horticulturae

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Potato Aeroponics: Effects of Cultivar and Plant Origin on Minituber Production

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Abstract: Aeroponics is a modern and soilless technology that is used for the efficient production of pre-basic seed potatoes, namely minitubers. This study aimed to evaluate the effects of the cultivar and type of planting material on the production of minitubers in the aeroponic facility in Guča, Serbia, at short, 7-day harvest intervals. Although aeroponic cultivation prolonged the vegetative cycle in all five investigated cultivars, the dynamics of minituber formation varied between genotypes. Two early maturing cultivars, Cleopatra and Sinora, quickly completed the vegetative cycle and formed a small number of minitubers, while the medium-late to late cultivars, Kennebec and Agria, steadily tuberized during the entire cultivation period in the aeroponic facility. The type of planting material affected the dynamics of minituber formation in three investigated cultivars. Sinora, Cleopatra, and Désirée's plants of in vitro origin reached the final number of minitubers and the vines started senescing much earlier than plants of minituber origin. Kennebec and Agria plants of in vitro origin produced the largest number of minitubers (53.8–54.5) and showed the highest yield (9.8–10.5 kg m^{-2}) during the cultivation period, while the heaviest minitubers were formed by Sinora plants of minituber origin (15.48 g). In addition, the temperature during pre-harvest periods significantly affected the number of tubers at harvests in Kennebec and Agria, and minituber mass in Désirée.

Keywords: pre-basic seed potato; soilles cultivation; *Solanum tuberosum*; virus-free

Citation: Broćić, Z.; Oljača, J.; Pantelić, D.; Rudić, J.; Momčilović, I. Potato Aeroponics: Effects of Cultivar and Plant Origin on Minituber Production. *Horticulturae* **2022**, *8*, 915. https://doi.org/10.3390/horticulturae8100915

Academic Editors: Sergio Ruffo Roberto and Luigi De Bellis

Received: 29 August 2022
Accepted: 30 September 2022
Published: 6 October 2022

1. Introduction

Potato, *Solanum tuberosum* L., is one of the most significant horticultural species globally, a staple food source for more than a billion people worldwide [1,2]. Production of this important crop is based on vegetative propagation by tubers. However, pathogens such as fungi and bacteria, and particularly viruses, can be easily transmitted from one vegetative generation to the next and, due to their accumulation in tubers through successive cycles of open field propagation, can cause potato degeneration and a significant reduction in the total and marketable yield [3]. Therefore, the usage of healthy planting material, pathogen-free seed tubers, is of utmost importance.

The current conventional seed potato production includes the following phases: propagation of pathogen-free plant material (microplants, microtubers) under aseptic conditions in vitro, acclimation and cultivation of pathogen-free plant material on a substrate in insect-free greenhouses to produce minitubers (pre-basic seed potato), and field planting of the minitubers to increase the volume of seed material (basic seeds and other categories of seeds) [4]. The low reproduction rate and variable size of minitubers are the main limitations in conventional production [5]. The number of minitubers produced in a substrate is normally 2–5 tubers per cultivated microplant [6,7]. Due to the low minitubers' production rate, a common practice in seed production programs is the production of the final seed tubers after three to five generations of propagation in the open field, with every cycle increasing the risk of pathogen infection. To overcome the problem of low efficiency in

minituber production, soilless technologies for the production of seed potato have been employed over the last 20 years. These technologies include hydroponics, semi-hydroponics, and aeroponics [4,8].

Aeroponics is a modern, soilless technology for the production of minitubers. In the aeroponic cultivation system, the underground parts (roots and underground stems) of potato plants are situated in a dark chamber, called the module, suspended in the air, and supplied with water and nutrients through a nutrient solution dispersed in the form of fine fog particles (30–100 microns), while the foliage is grown above the module under greenhouse conditions [4]. Minitubers are produced on underground stems, which are called stolons. Minituber production in aeroponics has significant advantages over other used systems or technologies. Firstly, tubers grown in an aeroponic system are well-protected from pests and soil-borne diseases since the systems are soilless and located in insect- and pest-free greenhouses. These systems enable the production of a high number of minitubers per plant during the growing period, which is usually prolonged for one to two months [9]. Depending on genotype/cultivar and aeroponic system, potato plants can produce 30–170 minitubers per production period [4,10]. Besides, successive harvesting allows minitubers to reach their desired and uniform size. Due to the recirculation of nutrient solution, minimal environmental pollution and efficient usage of space, aeroponic technology enables the production of potato minitubers, as well as cultivation of other vegetable and ornamental plants [11], in an environmentally friendly manner.

