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ÉLETTUDOMÁNYI EGYETEM

**HUNGARIAN UNIVERSITY OF AGRICULTURE
AND LIFE SCIENCES**

**Effect of Different Cultivation Technologies and
Nutrient Supply on the Yield and Phytonutrient
Composition of Various Sweet Potato Cultivars**

PhD. THESIS

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1. Background and objectives

Sweet potato (*Ipomoea batatas* (L.) Lam.), also known as batata, ranks among the top ten most important food crops in the world according to the global FAO ranking (FAO, 2024). It plays a particularly crucial role in tropical and subtropical regions, serving as a staple food source in many developing countries due to its high polyphenol and carotenoid content. However, its significance is growing in developed countries as well, where consumer preferences are moving in the direction of healthier food options (functional foods). Beyond its positive nutritional and gastronomic attributes, sweet potato is also valuable for its broad applicability in animal feed and industrial uses.

Globally, sweet potato production has reached substantial volumes, exceeding 92 million tons in recent years, with China accounting for approximately half of this amount (FAO, 2024). The largest cultivation areas are found in Asia, but Africa, the Americas, and Oceania also contribute significantly. Although sweet potato is grown on a smaller scale in Europe, countries such as Portugal and Spain are prominent suppliers for the European internal market. In Hungary, sweet potato cultivation has gained increasing popularity in recent years, partly due to rising consumer awareness of healthy diets and a growing interest in sustainable farming practices. Additionally, changing climatic conditions have made cultivation more effective, enhancing its competitiveness compared to other tuber crops in Hungary.

Despite the decades-long history of batata cultivation in Hungary, current production technologies are not yet standardized, and growers continue to face multiple challenges. There is a need to expand site- and variety-specific research to identify optimal agrotechnical solutions and develop technologies that correspond with the crop's ecological requirements. The first significant domestic efforts to introduce batata date back to the 1910s (Surányi, 1916), but more substantial cultivation experiments began in the second half of the 20th century, notably through the work of Lajos Horváth, who laid the foundation for Hungarian production techniques with his trials in Tápiószele. In recent years, researchers at the Faculty of Agriculture of the University of Szeged and the University of Debrecen have also conducted practical, technology-oriented studies and breeding activities to promote the local adaptation of the crop (Szarvas, 2021).

For effective cultivation, the application of proper agrotechnical practices is essential. These include soil preparation, the quality of planting material, the evaluation of different cultivation methods, and the development of appropriate

nutrient supply and fertilizer strategies. All these factors significantly influence both yield quantity and quality. Sweet potato varieties exhibit distinct physiological and agronomic traits, and examining these characteristics is crucial for successful site-specific production. With this research, I aimed to contribute to improving batata cultivation in Hungary by evaluating the physiological and compositional traits of different varieties under varying production technologies. Particular attention was given to carotenoids and anthocyanins as bioactive compounds with notable antioxidant properties and nutritional importance. The findings of this dissertation aim to support domestic producers in developing optimal cultivation guidelines and promote the wider adoption of sweet potato as a sustainable, healthy, and nutritious food crop in Hungary.

The primary objective of this research was to examine how different cultivation techniques specifically ridge and flat planting methods, as well as varying plant densities (single and double row) affect the yield and the carotenoid and anthocyanin content of orange-fleshed Beauregard and Ásotthalmi 12, purple-fleshed Purple, and white-fleshed Murasaki-29 batata cultivars. Furthermore, the study investigated how cultivation method, plant density, and nutrient supply influence the physiological parameters of the plants, with particular focus on relative chlorophyll content (SPAD index), chlorophyll fluorescence (Fv/Fm), a key parameter of photosynthetic efficiency, and the normalized difference vegetation index (NDVI). Another aim was to explore the relationships among these physiological parameters, yield, and the levels of carotenoids and anthocyanins. The study also sought to identify and quantify polyphenol, carotenoid, and anthocyanin compounds in different cultivars using HPLC analysis. A key objective of the dissertation was to evaluate the interactive effects of different potassium-, magnesium sulfate-, and sulfur-based basal and liquid fertilization strategies on the yield and bioactive compound content of the Beauregard and Purple cultivars.

2. Material and methods

The experimental work consisted of two main research components. The first part aimed to evaluate the agronomic performance of various batata cultivars under flat and ridge cultivation systems, combined with different plant densities. This study was conducted during the 2019–2020 growing seasons. The second part, carried out in 2021–2022, involved a comparative analysis of the efficiency of different predominant potassium fertilization methods, also including multiple sweet potato cultivars Beauregard and Purple.

2.1 Research location and Environmental Conditions

2.1.1 Soil Characteristics of the Experimental Site

The experimental field was located in Heves, Hungary (GPS: 47°37'12.7" N, 20°13'42.4" E). Based on the soil profile, the area is salt-free and lime-deficient, and falls under the coarse sand texture category. The soil type is skeletal soil (main type), shifting sand (type), and non-carbonate shifting sand (subtype). The humus content is very low, and pH varies from slightly acidic at the surface to slightly alkaline in deeper layers. Organic matter content is below 1%.

2.1.2 Climatic Conditions at the Experimental Site (2019–2022)

Meteorological data for the experimental site were analyzed based on data provided by HungaroMet. Significant inter-annual differences in weather conditions were observed. The year 2019 was warm and highly variable in precipitation, with 202.3 mm of rainfall and an average growing season temperature of 19.4 °C. In 2020, rainfall distribution was spatially and temporally inconsistent, with 348.5 mm during the growing season and an annual average temperature of 11.5 °C. In 2021, cooler early-season temperatures slowed initial development, but warm summer conditions and October warming improved harvest conditions; however, total rainfall remained low (118.8 mm). The 2022 season was extremely hot and dry (55.7 mm during the growing period), which significantly affected conditions, though sweet potato's heat tolerance partially compensated for the stress.

2.2 Effect of Cultivation Method and Plant Density on Different Sweet Potato Cultivars (2019–2020)

This part of the study evaluated the agronomic performance of four sweet potato cultivars: the internationally known Beauregard and Murasaki-29, and the Hungarian-bred Ásotthalmi 12 and Purple. The aim was to determine the effect of different cultivation methods and planting densities on yield and polyphenol and carotenoids parameters.

Two cultivation methods were used: flat-bed (F) and ridge planting (R), combined with two plant densities: single row (S) at 17,500 plants/ha and twin row (T) at 35,000 plants/ha. For Murasaki-29, only ridge cultivation was used, as field observations showed significant tuber epidermis damage during harvest under flat conditions. The experiment was set up with four replications, each plot containing 200 plants. The growing season lasted 120 days in both years.

2.3 Methodology of Field Trials for the different Nutrient Supplementation Experiment (2021–2022)

This experiment was conducted at the same site as the previous trial, in 2021 and 2022. A three-factorial design was used: the first factor was cultivar (Beauregard and Purple), the second was different N-P-K basal fertilization levels, and the third was liquid fertilization (plots with and without supplemental nutrient solution). The trial was replicated four times, with 200 sweet potato plants per plot. The active ingredient quantities applied to each treatment are listed in Table 1.

Table 1 The total amount of active substances fertilizer applied in the treatment

Field Experiment	Code	Pre-Planting Fertilizer (Total kg/ha)			Liquid Fertilizer (Total kg/ha)		
Variety		K ₂ O	N	P ₂ O ₅	K ₂ O	MgSO ₄	SO ₃
Experiment I							
Beauregard	BP-I	120	33	46	0	0	0
Beauregard	BL-I	120	33	46	797	33	66
Beauregard	BC	0	0	0	0	0	0
Experiment II							
Beauregard	BP-II	263	73	101	0	0	0
Beauregard	BL-II	263	73	101	797	33	66
Beauregard	BC	0	0	0	0	0	0
Experiment III							
Purple	LP-I	120	33	46	0	0	0
Purple	LL-I	120	33	46	797	33	66
Purple	LC	0	0	0	0	0	0
Experiment IV							
Purple	LP-II	263	73	101	0	0	0
Purple	LL-II	263	73	101	797	33	66
Purple	LC	0	0	0	0	0	0

2.4 Plant Physiological Measurements

During the growing season, the physiological condition of the sweet potato stands was assessed through chlorophyll fluorescence, relative chlorophyll content (SPAD index), and leaf reflectance measurements. SPAD measurements were performed using a SPAD 502 Plus meter on young but fully expanded upper canopy leaves, with 20 measurements per plot, aligned with the four-replication layout.

