Journal of Agricultural Sciences (Belgrade) https://doi.org/10.2298/JAS2404367O
Vol. 69, No. 4, 2024 UDC: 635.21:631.53.03
Pages 367-381 Original scientific paper

POTATO MINITUBER PRODUCTION BY AEROPONICS: EFFECTS OF GENOTYPE AND PLANT ORIGIN

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Abstract: Aeroponics is a modern farming technology for soilless potato cultivation that enables the efficient production of high-quality pre-basic seed potatoes (minitubers). In this system, the roots and underground stems (stolons) of the potato plants grow within closed modules, suspended in a fine mist of a nutrient-rich solution that continuously recirculates. This setup enables the formation of numerous minitubers with a length greater than 10 mm during the growing period. Our study aimed to evaluate the impact of the genotype and origin of the planting material on minituber production in an aeroponic facility in Guča, Serbia. Three potato cultivars were analyzed: Cleopatra, Kennebec, and Désirée, using two types of planting material: acclimated microplants and plants derived from the previous season's minitubers. The plants were cultivated aeroponically from late May to December 2019, with a planting density of 24 plants per square meter and harvest intervals of approximately 14 days. The Désirée cultivar produced the highest average number of minitubers per plant (19.89), followed by Kennebec (15.71) and Cleopatra (11.05). The average weight of minitubers was significantly greater in plants grown from last season's minitubers compared to plants grown *in vitro*. The Kennebec plants originating from minitubers achieved the highest yield of 10.27 kg per square meter. Additionally, the plants originating from minitubers consistently produced tubers throughout the entire cultivation period in the aeroponic growing system.

Key words: soilless production, potato cultivars, pre-basic seed potato.

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Introduction

Potato (*Solanum tuberosum* L.) is one of the world's most significant food crops, with commercial production based on vegetative propagation by seed tubers. In Serbia, the average potato yield in 2022 was 21.1 t ha-1 (Statistical Office of the Republic of Serbia, 2023), while in the developed countries of Europe and North America it can reach 40–50 t ha-1 (FAO, 2023). Low yields are due to various limitations, with the condition of the planting material being a key factor (Broćić et al., 2021). High-quality, healthy seed material derived from potatoes grown from minitubers (pre-basic seed potatoes) is essential for achieving high yields. Minituber production is a step between in vitro propagation of disease-free plant material (microplants, microtubers) under sterile conditions and seed production in the open field (Struik, 2007). Therefore, the minitubers serve as the initial material for field propagation, leading to the production of basic seeds and other seed categories (da Silva Filho et al., 2020).

Traditional seed potato production systems are based on the production of minitubers, grown on substrate in controlled environments, such as greenhouses. This process involves the acclimation and growth of plant material previously propagated in vitro. A low reproductive rate, usually 2–5 tubers per cultivated microplant, is considered the main limitation of substrate-based potato minituber production (Struik, 2007; Otazú, 2010; Broćić et al., 2021). To address this limitation, a common practice is the production of the final seed tubers (certified following the legislation of the respective country) after three to five generations of propagation in open field conditions (Struik and Wiersema 1999; Broćić et al., 2022). However, pathogens such as fungi, bacteria, and particularly viruses can easily be transmitted from one vegetative generation to the next, which increases the risk of infection with each subsequent reproduction cycle (Naik and Buckseth, 2018). To overcome these challenges in conventional seed production, soilless seed potato production technologies such as hydroponics, semi-hydroponics, and aeroponics have been developed over the last 20 years (Ritter et al., 2001; Mateus-Rodriguez et al., 2013). The world's largest potato producers are moving from conventional to high-tech seed production systems to improve seed quality and increase minituber production rates (Buckseth et al., 2022). In addition, researchers are making constant efforts to further develop innovative technologies for the rapid multiplication of minitubers.