To optimize aeroponic systems for minituber production, the effects of various factors were studied, including the type of planting material, planting density, harvest intervals, the composition of nutrient solution, regulation of fertigation, and temperature conditions in the aeroponic module [4]. Different types of plant material can be used for the aeroponic production of potato minitubers. Besides acclimatized microplants and microtubers from in vitro culture, the starting plant material may be rooted stem cuttings or shoots obtained from microplants after acclimatization, and rooted sprouts from previously collected minitubers [12–15]. Planting density (number of plants per m^2 of a module) may also vary, with the best plant productivity obtained at relatively low densities of 24–60 plants per m^2 [4,16–18]. Harvesting is an important practice in the aeroponic production of minitubers, which frequency depends on potato cultivar, the desired size of minitubers, and the duration of the growing season. Various harvest intervals were reported in the literature, ranging from 7 to 20 days [4,16,19].

The aim of this study was to evaluate the effects of the cultivar and type of planting material on the production of minitubers in the aeroponic facility in Guča, Serbia, at a relatively short, 7-day harvest interval. Five potato cultivars: Agria, Cleopatra, Désirée, Kennebec, and Sinora, were the objects of our study, and two types of planting material—acclimated microplants and plants originated from minitubers—were used. In addition, we investigated the effects of temperature in the aeroponic module during the pre-harvest period on the number and mass of minitubers at harvest.

2. Materials and Methods

2.1. Plant Material and Establishment of In Vitro Culture

Five commercial *S. tuberosum* L. cultivars, Agria, Cleopatra, Désirée, Kennebec, and Sinora, were used in experiments. These cultivars are popular and frequently used in commercial potato production in Serbia. Virus-free tubers were obtained from Arum Deč (Belgrade, Serbia) and HZCP-Srbija (Guča, Serbia). In vitro cultures were established from surface-sterilized tuber sprouts as described by Momčilović et al. [20]. Sprouts were transferred on the basal medium (BM), consisting of Murashige and Skoog salt mixture [21], Linsmaier and Skoog vitamins [22], 0.7% agar, 3% sucrose, 100 mg L^{-1} myo-inositol, and supplemented with 0.5 mg L^{-1} 6-benzylaminopurine (BAP; Sigma Aldrich, St. Louis, MO, USA). Shoots obtained on this medium gave rise to plantlets when transferred to BM without BAP. Microplants were grown in a climate-controlled room (21 °C, 16 h photoperiod, light flux 90 µmol m^{-2} s^{-1}) and were regularly subcultured every 30 days by single-node stem cuttings.

2.2. Preparation of Plants for Aeroponic Cultivation

Plants used for the aeroponic experiment were obtained from in vitro stock or sprouted minitubers. Microplants were transferred to the substrate of sand and perlite (4:1) and acclimated in the insect-free greenhouse 20 days before planting in aeroponics. Minitubers (aeroponic crop from the previous season) were also planted in the substrate of sand and perlite (4:1) 35 days before the obtained plants were transferred to the aeroponic module. Plants from both sources were regularly watered and treated with a nutrient solution before being transplanted into an aeroponic module on 11 May 2020. The roots of the plants were thoroughly washed with water, the stems were placed in the holders, and the holders were positioned in the module.