Chlorophyll fluorescence (Fv/Fm) was measured after dark adaptation using a PAM-2500 fluorometer, capturing a key parameter of photosynthetic efficiency. Measurements were taken near midday with 20 replicates per plot.

Leaf reflectance of the sweet potato canopy was measured using an ASD FieldSpec Handheld 2 Portable Spectroradiometer (Malvern Panalytical), on young, fully developed upper leaves. The device operates across the 325–1075 nm wavelength range. Four measurements were taken per plot.

2.5 HPLC-Analytical Measurements

Carotenoids and phenolic compounds were extracted and quantified using high-performance liquid chromatography (HPLC). For each treatment, 20 tubers were sampled, representing an average size range of 250–700 g. Carotenoid extraction followed the protocol of Daood et al. (2014), using a methanol–n-hexane mixture, with detection on a C30 column using a diode array detector (DAD). Compounds were identified based on reference standards and literature data. β -carotene yield was calculated in kg/ha based on concentration and tuber yield.

Phenolic compounds were extracted using a phosphoric acid–ethanol solution, and HPLC analysis was conducted on a C18 column with 1% phosphoric acid and acetonitrile gradient elution. Quantification was based on external standards; anthocyanins were expressed in delphinidin chloride equivalents. Total anthocyanin yield was also calculated in kg/ha based on concentration and total yield.

2.7 Statistical Analysis

Data collected from the experiments and treatment effects were analyzed using ANOVA. Tukey's test was applied for multiple comparisons of treatment means. Linear relationships between variables were assessed using Pearson correlation analysis. Graphs were generated using Scimago Graphica (v1.0.42, SCImago Lab, Granada, Spain), Microsoft Excel (v2108), and the 'metan' package in RStudio.

The effects of cultivation method and plant density on yield, compositional parameters, Fv/Fm, and SPAD values were evaluated using two- and three-way ANOVA to determine interaction effects. Tukey post hoc tests were applied for mean comparisons. Principal component analysis (PCA) was used to assess the relationships among SPAD, Fv/Fm, and the concentrations of key carotenoids and anthocyanins. Pearson correlation analysis was also applied to evaluate the association between physiological parameters measured at different time points and yield or SPAD values.

3. Results and Discussion

3.1. Agronomic Evaluation of the Beauregard

3.1.1 Effect of Different Cultivation Methods and Plant Density on the Yield of Beauregard

The RT treatment (ridge cultivation, twin-row planting) produced significantly higher yields for the Beauregard cultivar in both growing seasons. In 2019, the average yield was 38.79 t/ha, while in 2020 it was 40.87 t/ha. This indicates that the RT treatment resulted in 44%, 13%, and 42% higher yields in 2019 compared to the RS, FT, and FS treatments, respectively. Moreover, in the 2020 growing season, the RT treatment outperformed the RS, FT, and FS treatments by 61%, 55%, and 80%, respectively (Figure 1).

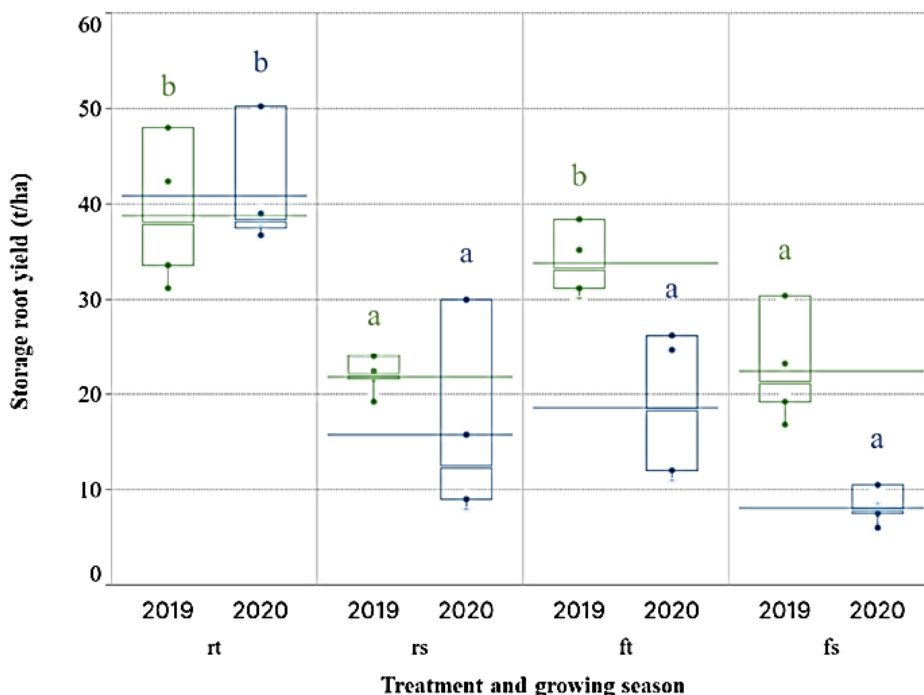


Figure 1. Effect of different cultivation technologies on the yield of Beauregard cultivar. Abbreviations: R – ridge, F – flat, T – twin row, S – single row. Color coding: green – 2019 growing season; blue – 2020 growing season. Different letters within each year indicate statistically significant differences between treatments ($p < 0.05$).

3.1.2 Effect of Cultivation Technology and Plant Density on Plant Physiological Changes in Beauregard

The highest SPAD values, indicating relative chlorophyll content, were recorded in RT treatments during both years, particularly in the first half of the season. SPAD values measured in 2020 were higher across all treatments compared to those from 2019, suggesting a favorable annual climatic effect.

In terms of chlorophyll fluorescence (F_v/F_m) in 2019, RS and RT treatments showed higher values. However, in the 2020 experiment, the differences between cultivation methods were less pronounced. These results indicate that plant density had a more substantial influence on F_v/F_m values than the cultivation method. The highest values were recorded in RT treatments in both years.

3.1.3 Analytical Determination of Polyphenol and Carotenoid Profile in Beauregard Sweet Potato

HPLC analysis of the Beauregard cultivar identified 11 carotenoids and several significant polyphenolic compounds. β -carotene was the dominant carotenoid, serving as the primary provitamin A with outstanding nutritional relevance. Other provitamin A-active compounds (e.g., α - and β -cryptoxanthin), as well as isomers and epoxidized forms formed by heat, light, or oxidation, were also present, potentially affecting bioavailability (Figure 2). Among the polyphenols, chlorogenic acid was predominant, alongside various caffeoyl- and feruloylquinic conjugates, and higher molecular weight di-esters (Figure 3). These compounds, especially hydroxycinnamic acid derivatives, possess broad antioxidant, antimicrobial, antidiabetic, and neuroprotective properties, playing important roles in the prevention of chronic diseases (Silva et al., 2015)

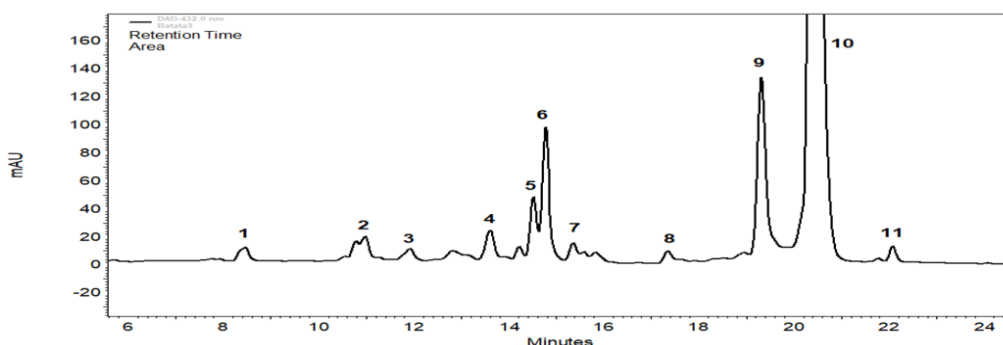


Figure 2 HPLC profile of carotenoids extracted from Beauregard sweet potato. 1: Mutatoxanthin, 2: Luteoxanthin, 3: Auroxanthin, 4: Apo-carotenoid, 5: β -carotene-epoxide, 6: α -cryptoxanthin, 7: β -cryptoxanthin, 8: ζ -carotene, 9: *cis*- β -carotene, 10: β -carotene, 11: γ -carotene.