Aeroponics is an advanced soilless potato growing technology that allows the production of a substantial quantity of healthy minitubers. The advantages of this technology, promoted by the International Potato Center (CIP), are high multiplication rates (up to 1:45), high production efficiency per area (>900 minitubers per m^2), as well as water, chemicals and energy savings (Mateus-Rodriguez et al., 2013). Aeroponics can potentially reduce the number of generations of seed multiplication in the field, with lower costs and maintain high phytosanitary quality (Nichols, 2005). In aeroponics, the leaves of the plants are exposed to the open air and light source of the greenhouse, while the roots and stolons grow suspended in a fine mist of nutrient solution inside the closed modules. The front part of the modules is hinged and can be opened for harvesting minitubers of the desired size (Otazú, 2010; Lakhiar et al., 2018; Andrade-Piedra et al., 2019). The full access to available oxygen and carbon dioxide in the modules stimulates the growth of roots and stolons, leading to increased plant growth rates and productivity. Consequently, a significant number of tubers larger than 10 mm can develop on the stolons during the growth period. The sequential collection of tubers is conducted at intervals of 7 to 15 days (Farran and Mingo-Castel 2006). The minitubers are harvested when they reach the desired size, usually > 3 g. The harvested minitubers are left to harden for about two weeks before being placed in refrigerators at 2–4°C to be utilized for planting in the next crop season. Multiplication rates in aeroponic systems are significantly higher than those obtained in conventional systems and can range from 36 to over 100 minitubers per plant depending on the cultivar (Ritter et al., 2001; Farran and Mingo-Castel, 2006; Tierno et al., 2014; Broćić et al. 2021).

In addition to acclimated microplants and microtubers obtained from in vitro culture, the starting plant material for aeroponic potato production can also include minitubers from aeroponic cultivation of the previous seasons (Broćić et al., 2021, 2022), shoots or rooted stem cuttings delivered from microplants after acclimatizations (Muthoni et al., 2017), and rooted sprouts previously separated from seed tubers (da Silva Filho et al., 2020). Previous research has shown that the aeroponic technology is potentially efficient for specific potato cultivars and that there is variability between cultivars in terms of production in an aeroponic system under uniform conditions (Mateus-Rodriguez et al., 2012; Chang et al., 2011).

Cleopatra, Kennebec, and Désirée are popular and frequently cultivated potato cultivar in Serbia. This study aimed to evaluate the dynamics of minituber production and the aeroponic performance (the number of minitubers per plant, minituber mass, and yield per square meter) of these three cultivars when two types of planting material, acclimated microplants and plants originating from the previous season's minitubers, were used. Our results provide valuable information for selecting cultivars/genotypes and the type of planting material that enables a high production level of pre-basic seed potatoes in aeroponics.

Material and Methods

Experimental setup and plant material

The experiment was conducted from late May to December 2019 in an aeroponic facility in Guča (Serbia). A completely randomized block design with three replications for each combination of cultivar and plant origin was used for this study, with each replication comprising ten plants. The study involved three potato cultivars: Cleopatra (early maturing), Kennebec and Désirée (late maturing). Two types of planting material were used in the experiment: acclimated microplants and plants derived from the minitubers harvested in the previous season. The potato microplants were *in vitro*-grown in environmentally-controlled conditions, (21 ºC, photoperiod 16 h, illumination 90 µmol m^{-2} s^{-1}, relative humidity 70%) and were transferred to nutritive medium every 30 days using single-node cuttings according to Momčilović et al. (2014). The potato microplants were planted in the substrate consisting of sand and perlite (4:1) and subsequently acclimated in the pest-free greenhouse for 25 days prior to their transfer to the aeroponic system (Figure 1A). Sprouted minitubers (obtained during the preceding season of aeroponic cultivation) were sown 10 days earlier in the same substrate, and 35 days later, the developed plants of similar size and physiological age compared to the acclimated microplants were transferred to aeroponics.

Figure 1. Potato production in an aeroponic facility. (A) Acclimation of potato microplants in the greenhouse. (B) Plants positioned in plant holders before transfer to the aeroponic module. (C) Aeroponic facility for potato cultivation. (D) Roots of plants, stolons and minitubers in the aeroponic module. Minitubers collected from aeroponically grown plants of (E) cv. Cleopatra, (F) cv. Désirée, and (G) cv. Kennebec.

The roots of the plants were rinsed with water, the stems were inserted into the holders, and the holders were placed in the module (Figure 1B). In the aeroponic system, the subterranean plant organs (roots and stolons) of the potato plants are suspended in the air and supplied with water and nutrients via a nutrient solution dispersed as fine fog particles (30–100 microns). Concurrently, the foliage develops above the module under controlled greenhouse conditions. The planting density within the aeroponic module was established at 24 plants per m^2 (Figure 1C). The minitubers were collected approximately every 14 days (from July to December), and the number of minitubers (tuber length ≥ 2 cm) per plant, along with the mass of the minitubers, were measured at the end of each harvest interval. In addition, the productivity parameters of the cultivars (minitubers per plant, minituber mass and yield per m^2) were measured at the end of the growing season.