2.3. Minituber Production in Aeroponic System

The experiment was conducted in 2020 (11 May–30 November) at RZ Plant & Potato Research Center aeroponic facility (Guča, Serbia), comprising an aeroponic system [23] placed in an insect-free, plastic greenhouse with screened ventilation openings. The aeroponic system was operated according to the fertigation regime previously described by Broćić et al. [24]. During the first 30 days after transplanting (DAT), the plants were treated with a nutritive solution that comprised Poly-Feed GG 11−44−11 with micronutrients (Haifa Group, Haifa, Israel) at a concentration of 1 g L^{-1}. From 30 DAT until the end of the cultivation period, plants were fertigated with nutritive solution based on Poly-Feed GG 11−12−33 + 2 MgO with micronutrients (Haifa Group, Haifa, Israel) in at a concentration of 1 g L^{-1}. The nutritive solution was replenished every 15 days. During the day, a nutrient solution was applied for 20 s every 5 min from 8:00 to 18:00, and during the night, for 20 s every 10 min from 18:00 to 8:00. The planting density in the aeroponic module was 24 plants per m^2. The experimental layout was a complete randomized block design with 3 replications for each cultivar-plant origin combination and 10 plants per replica. Except for the first harvest, the minitubers were collected at a 7-day interval (from June to December). Harvests were labeled I–XXIV considering all cultivars (Figure 1), although some cultivars were not forming minitubers at a particular harvest. The number of collected minitubers (tuber length $\geq$ 2 cm) per plant and mass of collected minitubers were measured at the end of each harvest interval. In addition, the cultivars' productivity parameters (total number of produced minitubers per plant, tuber mass, and yield) were quantified at the end of the cultivation period. For calculating the yield, three replications (yield of 24 plants m^{-2}) were used.

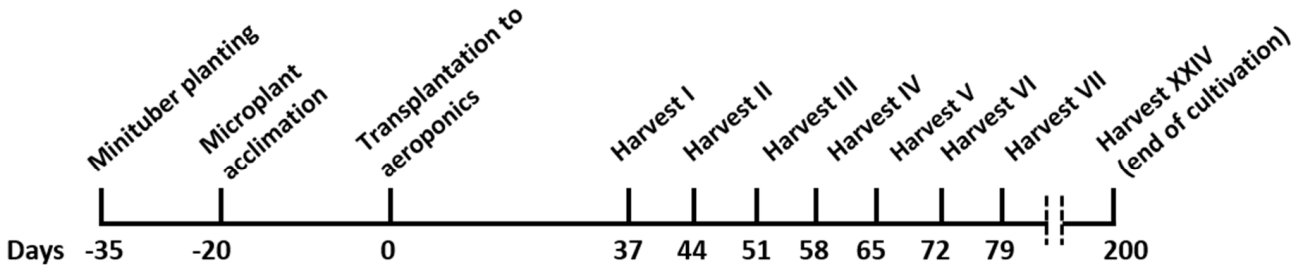

Figure 1. Scheme of the activities before and during aeroponic cultivation of the potato cultivars.

During the experiment, the temperature was recorded in the module (root zone) and greenhouse (haulm and leaves zone). The average daily temperatures are presented in Figure 2.