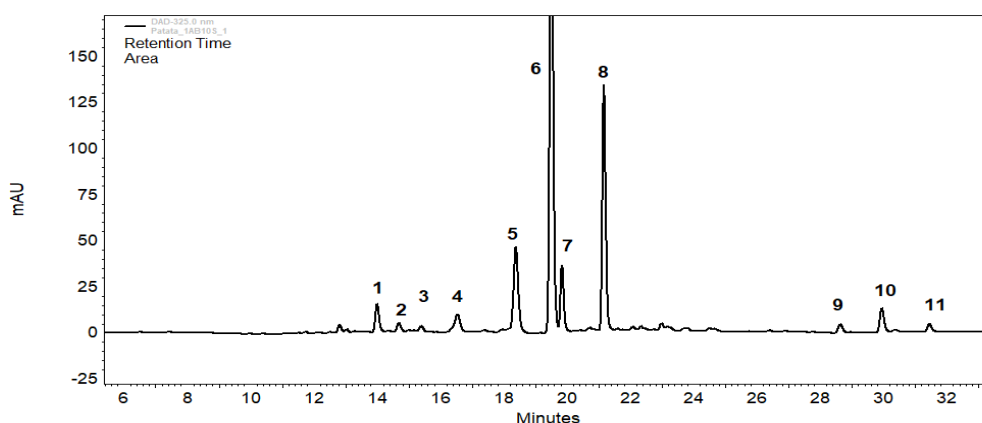


Figure 3. HPLC profile of polyphenols extracted from Beauregard sweet potato. 1: feruloylquinic acid, 2: caffeic acid, 3: coumaric acid, 4 & 5: caffeoylquinic acid isomers, 6: chlorogenic acid, 7: di-caffeoylquinic acid, 8: di-feruloylquinic acid, 9: di-caffeoyl glucoside, 10: di-caffeoyl, 11: di-feruloyl.

3.1.4 Effect of Cultivation Methods and Plant Density on Carotenoid Content in Beauregard Sweet Potato

The levels of minor carotenoids, such as cis- β -carotene and ζ -carotene, did not show significant differences across cultivation treatments. Similar findings have been reported in other studies where planting density and cultivation method did not significantly affect cis- β -carotene or ζ -carotene levels (Islam et al., 2016).

In single-row setups, β -carotene content remained similarly high. Compared to the RS treatment with the highest carotenoid concentration, the RT and FT treatments showed 19% and 31% lower concentrations, respectively.

In the 2020 growing season, the highest β -carotene and total carotenoid levels were observed in single-row ridge treatments, consistent with the previous year. The RS treatment exceeded RT, FT, and FS treatments by 12%, 6%, and 15% in β -carotene content, respectively.

Overall, the two-year analysis suggests that the examined treatment combinations significantly influenced carotenoid parameters important for food quality, with plant density being the more decisive factor. Single-row ridge cultivation produced the highest carotenoid levels in both years (Table 2). Interestingly, the RT treatment yielded the highest β -carotene output per plant in both years, despite the RS samples having the highest concentration.

Table 2. Effect of different cultivation methods and plant density on the carotenoid content of sweet potato tubers ($\mu\text{g/g}$). Different letters within the same growing season indicate statistically significant differences between treatments ($p < 0.05$); ns = not significant.

2019	β -carotene	cis- β -carotene	ζ -carotene	total carotenoid
FS	244,5 \pm 37,6 a	16,5 \pm 17,7 a	1,7 \pm 0,2 a	283,9 \pm 21,4 ab
FT	170,9 \pm 17,0 b	14,0 \pm 3,3 a	1,0 \pm 0,2 a	202,0 \pm 16,0 c
RS	247,0 \pm 2,0 a	21,7 \pm 7,9 a	1,4 \pm 0,6 a	297,4 \pm 18,2 a
RT	200,9 \pm 34,7 ab	18,4 \pm 3,1 a	1,5 \pm 0,4 a	240,8 \pm 36,2 bc
P-value	<0,01	ns	ns	<0,001
2020	β -carotene	cis- β -carotene	ζ -carotene	total carotenoid
FS	204,6 \pm 17,0 a	8,8 \pm 2,5 a	1,3 \pm 0,3 a	232,0 \pm 19,6 a
FT	232,6 \pm 9,6 a	15,23 \pm 2,4 a	1,7 \pm 0,3 a	262,8 \pm 9,0 a
RS	242,1 \pm 32,2 a	11,6 \pm 7,6 a	1,6 \pm 1,1 a	276,1 \pm 37,9 a
RT	212,1 \pm 6,0 a	10,6 \pm 3,3 a	1,5 \pm 0,2 a	239,2 \pm 6,7 a
P-value	ns	ns	ns	ns

3.1.5 Effect of Cultivation Method, Plant Density, and Year on Yield and Compositional Parameters of the Beauregard Cultivar

Based on the variance analysis (ANOVA), yield, β -carotene and total carotenoid content, as well as physiological parameters, were significantly affected by plant density, cultivation method, and year effect. Among these, plant density emerged as the most influential factor, significantly impacting not only yield but also compositional and physiological traits such as SPAD index and Fv/Fm.

Cultivation method primarily influenced yield and photosynthetic performance, while year-to-year differences were attributed to climatic variability. Detailed results of the ANOVA test are presented in Figure 4.

Factor	Yield	β -carotene	total carotenoid	β -carotene yield	cis- β -carotene	ζ -carotene	Fv/FM	SPAD
Year effect	**	n.s.	n.s.	**	*	n.s.	**	***
Cultivation method	***	n.s.	*	***	n.s.	n.s.	***	n.s.
Plant density	***	***	***	***	n.s.	n.s.	**	***
Year \times Cultivation method	**	n.s.	n.s.	**	n.s.	n.s.	***	n.s.
Year \times Plant density	n.s.	**	***	**	n.s.	n.s.	n.s.	.
Cultivation method \times Plant density	*	n.s.	n.s.	*	n.s.	n.s.	n.s.	*
Year \times Cultivation method \times Plant density	n.s.	*	**	n.s.	n.s.	n.s.	n.s.	n.s.

Legend:

- *** $p < 0,001$
- ** $p < 0,01$
- * $p < 0,05$
- . $p < 0,1$ (marginal significant)
- n.s. non significant ($p \geq 0,1$)

Figure 4. Effects of different cultivation technologies on the compositional quality, yield, and physiological parameters of the Beaugard cultivar – ANOVA results.

3.2 Results of the Agronomic Evaluation of the Ásotthalmi-12 Cultivar

3.2.1 Effect of Different Cultivation Methods and Plant Density on Yield Performance

The effect of four different cultivation methods on the average yield per hectare across the two experimental years is presented in Figure 5.

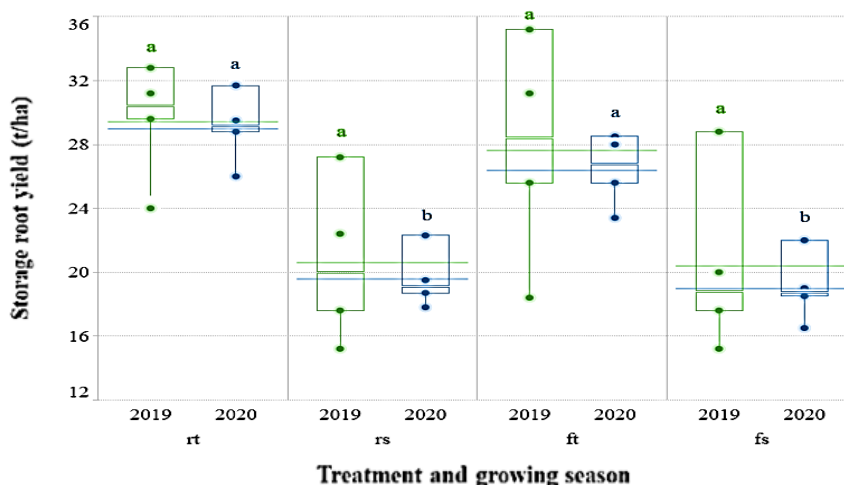


Figure 5. Effect of different cultivation technologies on the yield of Ásotthalmi-12 sweet potato cultivar (green: 2019, blue: 2020). Abbreviations: R – ridge, F – flat, T – twin row, S – single row. Different letters within each growing season indicate statistically significant differences between treatments ($p < 0.05$).