Temperature conditions

During the experiment, temperature measurements were made in the greenhouse (in the area of the plant leaves) and in the aeroponic module, where the plant roots and stolons were located. The average daily temperatures are presented in Table 1.

Table 1. Daily average temperatures in the greenhouse and aeroponic module during the experimental period in 2019.

Month	Greenhouse	Aeroponic module
	Temperature (°C)	
June	27.8	23.4
July	25.1	21.9
August	27.3	22.6
September	23.2	19.3
October	20.0	17.0
November	15.7	13.4
Average	23.2	19.6

Statistical analysis

Statistical analysis was performed using STATISTICA 12 (StatSoft, Inc. 1984-2014, Tulsa, OK, USA). The mean minituber mass or number of tubers per plant was correlated with harvest time. A regression analysis was performed and the Pearson's correlation coefficient was calculated. The data regarding the total number of minitubers per plant, the mass of minitubers and yield were analyzed using a two-factor analysis of variance (ANOVA) with plant origin and genotype (cultivar) as the categorical predictors. To compare the means, the Tukey's multiple comparison test was used at the significance level of $p < 0.05$.

Results and Discussion

Cultivation of potato plants in aeroponics

The research study involved plants from three commercial potato cultivars (Cleopatra, Désirée, and Kennebec), obtained from two types of planting materials: microplants and minitubers. The microplants were acclimated in the greenhouse for 25 days prior to transfer into the aeroponic system, while the plants developed from sprouted minitubers were transferred to the aeroponic modules 35 days after tuber-sowing. These plants differed in some morphological and anatomical traits before and after relocation to aeroponics. Plants developed from minitubers possessed a vigorous primary shoot, greener foliage and a larger root system compared to those originating from the *in vitro* culture, which produced multiple shoots.

The temperature, as a significant factor for the initiation, development and enlargement of potato tubers, was recorded in both the aeroponic system and the greenhouse throughout the duration of the experiment (Table 1). In the aeroponic module, the daily average temperatures ranged from 22.6 ºC in the initial stage of the growing period to 16.6 ºC in the second stage of the growing period, which is close to the optimal temperature range for potato tuberization (14–22°C). The temperatures in the insect-free greenhouse were higher, namely 26.7°C in the initial phase of the growing period and 19.7°C in the second phase, but adequate for foliage growth due to the different temperature requirements.

Dynamics of minituber production in aeroponics

The dynamics of minituber production varied among the three genotypes studied. For plants originating from minitubers, tuberization in the Cleopatra, Kennebec, and Désirée cultivars began 30 days after planting in the aeroponic system, and tuber formation occurred consistently throughout the entire cultivation period (Figure 2A). However, Kennebec and Désirée exhibited more vigorous tuberization, producing a significantly higher number of minitubers compared to Cleopatra. The highest numbers of minitubers were recorded in Kennebec and Cleopatra plants at the seventh harvest and in Désirée at the final, tenth harvest. Overall, a greater number of minitubers was collected from Cleopatra plants during the mid-point of the cultivation period, whereas Kennebec and Désirée produced more minitubers in the latter half of the growing season (Figure 2A). Both Kennebec and Désirée are classified as medium-late to late cultivars, with a vegetative cycle of 120–135 days, which was extended by 35 days due to aeroponic cultivation.