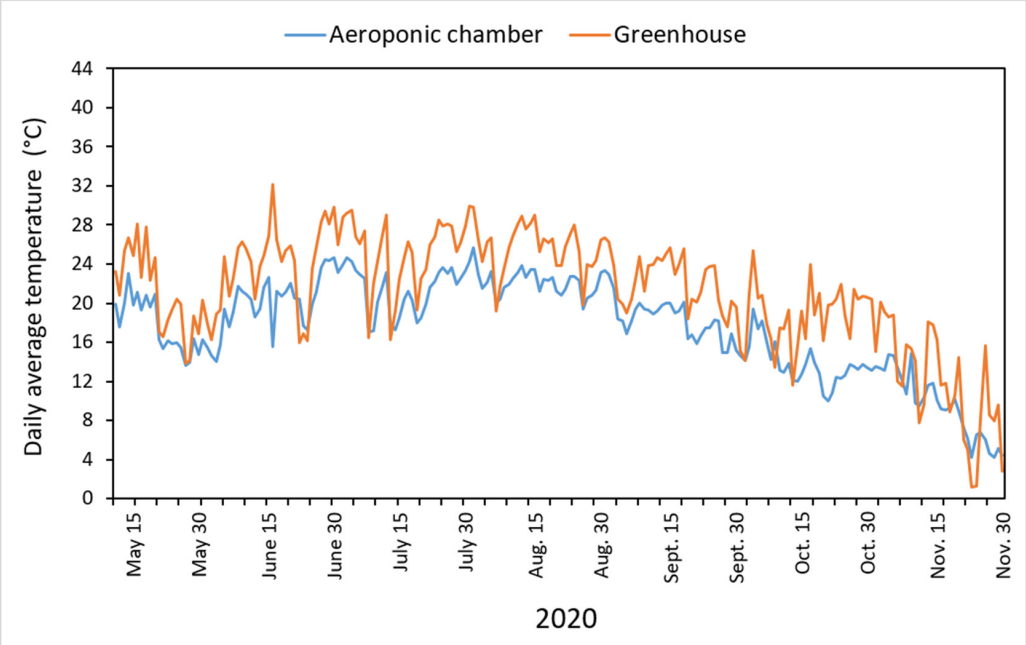

Figure 2. Temperature conditions in the aeroponic module and plastic greenhouse during the experimental period in 2020.

2.4. Statistical Analysis

Statistical analysis was performed using STATISTICA 12 (StatSoft, Inc. 1984–2014, Tulsa, OK, USA). The data regarding the number of minitubers per plant, the minituber's mass, and yield were analyzed using a two-factor analysis of variance (ANOVA) with plant origin and cultivar as the categorical predictors. After that, a secondary statistical analysis, such as homogeneity of variances (Levene's test), was conducted, and the means of the factors combination were compared using Tukey's multiple comparison test. The mean number of minitubers per plant or minituber mass at each harvest was correlated with a cumulative thermal time during the pre-harvest period (the sum of daily average temperatures for 7 days preceding harvest). Regression analysis was conducted and Pearson's correlation coefficient was calculated. For the data analysis, the significance level $p < 0.05$ was used.

3. Results

3.1. Potato Cultivation in an Aeroponic System

Plants of five potato cultivars, Agria, Cleopatra, Désirée, Kennebec, and Sinora, of different origins (in vitro culture or minitubers), were used in experiments. Microplants were acclimated in the greenhouse for 20 days before being transferred to an aeroponic system (Figure 3A). Plants developed from sprouted minitubers (Figure 3B) were transplanted to aeroponics 35 days after tuber-sowing. Plants of different origins varied in some morpho-anatomical features before transfer to aeroponics; plants that originated from in vitro culture formed several shoots, while plants developed from minitubers possessed one, rarely two, vigorous primary shoots (Figure 3A,B). During aeroponic cultivation, plants originated from minitubers developed more verdant foliage and a bulkier root system than acclimated microplants. Regardless of origin, tuberization of Cleopatra and Sinora plants started approximately 21 DAT to aeroponics, while Agria, Désirée, and Kennebec plants began producing tubers 28–35 DAT.