In 2019, no statistically significant differences were observed between treatments. However, higher average yields were recorded in twin-row plantings, regardless of cultivation method. The highest average yield was observed in the RT treatment at 29.4 t/ha, which was 30.61% higher than the lowest yield on FS treatment.

In 2020, the RT and FT twin-row systems also outperformed the RS and FS single-row treatments, confirming the pattern observed in 2019. In this year, the RT treatment again produced the highest yield (29 t/ha), which was 9.5%, 31.9%, and 32.5% higher than the FT, FS, and RS treatments, respectively.

However, literature also includes studies reporting contradictory results regarding the advantage of ridge cultivation. For instance, one agronomic study found that flat-bed cultivation yielded on average 26% more than ridge planting. The same study also noted a positive effect of increased planting density, where increasing plant population from 33 333 to 44 444 plants/ha resulted in a comparable average yield increase of 44% (Pepó, 2020). Similarly, another Hungarian study conducted in Deszk reported that flat cultivation resulted in a significantly 10% higher yield (Szarvas A., 2021).

3.2.2 Effect of Cultivation Technology and Plant Density on Plant Physiological Changes

3.2.2.1 Relative Chlorophyll Content (SPAD Index)

Across both years, the highest SPAD values for the Ásotthalmi-12 cultivar were measured in the RT treatment (which also produced the highest yield), while the lowest values were observed in the FS combination. A domestic study showed similar results: SPAD values of the Ásotthalmi-12 sweet potato correlated strongly with yield, particularly based on measurements taken in the first half of the growing season (Pepó, 2020).

3.2.2.2 Chlorophyll Fluorescence measurement result in Ásotthalmi-12

The seasonal pattern of Fv/Fm values in Ásotthalmi-12 showed similarities to those recorded in the Beauregard cultivar. The highest Fv/Fm values were observed under RT treatment across both growing seasons for both cultivars. In 2019, ridge cultivation proved more favorable for both cultivars. In 2020, the RT treatment again produced the highest Fv/Fm values, followed by FT in both cultivars. Another study (Yakubu et al., 2024) also found ridge cultivation superior in terms of physiological performance compared to flat-bed systems.

3.2.3 Analytical Determination of Polyphenol and Carotenoid Profiles in Ásotthalmi-12

HPLC analysis enabled detailed identification of the carotenoid and polyphenol profiles of the Ásotthalmi-12 sweet potato cultivar. Between 8–30 minutes, nine carotenoid peaks were observed in the chromatogram. The dominant peak at

minute 25 indicated the presence of β -carotene. Additional compounds included epoxidized and cis-isomer forms, indicating the cultivar's high nutritional value (Figure 6).

The polyphenol profile also revealed nine distinct peaks, with chlorogenic acid being dominant. Other compounds included various mono- and di-esters of caffeoyl- and feruloylquinic acids, and glucose-bound conjugates. The complex polyphenolic structure of the *Asotthalmi-12* cultivar provides particularly valuable antioxidant potential (Figure 7).

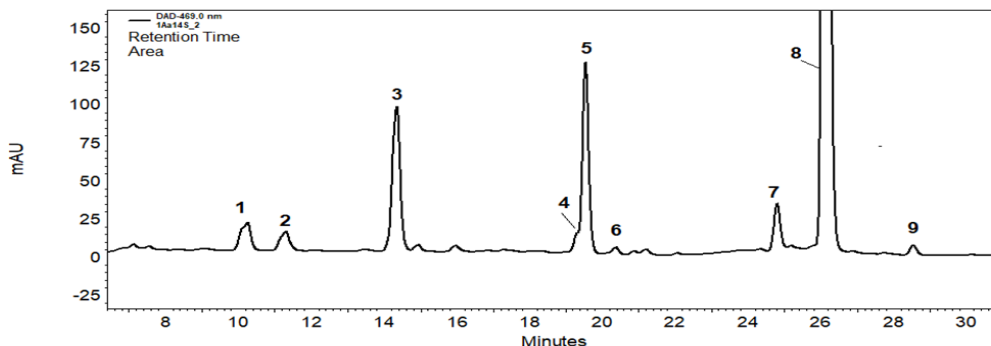


Figure 6. HPLC profile of carotenoids extracted from *Asotthalmi-12* sweet potato: 1 – Mutatoxanthin, 2 – Luteoxanthin, 3 – β -carotene diepoxide, 4 – β -carotene epoxide, 5 – α -cryptoxanthin, 6 – β -cryptoxanthin, 7 – cis- β -carotene, 8 – β -carotene, 9 – γ -carotene.

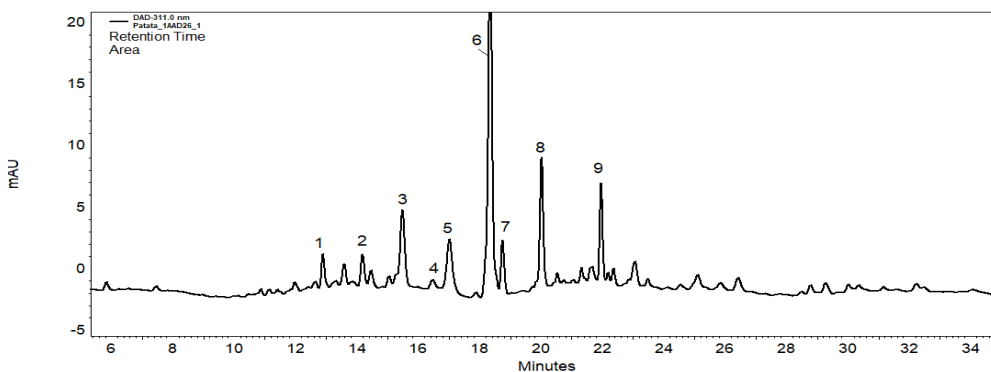


Figure 7. HPLC profile of polyphenols extracted from *Asotthalmi-12*: 1 – feruloylquinic acid, 2 – caffeic acid, 3 – coumaric acid, 4–5 – caffeoylquinic acid isomers, 6 – chlorogenic acid, 7 – di-caffeoylquinic acid, 8 – di-feruloylquinic acid, 9 – di-caffeoyl-glucoside.

3.2.3 Effect of Cultivation Method and Plant Density on the Carotenoid Content of *Asotthalmi-12* Sweet Potato

Based on the results from the two study years, single-row cultivation led to significantly higher β -carotene and total carotenoid content compared to twin-row arrangements, regardless of the cultivation method. The higher phytochemical values were primarily associated with lower plant density, while the cultivation method (flat or ridge) did not have a significant impact on phytonutrient

composition. For minor carotenoids such as *cis*- β -carotene and ζ -carotene, neither factor caused statistically significant differences.

On the other hand, treatments resulting in higher yields—such as the RT treatment—were associated with lower carotenoid content, suggesting an inverse relationship between yield and bioactive compound concentration (Table 3).

Table 3. Effect of cultivation method and plant density on the carotenoid content ($\mu\text{g/g}$) of *Ásothalmi-12* sweet potato tubers. Different letters within each growing season indicate statistically significant differences between treatments ($p < 0.05$); ns = not significant.