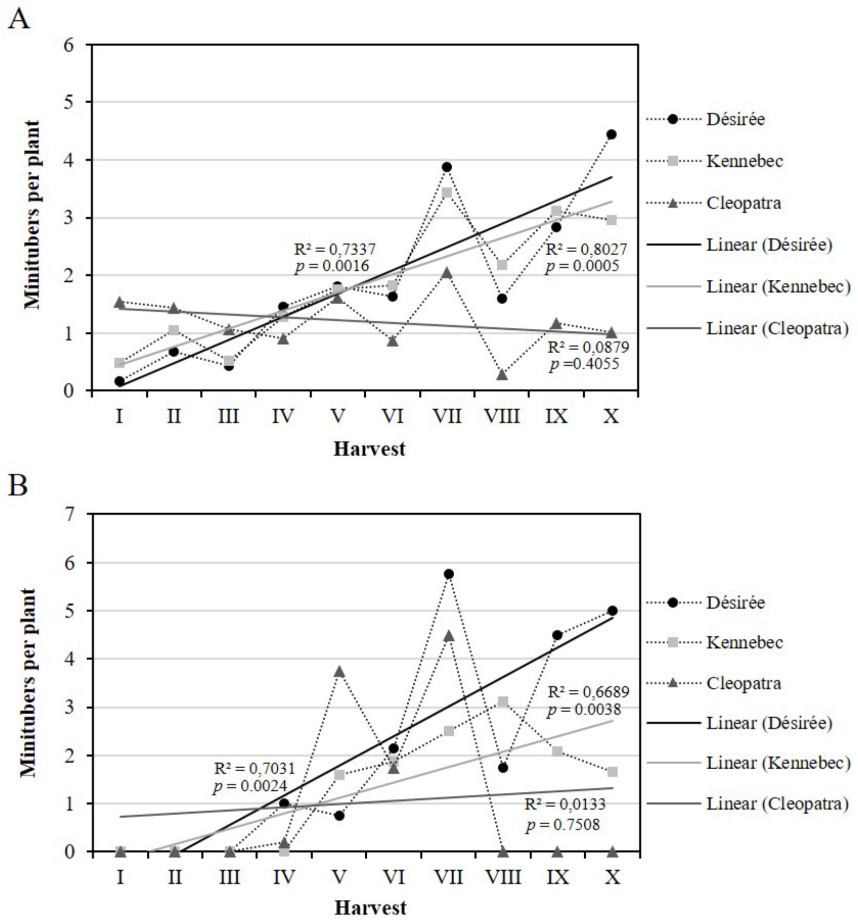

Figure 2. The dynamics of minituber formation for three potato cultivars grown in the aeroponic system**.** (A) Plants of minituber origin. (B) Plants of *in vitro* origin.

For plants of *in vitro* origin, the first minitubers from the investigated cultivars were harvested at the end of August (harvest IV). The Cleopatra cultivar was the first to complete its vegetative cycle at the end of October, with the final number of minitubers collected at the seventh harvest (Figure 2B). This cultivar is characterized as early-maturing, with a vegetation period of 85–100 days in open field production in Serbia; in particular, this developmental trait appears unaffected by aeroponic cultivation. In the Kennebec plants of *in vitro* origin (Figure 2B), the highest number of minitubers was collected during the middle of the growing period (harvests V–VIII), while Désirée exhibited maximum values for the number of minitubers per plant in the second half of the cultivation period (harvests VI-X). Additionally, Désirée produced a significantly larger number of minitubers compared to the other two cultivars.

