vrednosti od 1 do 9 mogu se izražavati slovima, a ostali brojevi isključivo numerički.

Nomenklatura

Celokupna nomenklatura (hemijska i biohemijska, taksonomska, genetička itd.) mora biti usklađena sa međunarodnim kodeksima i komisijama, kao što su *International Union of Pure and Applied Chemistry, IUPAC-IUB Combined Commission on Biochemical Nomenclature, Enzyme Nomenclature, International Code of Botanical Nomenclature, International Code of Nomenclature of Bacteria* itd.

Formule

Sve formule i jednačine u radu moraju biti urađene pomoću programa „Word Equation". Pri pisanju formula, radi preglednosti, ostaviti dovoljno praznog prostora oko same formule. Subskripti i superskripti treba da budu jasni. Prilikom pisanja jednačina treba dati smisao svih simbola odmah posle jednačine u kojoj se simbol prvi put koristi. Jednačine treba da budu numerisane arapskim brojevima, serijski u zagradama, na desnoj strani linije. Svaka jednačina mora biti pomenuta u tekstu kao Eq. (1), Eq. (2), itd.

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