2019	β -carotene	<i>cis</i> - β -carotene	ζ -carotene	total carotenoid
RT	87,5 \pm 17,2 a	11,9 \pm 13,1	1,2 \pm 0,3	126,1 \pm 28,1 a
RS	142,7 \pm 16,3 b	3,2 \pm 0,5	1,4 \pm 0,5	181,5 \pm 17,1 b
FT	92,8 \pm 14,1 a	5,9 \pm 1,6	1,3 \pm 0,2	125 \pm 16,4 a
FS	133,7 \pm 14,6 b	3,6 \pm 0,8	1,9 \pm 0,7	175,5 \pm 20,1 b
<i>P</i> -value	<0,05	ns	ns	<0,05
2020	β -carotene	<i>cis</i> - β -carotene	ζ -carotene	total carotenoid
RT	94,5 \pm 17,1 a	4,8 \pm 13,1	1,6 \pm 0,3	113,6 \pm 19,8 a
RS	171,2 \pm 25,5 b	3,1 \pm 0,5	2,2 \pm 0,6	184,8 \pm 23,4 b
FT	112,7 \pm 32,6 ab	3,3 \pm 2,3	1,5 \pm 0,6	127,4 \pm 31,9 ab
FS	151,1 \pm 37,1 ab	2,3 \pm 0,8	2,1 \pm 0,7	171 \pm 35,8 ab
<i>P</i> -value	<0,05	ns	ns	<0,05

3.2.4 Effect of Cultivation Method, Plant Density, and Year on the Yield and Nutritional Parameters of the *Ásothalmi-12* Cultivar

The yield of the *Ásothalmi-12* sweet potato cultivar was significantly influenced by plant density ($p < 0.001$), with twin-row planting resulting in higher yields compared to single-row configurations. Cultivation method and year did not show a statistically significant effect on yield.

Plant density also had a significant influence on β -carotene and total carotenoid content ($p < 0.001$), with higher values recorded in single-row treatments.

The SPAD index was most strongly affected by year ($p < 0.001$), followed by cultivation method ($p < 0.01$) and plant density ($p < 0.05$). In the case of chlorophyll fluorescence (*Fv/Fm*), both cultivation method and plant density had significant effects ($p < 0.01$).

Interaction effects indicated that yield and compositional parameters were shaped by independent factors, whereas plant physiological parameters were influenced by the combined effects of year and plant density (Table 8.). Literature findings (Pepó, 2020) confirm the significant role of year in shaping SPAD values and yield outcomes.

Factor	Yield	β -carotene	total carotenoid	β -carotene yield	cis- β -carotene	ζ -carotene	Fv/FM	SPAD
Year effect	n.s.	*	n.s.	.	n.s.	*	*	***
Cultivation method	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	**	**
Plant density	***	***	***	n.s.	.	*	**	*
Year \times Cultivation method	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Year \times Plant density	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	*	***
Cultivation method \times Plant density	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Year \times Cultivation method \times Plant density	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Legend:

- *** $p < 0,001$
- ** $p < 0,01$
- * $p < 0,05$
- . $p < 0,1$ (marginal significant)
- n.s. non significant ($p \geq 0,1$)

Figure 8. ANOVA results showing the effects of different cultivation methods on the compositional quality, yield, and physiological parameters of the *Ásothalmi-12* cultivar.

3.3 Results of the Agronomic Evaluation of the Purple Cultivar

3.3.1 Effect of Different Cultivation Methods and Plant Density on Yield Performance

The impact of four different cultivation methods on the average yield per hectare for the Purple cultivar across the two experimental years is shown in Figure 9.

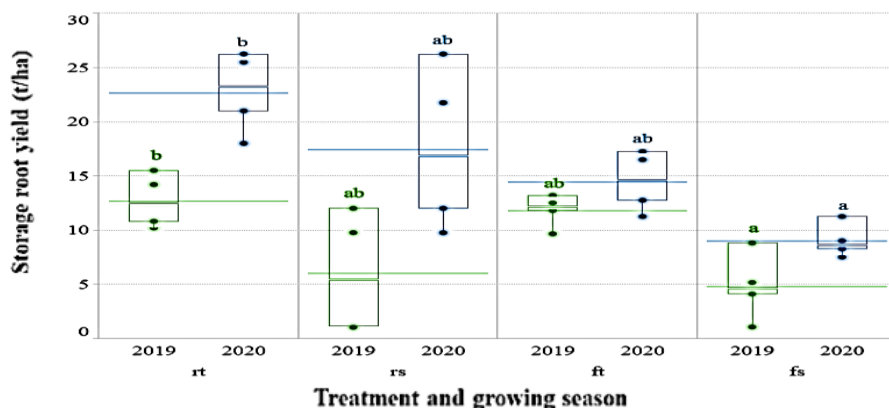


Figure 9. Effect of different cultivation methods on the yield of the Purple sweet potato cultivar (green: 2019, blue: 2020). Abbreviations: R – ridge, F – flat, T – twin row, S – single row. Different letters within each growing season indicate statistically significant differences between treatments ($p < 0.05$).

Unlike the other cultivars, the Purple variety achieved considerably better yields during the 2020 growing season than in 2019, which had a higher mean temperature. In 2019, the average yield was 8.80 t/ha, while in 2020 it nearly doubled to 15.89 t/ha. Despite this improvement, the Purple cultivar consistently produced lower average yields compared to the orange-fleshed varieties (Beauregard and *Ásothalmi-12*).

Taking both years into account, the RT treatment produced significantly higher yields than all other treatments, with 12.68 t/ha in 2019 and 22.69 t/ha in 2020.

4.3.2 Effect of Cultivation Technology and Plant Density on Plant Physiological Changes

For the Purple cultivar, no statistically significant differences in SPAD index values were observed between the different cultivation methods. SPAD values showed high variability.

The year effect had a notable influence on Fv/Fm values. However, there were no statistically significant differences between average Fv/Fm values of the various treatments within the same year. Similar findings were reported in previous studies, where 18.5–55.1% of variation in Fv/Fm values was attributed to environmental factors rather than cultivation method (Krochmal-Marczak et al., 2019).

4.3.3 Analytical Determination of the Anthocyanin Profile of Purple Sweet Potato

HPLC analysis of the anthocyanin profile of the Purple sweet potato identified 17 compounds, mainly built on cyanidin (Cy), peonidin (Peo), and pelargonidin (Pg) aglycones. These were conjugated with various sugar residues (glucose, sophoroside) and organic acids (p-hydroxybenzoic acid, caffeic acid, ferulic acid), often occurring in acylated forms (Figure 10).

The acylated anthocyanins, especially those based on cyanidin and peonidin glycosides, exhibited high chemical stability and antioxidant activity. These compounds contribute to the scavenging of free radicals and exhibit anti-inflammatory effects, which enhances the functional food value of sweet potato.

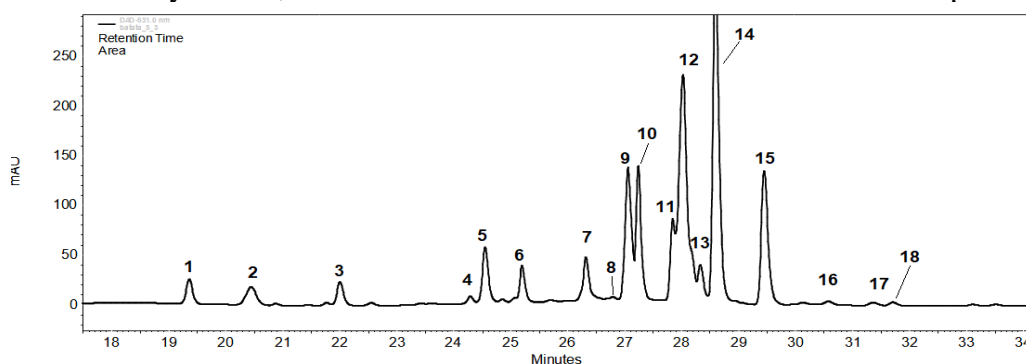


Figure 10. HPLC profile of anthocyanins extracted from Purple sweet potato. Abbreviations: Cy – cyanidin; Pg – pelargonidin; Peo – peonidin; p-hb – p-hydroxybenzoic acid; caf – caffeic acid; fer – ferulic acid; soph – sophoroside; glc – glucose; rt – rutinoside.