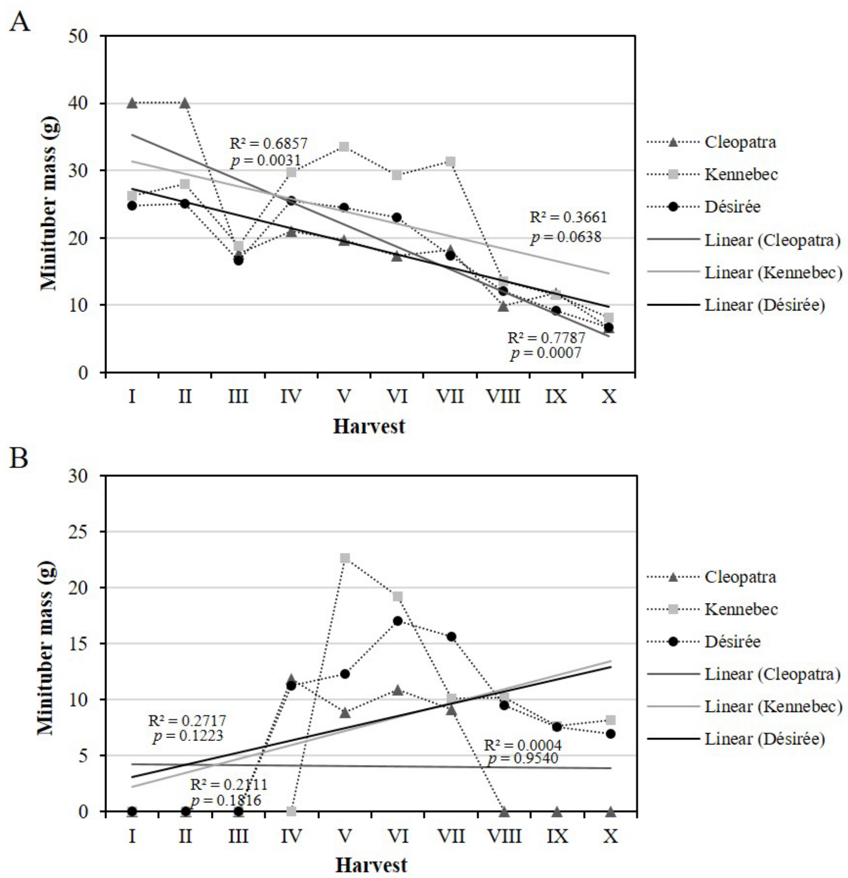

Figure 3. The dynamics of minituber mass per harvest for three potato cultivars grown in the aeroponic system. (A) Plants of minituber origin. (B) Plants of *in vitro* origin.

The heaviest tubers formed by Cleopatra plants of minituber origin (Figure 3A) were recorded early in the cultivation period (harvests I and II), while the mass of minitubers decreased in later harvests during the second half of the growing period. These findings align with those of Broćić et al. (2019a). In contrast, for Cleopatra plants of *in vitro* origin, only four harvests were conducted during the middle of the cultivation period (Figure 3B). The highest masses of minitubers for both Kennebec and Désirée, produced by plants of both origins (Figure 3A and B), were recorded in the middle of the cultivation period (harvests V–VII). According to Broćić et al. (2022), the decrease in minituber mass during the final harvests is likely due to rapid maturation caused by low environmental temperatures, a phenomenon also noted in our experiment (Table 1, Figure 3).

Effects of cultivar and plant origin on minituber production

The results of the two-way ANOVA showed the significant effect of both factors, genotype (cultivar) and plant origin, on minituber production in an aeroponic system (Table 2).

Table 2. Results of the two-way ANOVA for production of potato minitubers in an aeroponic system.

Parameter	Factor	df	SS	MS	F	*p*	Sig.
Number of minitubers per plant	Genotype	2	231.977	115.989	50.269	1.47×10^{-6}	***
	Plant origin	1	15.290	15.290	6.627	2.44×10^{-2}	*
	Genotype x plant origin	2	45.408	22.704	9.840	2.95×10^{-3}	**
Minituber mass	Genotype	2	46.570	23.285	12.540	1.15×10^{-3}	**
	Plant origin	1	319.286	319.286	171.949	1.79×10^{-8}	***
	Genotype x plant origin	2	6.803	3.401	1.832	2.02×10^{-1}	-
Yield per m^2	Genotype	2	40.8498	20.4249	38.789	5.78×10^{-6}	***
	Plant origin	1	67.6076	67.6076	128.395	9.14×10^{-8}	***
	Genotype x plant origin	2	12.6105	6.3052	11.974	1.38×10^{-3}	**

Note: SS, MS, df, and F are test parameters; * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

The investigated factors significantly affected the total number of minitubers per potato plant. A significant two-way interaction between genotype and plant origin indicated that the effect of plant origin varied among cultivars (Table 2). The highest number of minitubers per plant (20.90) was recorded in Désirée plants of *in vitro* origin, while the lowest number (10.22) was observed in the Cleopatra cultivar of the same origin (Figure 4). Similar findings for the Désirée and Cleopatra cultivars were reported by Broćić et al. (2018). The Désirée and Kennebec plants, derived from minitubers, produced a significantly larger number of minitubers compared to the Cleopatra plants. A post-hoc analysis revealed that plant origin did not significantly affect the number of minitubers formed in Cleopatra and Désirée, while it resulted in significant changes in Kennebec (Figure 4). Specifically, Kennebec plants originating from minitubers formed a significantly higher number of minitubers than those of *in vitro* origin. The results of the present study are not in accordance with some previous findings (Muthoni et al., 2017). Muthoni et al. (2017) investigated how different planting materials (microplants, stem cuttings, and minitubers) impact the productivity of two potato cultivars, Asante and Tigoni, in an aeroponic minituber production system. The authors reported that potato plants derived from *in vitro* sources produced a significantly higher quantity of minitubers compared to plants derived from minitubers, 1.64- and 1.84-fold higher in the first and the second growing season, respectively. However, the results were not shown separately for each cultivar, Asante and Trigoni.