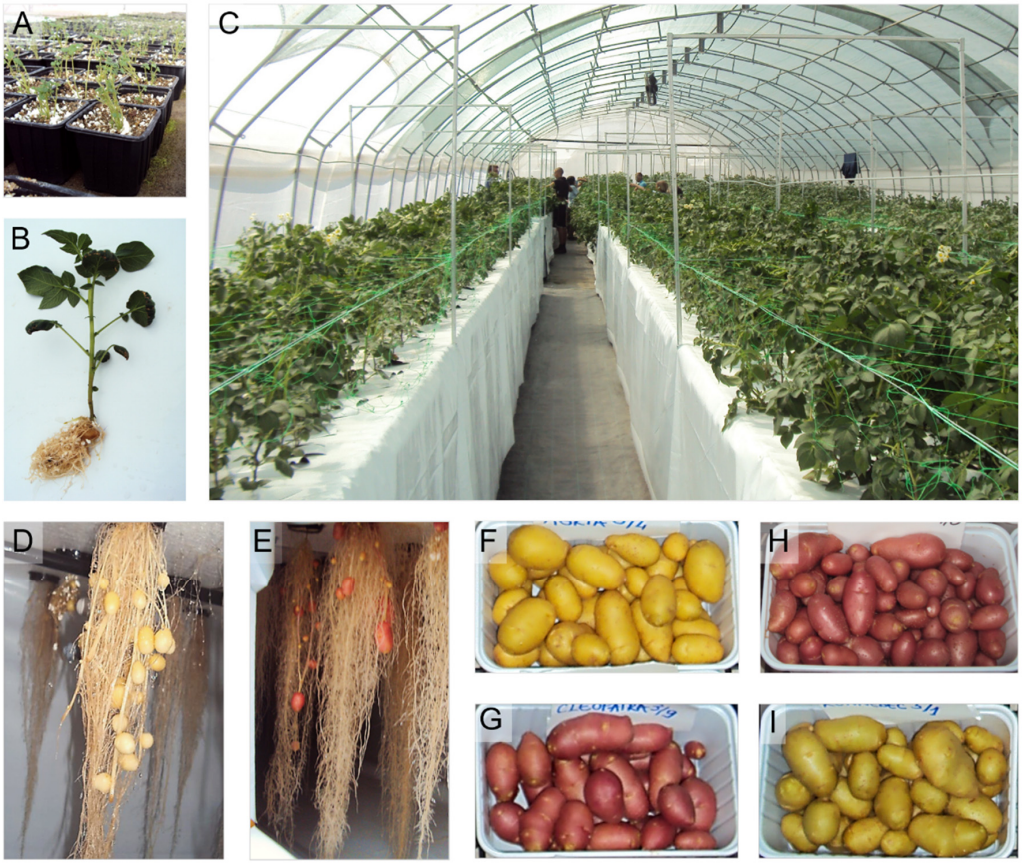

Figure 3. Cultivation of potato in the aeroponic system. (**A**) Plants obtained from in vitro culture at the beginning of acclimation in the greenhouse; (**B**) Plants developed from sprouted minitubers before transfer to aeroponic system; (**C**) Aeroponic facility for minituber production in Guča, Serbia; (**D**,**E**) Aboveground parts of potato plants with formed minitubers; Minitubers collected from (**F**) cv. Agria, (**G**) cv. Cleopatra, (**H**) cv. Désirée, and (**I**) cv. Kennebec plants.

3.2. Dynamics of Minituber Formation

The dynamics of minituber formation differed between the five investigated cultivars. In mid-June (I harvest), minitubers were formed only by Cleopatra's and Sinora's plants regardless of their origin. The first minitubers of Agria and Désirée were collected in the second half of June (II harvest), while the first Kennebec minitubers were gathered at the end of June 2020 (III harvest).

Concerning plants of minituber origin, the highest rates of minituber formation in Sinora and Cleopatra were measured after the III–X harvest, in Désirée after the V–XIII harvest, and Kennebec and Agria after the XIV harvest (Figure 4A). Plants of Sinora and Cleopatra quickly reached the final number of formed tubers (X–XI harvest). Agria and Kennebec plants were steadily forming tubers during the cultivation period (Figure 4A). Regardless of the type of planting material, Agria and Kennebec formed the highest number of minitubers during the final harvest interval (between the XXIII and XXIV harvest).