1. peak: Cy3-soph-5-glc; **2. peak:** Cy3-rt-5-glc; **3. peak:** Cy3-p-hb-soph-5-glc; **4. peak:** Cy3-(6'''-caf soph)-5-glc; **5. peak:** Pg-3-soph-5-glc; **6. peak:** Peo3-caf soph-5-glc; **7. peak:** Pg3-caf soph-5-glc; **8. peak:** Cy3-fer soph 5-glc; **9. peak:** Peo3-p-hb-soph; **10. peak:** Peo3-(6'''-caf soph)-5-glc; **11. peak:** Peo3-fer soph-5-glc; **12. peak:** Pg3-fer soph-5 glc; **13. peak:** Peo3-caf soph-5-glc; **14. peak:** Peo3-dicaf soph-5-glc; **15. peak:** Peo3-caf-p-hb soph-5 glc (polymer); **16. peak:** Cy3-caf-fer soph-5-glc; **17. peak:** Peo3-caf-fer soph-5-glc; **18. peak:** Pg3-caf-fer soph-5-glc

4.3.4 Effect of Cultivation Method and Plant Density on Anthocyanin Content in Purple Sweet Potato

Differences in cultivation method and plant density did not have a statistically significant effect on the anthocyanin content of the Purple cultivar. In contrast, the year effect had a significant influence on anthocyanin accumulation, as confirmed by multifactorial ANOVA.

Since the cultivation treatments did not result in significant differences in anthocyanin concentration, the total anthocyanin yield was primarily determined by the quantity of tuber yield per plant. Among the treatments, the RT, RS, and FT treatments showed higher total anthocyanin yields compared to the FS treatment.

4.3.5 Effects of Cultivation Method, Plant Density, and Year on the Anthocyanin and Yield of the Purple Cultivar

According to the ANOVA results, the yield of the Purple cultivar was significantly influenced by plant density ($p < 0.001$), cultivation method ($p < 0.01$), and year ($p < 0.001$). Anthocyanin accumulation was most strongly affected by the year effect ($p < 0.05$). Among the cultivation factors, only plant density had a significant effect on total anthocyanin yield per hectare ($p < 0.001$). No significant effects were found on the SPAD index from any of the tested factors. In contrast, chlorophyll fluorescence (Fv/Fm) was significantly affected by cultivation method and year (both $p < 0.001$), while plant density also had a moderate but statistically significant effect ($p < 0.05$). Among the interaction effects, the combined influence of year, cultivation method, and plant density significantly affected Fv/Fm values ($p < 0.01$) (Figure 11). This confirms the complex interaction between environmental and agronomic factors in shaping the physiological responses of the cultivar.

Factor	Yield	Total anthocyanin	Total anthocyanin yield	Fv/FM	SPAD
Year effect	***	*	n.s.	***	n.s
Cultivation method	**	n.s.	n.s	***	.
Plant density	***	n.s.	***	*	n.s
Year × Cultivation method	*	n.s.	.	**	n.s.
Year × Plant density	n.s	n.s.	.	*	n.s
Cultivation method × Plant density	n.s	n.s.	n.s.	*	n.s
Year × Cultivation method × Plant density	n.s	n.s.	n.s	**	n.s.
Legend: <ul style="list-style-type: none"> • *** $p < 0,001$ • ** $p < 0,01$ • * $p < 0,05$ • . $p < 0,1$ (marginal significant) • n.s. non significant ($p \geq 0,1$) 					

Figure 11. ANOVA results showing the effects of different cultivation technologies on the fitonutrient, yield, and physiological parameters of the Purple cultivar.

4.4 Results of the Different cultivation method of the Murasaki-29

4.4.1 Effect of Plant Density on Yield

For the Murasaki-29 cultivar, no statistically significant differences in yield were found between single-row and twin-row cultivation methods in either year. In 2019, single-row cultivation resulted in a 16.6% higher yield, while in 2020, twin-row planting produced 20.5% higher yields.

Greater variability in yield was observed under the single-row treatments. Overall, twin-row cultivation offered more stable yield performance for the Murasaki-29 cultivar. However, based on yield results alone, the use of twin-row planting does not appear economically justified for this cultivar.

4.4.2 Effect of Plant Density on Physiological Parameters

During both vegetation periods, no statistically significant differences were observed between the different treatments in terms of SPAD index values when comparing single- and twin-row ridge cultivation of the Murasaki-29 cultivar (Figure 29). According to the ANOVA test of chlorophyll fluorescence (Fv/Fm) measurements, twin-row treatments resulted in significantly higher Fv/Fm values ($p < 0.05$) than single-row treatments.

4.4.3 Effect of Plant Density and Year on Yield and Physiological Parameters

The two-way ANOVA showed no significant effect of plant density, cultivation method, or year on yield performance or SPAD values for the Murasaki-29 cultivar. However, the interaction between plant density and year had a statistically significant effect on the SPAD index ($p < 0.05$). In contrast, changes in chlorophyll fluorescence (Fv/Fm) were significantly influenced by plant density alone.

4.5 Effects of Different Fertilization level on the Yield and Carotenoids, and Phenolic content of Beauregard and Purple Sweet Potato

4.5.1 Effect of Different Fertilization level on the Yield of Beauregard

Across the 2021–2022 seasons, the treatments tested in Experiments I and II resulted in the following average tuber yields per plant:

- BP-I: 0.95 kg/plant
- BL-I: 1.13 kg/plant
- BP-II: 1.09 kg/plant
- BL-II: 1.13 kg/plant

The highest average yields were recorded in the BL-I and BL-II treatments (1.13 kg/plant). Compared to these, the BP-II treatment yield was 3.5% lower, while

BP-I was 16% lower. In contrast, the zero-control BC treatment resulted in 29.65% lower yield (Figure 12).

In a study conducted in Egypt, the highest yield was achieved with a K₂O fertilization level of 357.2 kg/ha, resulting in tuber yields ranging from 1.19 to 1.49 kg/plant (El-Baky et al., 2010). Another study from Brazil reported the best marketable yield when potassium fertilizers were applied either 30 or 60 days after planting, in the form of KCl or K₂SO₄ (da Silva et al., 2022). According to Harvey et al. (2022), economically viable yields for Beauregard can be achieved with less than 174 kg/ha of K₂O, although maximum potassium accumulation in leaves and tubers was observed at 269 and 404 kg/ha K₂O, respectively.

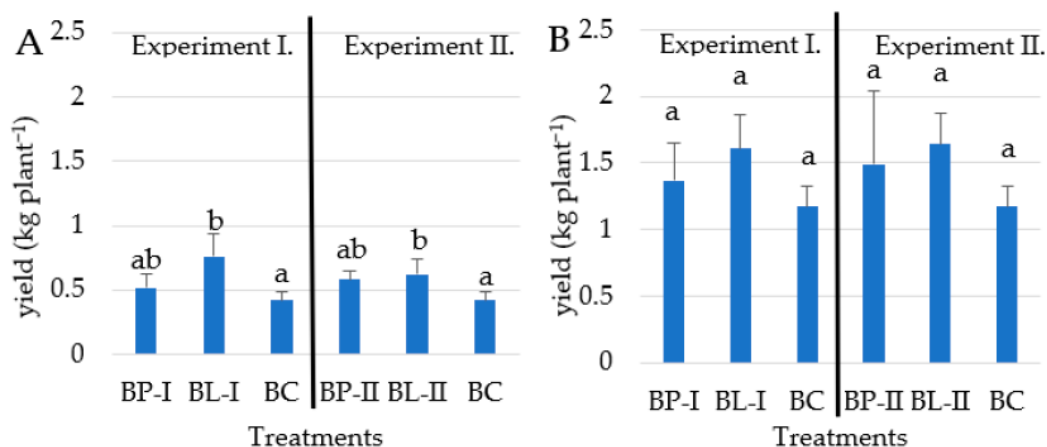


Figure 12. The effect of different fertilizer dosages on the Beauregard yield in 2021 (A) and 2022 (B) growing seasons ($n = 4$). Error bars represent SD. Different letters indicate statistical differences ($p < 0.05$)

4.5.2 Effect of Different Potassium-Predominant Fertilizer Treatments on the Carotenoid Content of the Beauregard

In the carotenoid profile of the Beauregard sweet potato, β -carotene was the dominant compound, accounting for 87% of the total carotenoid concentration. In 2021, the second most abundant compound after β -carotene was α -cryptoxanthin, while in 2022, luteo-chrome followed in concentration.