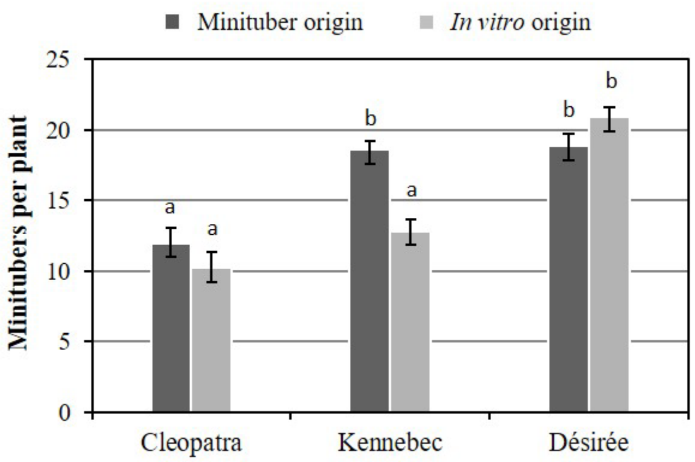

Figure 4. Effects of cultivar and plant origin on the formation of minitubers in an aeroponic system.

The mass of minitubers was also affected by the genotype and plant origin (Table 2). Plants of *in vitro* origin produced tubers of similar mass across all three investigated cultivars, while Kennebec plants from minituber origin yielded significantly heavier tubers than both Désirée and Cleopatra (Figure 5). Furthermore, the average mass of minitubers for all cultivars was significantly higher in plants originating from minitubers compared to those of *in vitro* origin (Figure 5), which is consistent with the findings of Broćić et al. (2023). In agreement with our previous research from the 2018 season (Broćić et al., 2019a, 2019b), Kennebec plants of minituber origin had the highest average mass of minitubers at 23.01 g, while Cleopatra and Désirée plants of minituber origin achieved a mass of 18.46 g. These minituber masses are notably higher than those reported by other researchers (Farran and Mingo-Castel, 2006; Struik 2007; Rykaczewska 2016) and in our previous studies (Broćić et al., 2018, 2019a, 2019b).

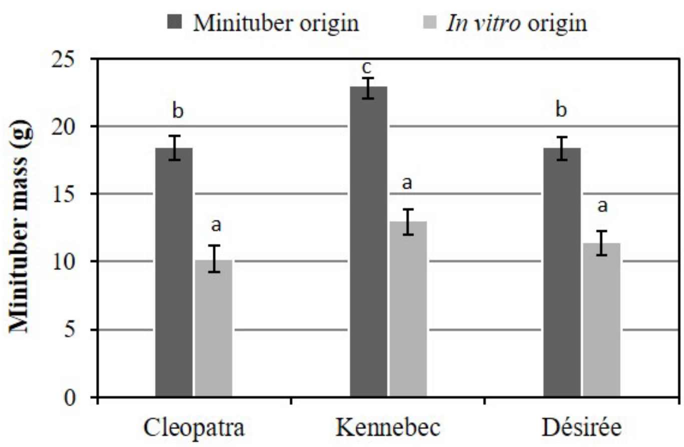

Figure 5. Effects of cultivar and plant origin on the minituber mass in an aeroponic system.

The yield was influenced by the factors of genotype and plant origin. A significant two-way interaction between genotype and plant origin revealed that the impact of plant origin varied among the cultivars (Table 2). Specifically, there was no significant difference in yield between the plants of minituber origin of the Désirée and Kennebec cultivars, while the Cleopatra plants of minituber origin produced a notably lower average yield compared to the other cultivars (Figure 6).