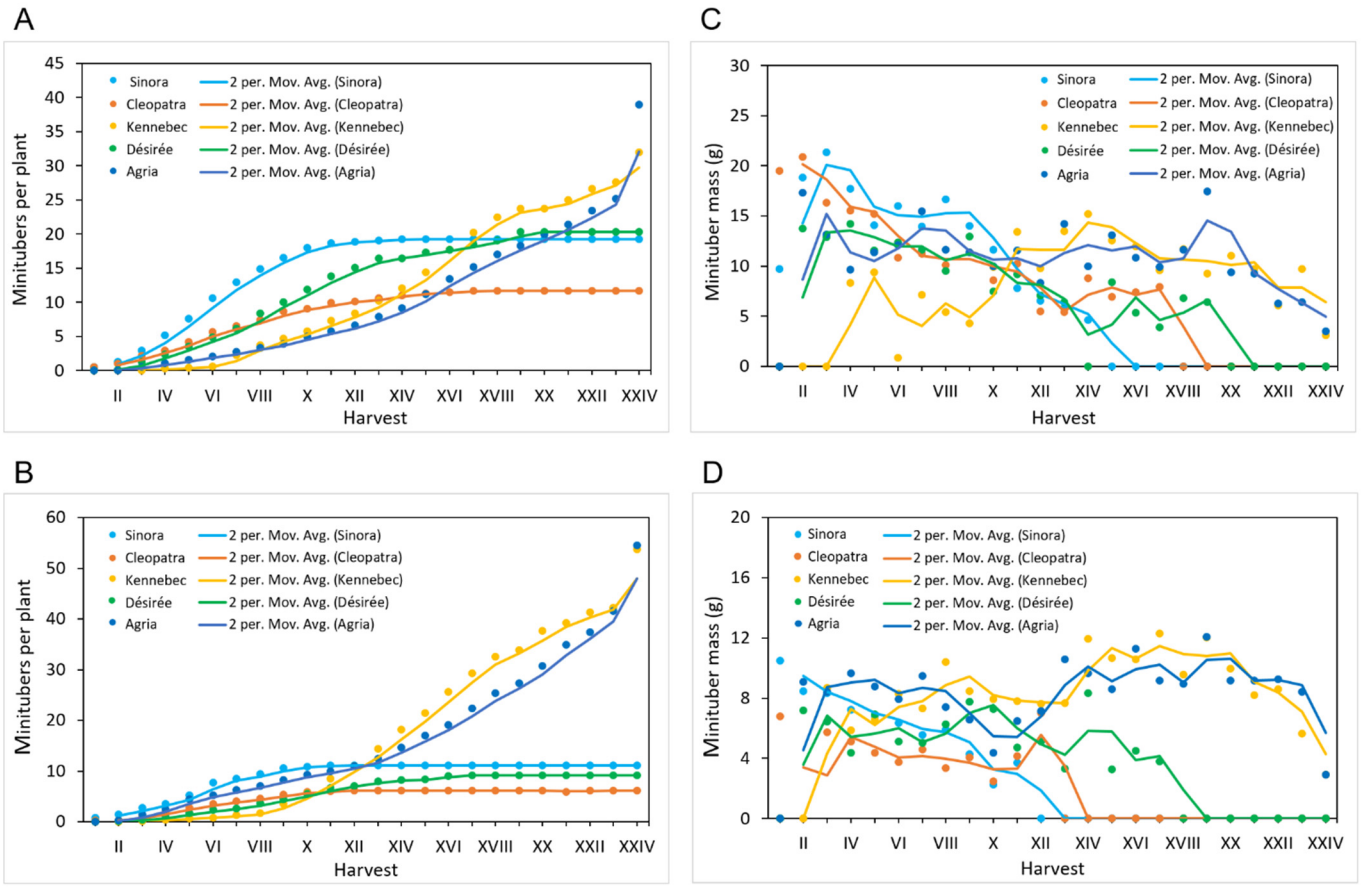

Figure 4. The dynamics of minituber formation and tuber mass at harvest for five potato cultivars grown in the aeroponic system. (**A**) The cumulative number of formed and collected minitubers per plant of minituber origin; (**B**) The cumulative number of minitubers per plant of in vitro origin; (**C**) Minituber mass per harvest—plants of minituber origin; (**D**) Minituber mass per harvest—plants of in vitro origin. Data represent the mean values of the three replicates (n = 10).

Considering plants of in vitro origin, rates of minituber formation were minor in Sinora, Cleopatra, and Désirée and the final number of 6–11 minitubers per plant was reached in the first half of the cultivation period (Figure 4B). Sinora, Cleopatra, and Désirée's plants of in vitro origin formed their final number of tubers, respectively, at 3, 5, and 2 harvest intervals earlier than plants of minituber origin. This was in concordance with the observed faster senescing of in vitro originated plants compared to plants of minituber origin. Conversely, Agria and Kennebec plants of in vitro origin had the highest tuberization rates in the second half of the cultivation period. The final number of minitubers collected per plant of these cultivars were 53.8 and 54.5 in Kennebec and Agria, respectively.

Although the fresh mass of collected minitubers at the harvests varied during the cultivation period for each investigated cultivar, some regularities were observed. Considering Sinora, Cleopatra, and Désirée plants of both minituber and in vitro origin, the highest masses of tubers were recorded at the first several harvests (Figure 4C,D). Conversely, the highest masses of Kennebec minitubers at harvest were measured in the second half of the cultivation period. Agria plants of both origins showed the least variation in the microtuber mass between harvests. The observed drop in Agria and Kennebec minituber mass at the last harvest most likely resulted from the fast maturation caused by low temperatures in the environment.