During the first experiment, the BL-I split fertilization treatment resulted in a significantly higher total carotenoid content (198.8 $\mu\text{g/g}$) compared to the zerocontrol plots in 2021. In 2022, the BL-I treatment again showed the highest value (159.1 $\mu\text{g/g}$), although the difference was not statistically significant in that year.

In the second experiment, the BL-II treatment, which also applied potassium in a split dose, resulted in a significantly higher total carotenoid concentration in 2022 (205.4 $\mu\text{g/g}$) compared to both the control (149.5 $\mu\text{g/g}$) and the BP-II treatment (126 $\mu\text{g/g}$). Favorable weather conditions in that season likely contributed to improved nutrient uptake and higher carotenoid accumulation.

In terms of total carotenoid yield per plant, the best results in both years were achieved in the BL-I and BL-II treatments. In 2022, the total carotenoid yield

reached 256.2 mg/plant in the BL-I treatment, and 336.9 mg/plant in the BL-II treatment, which represented a 51.9% increase compared to the control. Overall, the application of potassium-rich fertilizers in split doses had a positive effect on total carotenoid accumulation and β -carotene content, a finding that is also supported by relevant literature.

4.5.3 Effect of Different Potassium-Predominant Fertilizer Treatments on the Yield of the Purple sweet potato cultivar

Based on the average values from 2021 and 2022 in Experiments III and IV, the LL-I treatment provided the highest tuber yield per plant for the Purple cultivar, with a value of 0.40 kg/plant. Compared to this, yields from other treatments were lower as follows: the LP-II treatment produced 21.25% less, LP-I was 22.5% lower, LL-II was 26.25% lower, and the zero-control (LC) treatment yielded 47.5% less than LL-I. The LL-I treatment, which provided balanced nutrient supply, proved to be the most effective in maximizing yield for the purple-fleshed cultivar (Experiment III) (Figure 13). According to a previous study by Jian et al. (2001), the optimal sweet potato yield was achieved by applying 150–300 kg K_2O /ha. In contrast, the highest yields in our experiments were obtained at even higher potassium fertilizer doses. However, the efficiency and uptake of these nutrients are highly dependent on specific soil characteristics.

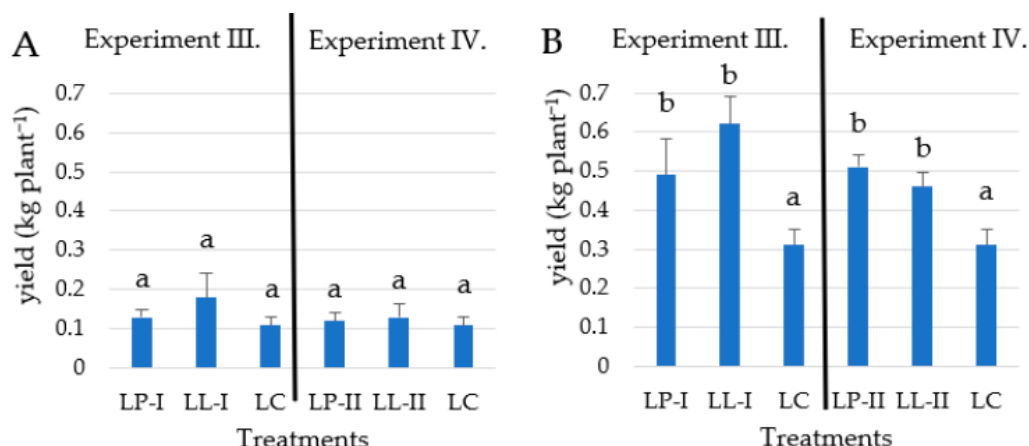


Figure 13. The effect of different fertilizer doses on the yield of Purple in the 2021 (A) and 2022 (B) growing seasons ($n = 4$). Different letters indicate significant differences at $p < 0.05$ level.

4.5.4 Effect of Different Fertilizer level on Total Anthocyanin Content and Yield in the Purple Cultivar

The analysis of Experiments III and IV indicated that anthocyanin concentration was primarily influenced by abiotic stress. During the unfavorable 2021 growing season, the control plots (LC) exhibited significantly higher anthocyanin concentrations (620.2 $\mu\text{g/g}$) compared to the fertilized plots. In 2022, due to more favorable weather conditions, anthocyanin levels decreased across all treatments.

Even in 2022, with the exception of Experiment III, the highest anthocyanin concentration was still measured in the zero-control (LC) treatment. This suggests that nutrient supplementation did not significantly enhance anthocyanin concentration. However, the LL-I treatment produced the highest total anthocyanin yield per plant in both years, with 97.2 mg/plant in 2021 and 322.9 mg/plant in 2022 (Figure 14). There was no strong correlation between total yield and anthocyanin concentration, as confirmed by statistical analysis ($R = 0.21$). Thus, anthocyanin accumulation in the Purple cultivar appeared to be influenced more by environmental stress factors than by the intensity of nutrient supply. This is consistent with previous findings indicating that anthocyanin biosynthesis in purple-fleshed sweet potato is strongly affected by abiotic stress (Hu et al., 2024).

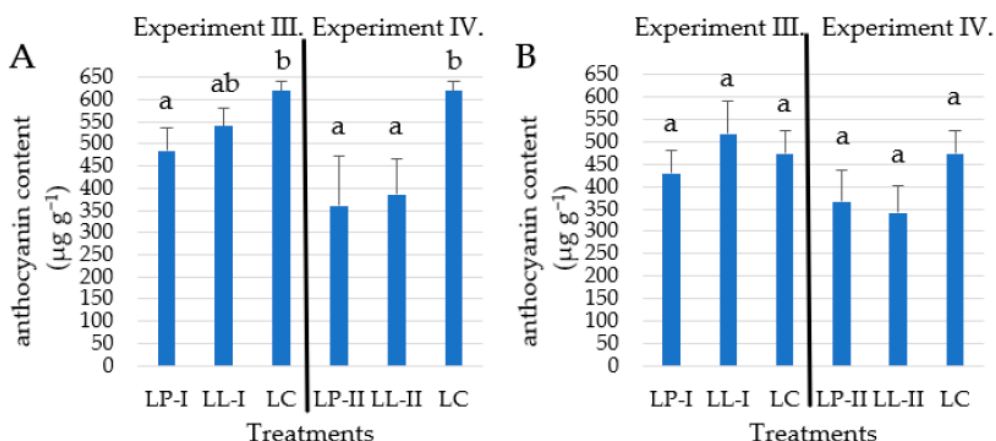


Figure 14. Total anthocyanin content of storage roots of Purple in the 2021 (A) and 2022 (B) growing seasons ($n = 4$). Different letters indicate significant differences at $p < 0.05$ level.

4.6 Correlation Analysis Between Measured Parameters

Based on the Pearson correlation analysis, a significant positive relationship was found between SPAD index values and yield in the Beauregard cultivar across the 2019–2022 data set. This indicates that higher relative chlorophyll content is associated with better photosynthetic performance and, consequently, higher yields.

However, no significant correlation was found between SPAD values and total carotenoid content, suggesting that carotenoid accumulation in tubers is not directly dependent on chlorophyll concentration (Table 4).

In the case of the Purple cultivar, the correlation between SPAD values and yield was even stronger than in Beauregard, implying a greater photosynthetic capacity in this genotype. Additionally, a significant positive correlation was observed between SPAD values and total anthocyanin content in the Purple cultivar, supporting previous research indicating that anthocyanins act as antioxidants during abiotic stress conditions (Naing et al., 2021).

When analyzing the 2021–2022 seasons separately, no significant correlations were detected between yield, compositional parameters, and physiological variables in any cultivar. Correlations only became evident when data were aggregated across multiple years.