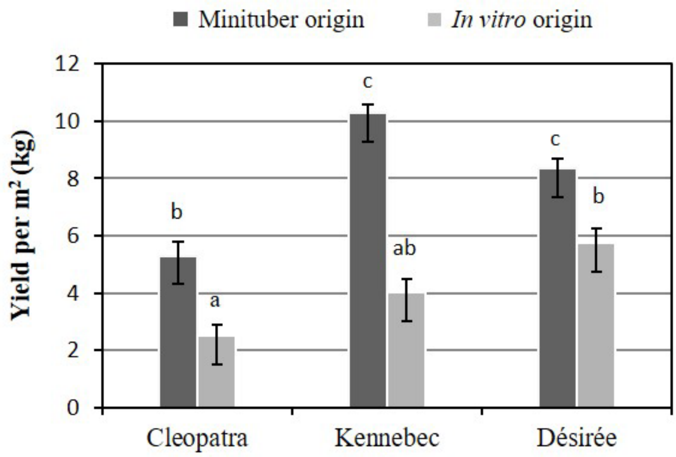

Figure 6. Effects of cultivar and plant origin on the minituber yield in an aeroponic system.

The Kennebec, Désirée, and Cleopatra plants of minituber origin produced higher yields per square meter compared to those from *in vitro* origin. The highest yield was observed in the Kennebec plants from minitubers, with 10.27 kg/m², followed by Désirée with 8.35 kg/m² and Cleopatra with 5.30 kg/m², both from the same origin (Figure 6).

Conclusion

The findings of our study indicate different performance of three potato cultivars in aeroponic cultivation, as well as a significant impact of the origin of the plant material on minituber production. The cultivars Kennebec and Desiree are classified as medium-late to late varieties with a growing season of 120–135 days that was extended by aeroponic cultivation to approximately 150 days. However, the life-cycle length of the Cleopatra cultivar depended on the plant origin. This cultivar is characterized as an early ripening plant with a vegetation period of 85–100 days in open-field cultivation in Serbia, and this period was prolonged only for plants derived from minitubers during aeroponic cultivation. Kennebec and Désirée were more productive than the Cleopatra cultivar during aeroponic cultivation in the 2019 season. Désirée and Kennebec plants derived from minitubers formed a significantly higher number of minitubers than Cleopatra plants, while Désirée plants of *in vitro* origin formed a significantly larger number of tubers compared to the other two cultivars of the same origin. Furthermore, the average mass and yield of minitubers of all the cultivars were significantly higher in plants grown from minitubers harvested in the previous season than in plants of *in vitro* origin.

Acknowledgments

This research was funded by the Ministry of Science, Technological Development and Innovation of the Republic of Serbia, contract numbers: 451-03-65/2024-03/200116 and 451-03-66/2024-03/200007.

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Received: September 6, 2024
Accepted: November 24, 2024

AEROPONSKA PROIZVODNJA MINI KRTOLA KROMPIRA: UTICAJ GENOTIPA I POREKLA SADNOG MATERIJALA

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R e z i m e

Aeroponika je savremena poljoprivredna tehnologija gajenja krompira bez zemljišta, koja omogućava efikasnu proizvodnju visokokvalitetnog predosnovnog semenskog krompira (mini krtola). U ovom sistemu, koreni i podzemna stabla (stolone) biljaka krompira rastu unutar zatvorenih modula, suspendovani u finoj magli rastvora bogatog hranljivim materijama koji neprekidno cirkuliše. Ova postavka omogućava formiranje brojnih mini krtola dužine veće od 10 mm tokom perioda rasta. Naša studija je imala za cilj da proceni efekat genotipa i porekla sadnog materijala na proizvodnju mini krtola u aeroponskom objektu u Guči, Srbija. Analizirane su tri sorte krompira: kleopatra, kenebek i dezire, upotrebom dve vrste sadnog materijala: aklimatizovanih mikrobiljaka i biljaka dobijenih od mini krtola iz prethodne sezone. Biljke su uzgajane aeroponski od kraja maja do decembra 2019. godine, sa gustinom sadnje od 24 biljke po kvadratnom metru i intervalima žetve od približno 14 dana. Sorta dezire produkovala je najveći broj mini krtola po biljci (19,89), zatim kenebek (15,71) i kleopatra (11,05). Prosečna težina mini krtola bila je značajno veća kod biljaka gajenih od prošlosezonskih mini krtola u odnosu na biljke *in vitro* porekla. Najveći prinos od 10,27 kg po kvadratnom metru, ostvarile su biljke sorte kenebek poreklom od mini krtola. Pored toga, biljke poreklom od mini krtola su postojano proizvodile krtole tokom čitavog perioda gajenja u aeroponskom sistemu.

Ključne reči: uzgoj bez zemljišta, sorte krompira, predosnovni semenski krompir.

Primljeno: 6. septembra 2024.
Odobreno: 24. novembra 2024.

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