Table 4. *Pearson Correlation Coefficients Between SPAD Index, Yield, and Compositional Parameters*

Cultivar	SPAD values	
	Beauregard	Purple
Yield (t/ha)	0,327*	0,403**
Total carotenoids (µg/g)	NS	NR
Total anthocyanins (µg/g)	NR	0,512***
*** correlation level LSD _{0,001} , ** correlation level LSD _{0,01} , * correlation level LSD _{0,05} , NS = non significant, NR= not relevant		
Based on the 2019-2020-2021-2022- growing season		

5 Conclusion and Recommendations

The yield and compositional parameters of the investigated sweet potato cultivars were significantly influenced by cultivation method, plant density, and year effect. In the case of the Beauregard cultivar, plant density proved to be a more decisive factor than cultivation method. The highest yield was obtained under the RT treatment (ridge cultivation with twin rows at 35,000 plants/ha) in both growing seasons. SPAD index values were also highest under RT treatment, although not all measurement dates showed statistically significant differences. Chlorophyll fluorescence values (Fv/Fm) were also superior under RT, but no strong correlation was observed between SPAD and Fv/Fm values. The weak correlation between these two physiological parameters suggests that they reflect distinct physiological processes.

A negative correlation was observed between β -carotene content and yield, indicating that higher yields were associated with a decrease in tuber compositional quality. From a yield-maximization perspective, the RT technology (ridge, twin row) is recommended. For the Ásothalmi-12 cultivar, plant density was again the most critical factor, with the RT twin-row technology (35,000 plants/ha) producing the highest yield. However, β -carotene content decreased significantly with increased plant density. The year effect was also pronounced, influencing both compositional and physiological traits. A negative correlation was found between Fv/Fm values and carotenoid content, suggesting that carotenoids may play a protective antioxidant role under stress conditions. For yield optimization, RT technology is again recommended; however, if the goal is to increase carotenoid concentration, single-row cultivation is more advantageous. In the case of the Purple cultivar, yield was primarily determined by plant density and, secondly, by cultivation method. The RT treatment consistently provided the highest yield. Among compositional parameters, anthocyanin accumulation was most strongly affected by the year, depending more on environmental conditions than on cultivation technology. Total

anthocyanin yield per hectare can be increased by applying high-density twin-row systems.

SPAD index values were more influenced by year than by nutrient supply. In this cultivar, lower Fv/Fm values in response to abiotic stress were accompanied by enhanced anthocyanin biosynthesis, indicating an adaptive physiological defense mechanism. From a yield standpoint, twin-row cultivation is also recommended for the Purple cultivar. For the Murasaki-29 cultivar, twin-row planting resulted in higher photosynthetic activity based on Fv/Fm values; however, this did not lead to a significant yield increase. Therefore, single-row cultivation is economically more justifiable for this variety.

Based on analytical assessments, the Beauregard, Ásotthalmi-12, and Purple cultivars all exhibit outstanding nutritional and functional value. The Beauregard and Ásotthalmi-12 cultivars were rich in β -carotene and had a diverse carotenoid profile, while their complex polyphenol composition—particularly caffeoyl- and feruloylquinic acid derivatives—suggests substantial antioxidant potential. The anthocyanin profile of the Purple cultivar included 17 compounds, including acylated, diacylated, and polymerized cyanidin-, peonidin-, and pelargonidin-based glycosides, further enhancing its biological value in terms of antioxidant capacity and chemical stability. These cultivars are therefore particularly suitable for use as functional food sources.

In the Beauregard nutrient supplementation trials, potassium-rich, split-dose fertilization systems (BL-I, BL-II) resulted in the highest yields and total carotenoid content. Continuous nutrient supply combined with liquid fertilization was more effective than single basal fertilization. Economically, the BL-I treatment is recommended; in the absence of fertigation, the BP-II technology may serve as a suitable alternative for growers. The various nutrient supply systems had their most significant effect on total carotenoid concentration.

For the Purple cultivar, the LL-I treatment (split application of potassium-dominant nutrients) proved most effective for yield enhancement, producing a 47.5% increase compared to the control. However, excessively high potassium doses (LP-II) led to yield depression. Regarding anthocyanin content, nutrient supply had no significant effect, while the year effect was the dominant influencing factor.

According to the correlation analyses, moderate positive relationships were found between SPAD values and yield for Beauregard, whereas for Purple, stronger correlations were observed among SPAD values, yield, and anthocyanin content. These results suggest that the accumulation of antioxidant compounds is more likely a stress response than an indicator of increased yield performance.

6 New Scientific Findings

1. For the Beauregard and Purple cultivars, ridge cultivation combined with a planting density of 35,000 plants/ha resulted in significantly higher yields compared to 17,500 plants/ha under both ridge and flat cultivation. Based on the magnitude of yield differences, doubling the amount of planting material under the examined conditions is considered economically justified.
2. In the Beauregard and Ásotthalmi-12 cultivars, a negative correlation was observed between yield and β -carotene content, indicating that increases in yield were accompanied by a decline in β -carotene concentration.
3. In the Ásotthalmi-12 cultivar, reducing the planting density to half (17,500 plants/ha in single rows) resulted in an average 30.9% increase in total carotenoid content compared to twin-row plots with 35,000 plants/ha. Based on these results, this planting method is particularly recommended in cultivation practices aiming to enhance carotenoid levels.
4. In the Beauregard cultivar, no relationship was found between SPAD index values and tuber carotenoid content, suggesting that leaf chlorophyll content is not directly linked to carotenoid biosynthesis and accumulation in the storage roots.
5. Changes in plant density (from 17,500 to 35,000 plants/ha), as well as the use of ridge versus flat cultivation, did not significantly affect the cis- β -carotene or ζ -carotene content in the tubers of the Beauregard and Ásotthalmi-12 cultivars.
6. In the Beauregard cultivar, K₂O supply had no significant effect on the concentration of cis- β -carotene, cis-luteochrome, or mutatochrome carotenoids.
7. For the Hungarian-bred Ásotthalmi-12 sweet potato, the carotenoid and polyphenol profiles were successfully characterized using HPLC analytical methods. A total of nine carotenoids and nine polyphenolic compounds were identified. On average, β -carotene accounted for 81.4% of the total carotenoid content, making it the dominant compound in the profile.
8. For the Hungarian-bred Purple sweet potato, its anthocyanin profile was comprehensively identified via HPLC analysis. A total of 17 distinct anthocyanin compounds were detected, with cyanidin, peonidin, and pelargonidin being the dominant aglycones.
9. In the Purple cultivar, the LL-I treatment—a split application of potassium-dominant base and nutrient solution fertilization at a total active ingredient dose of 917 kg/ha K₂O—resulted in the highest yield among all tested K₂O dose levels and application methods.
10. The total anthocyanin content in the Hungarian-bred Purple cultivar decreased under the LL-II treatment (averaging 360.67 $\mu\text{g g}^{-1}$) involving 1060 kg/ha of base and fertigation fertilizer, compared to the untreated

control (LC, averaging 546.66 $\mu\text{g g}^{-1}$). Therefore, this method is not suitable for enhancing anthocyanin concentration.

11. In the Purple cultivar, leaf relative chlorophyll content (SPAD index) showed a significant positive correlation with yield ($r = 0.4$, $p < 0.01$), and also with total anthocyanin content ($r = 0.51$, $p < 0.001$) among compositional parameters.

4. Publications

Q1-publications:

- Balázs, V., Helyes, L., Daood, H. G., Pék, Z., Ilahy, R., Neményi, A., Égei, M., & Takács, S. (2024). Cultivation Technology and Plant Density Affecting the Yield and Carotenoid Content of Beauregard Sweet Potato. *Agronomy* 2024, Vol. 14, Page 2485, 14(11), 2485.
DOI: 10.3390/AGRONOMY14112485
- Balázs, V., Helyes, L., Daood, H. G., Pék, Z., Ilahy, R., Neményi, A., Égei, M., & Takács, S. (2024). Cultivation Technology and Plant Density Affecting the Yield and Carotenoid Content of Beauregard Sweet Potato. *Agronomy* 2024, Vol. 14, Page 2485, 14(11), 2485.
- DOI: 10.3390/AGRONOMY14112485

Article

- Balázs, V., Helyes, -Lajos, Pék, Z., Neményi, -András, Takács, -Sándor, Égei, M., & Daood, -Hussein G. (2021). Effect of different production types on the yield and β -carotene content of sweet potato /cultivar Ásotthalmi- 12/. *Acta Agraria Debreceniensis*, 1, 45–49.
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