3.3. Effects of Cultivar and Plant Origin on Minituber Production

Results of the two-way ANOVA in Table 1 show the effects of cultivar, plant origin, and their interaction on minitubers production in an aeroponic system. The results of Levene's test indicate that the assumption of homogeneity of variances was not violated (Table 2). Therefore, conventional parametric statistics were used for further analysis, and the result of the analysis of variance is presented in Table 3, indicating significant differences between groups in their mean score achievement on the number and mass of minitubers, as well as yield. The significant differences in the mean achievement score of the groups were further investigated by the analysis of multiple comparisons using the post-hoc test, and the results are shown in Table 4.

Table 1. Two-way ANOVA results of the influence of cultivar, plant origin, and interaction between cultivar and plant origin on potato minituber production in an aeroponic system based on a factorial experiment.

Parameter	Factor	df	SS	MS	F	*p*	Sig.
Number of minitubers per plant	Cultivar	4	7151.313	1787.828	109.663	2.63×10^{-13}	***
	Plant origin	1	74.419	74.419	4.565	4.52×10^{-2}	*
	Cultivar × Plant origin	4	1569.557	392.389	24.069	2.10×10^{-7}	***
Minituber mass	Cultivar	4	68.338	17.084	8.505	3.53×10^{-4}	***
	Plant origin	1	145.332	145.332	72.350	4.46×10^{-8}	***
	Cultivar × Plant origin	4	58.251	14.563	7.250	8.88×10^{-4}	***
Yield per m^2	Cultivar	4	244.702	61.176	60.483	6.90×10^{-11}	***
	Plant origin	1	9.905	9.905	9.792	5.28×10^{-3}	**
	Cultivar × Plant origin	4	78.390	19.598	19.376	1.18×10^{-6}	***

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

Table 2. Test of homogeneity of variance.

Parameter	Levene's Statistic	df1	df2	*p*	Sig.
Number of minitubers per plant	2.475	9	20	0.068	ns
Minituber mass	0.833	9	20	0.595	ns
Yield per m^2	1.845	9	20	0.147	ns

Note: ns—non-significant at 0.05 probability level.

Table 3. The analysis of variance.

Parameter	Factor	df	SS	MS	F	*p*	Sig.
Number of minitubers per plant	Between groups	9	8795.288	977.254	59.943	0.000	***
	Within groups	20	326.060	16.303			
	Total	29	9121.348				
Minituber mass	Between groups	9	271.920	30.213	15.041	0.000	***
	Within groups	20	40.175	2.009			
	Total	29	312.095				
Yield per m^2	Between groups	9	332.941	36.993	36.568	0.000	***
	Within groups	20	20.233	1.012			
	Total	29	353.173				

Note: *** $p < 0.001$.

Table 4. Mean comparison for the effects of cultivar and plant origin interaction on the potato minituber production in an aeroponic system.

Cultivar	Plant Origin	Minitubers per Plant	Minituber Mass (g)	Yield per m^2 (kg)
Sinora	Minituber	19.33 ^{bc}	15.48 ^{a}	7.17 ^{cd}
	In vitro	11.16 ^{ab}	8.07 ^{cde}	2.17 ^{ab}
Cleopatra	Minituber	11.75 ^{ab}	12.28 ^{ab}	3.43 ^{ab}
	In vitro	6.25 ^{a}	4.54 ^{e}	0.69 ^{a}
Désirée	Minituber	20.37 ^{bc}	9.51 ^{bcd}	4.61 ^{bc}
	In vitro	9.16 ^{ab}	5.69 ^{de}	1.23 ^{a}
Kennebec	Minituber	28.58 ^{cd}	10.57 ^{bc}	7.32 ^{cd}
	In vitro	53.79 ^{e}	8.13 ^{cde}	10.50 ^{e}
Agria	Minituber	39.08 ^{d}	8.17 ^{cde}	7.56 ^{d}
	In vitro	54.50 ^{e}	7.57 ^{cde}	9.76 ^{de}

Note: Homogeneous groups are labeled with the same letter (a–e).

The total number of minitubers formed per potato plant was affected by the investigated factors of cultivar and plant origin (Table 1). A significant two-way interaction of cultivar: plant origin (Table 1) indicated that the effect of plant origin differs between the cultivars. Indeed, the post-hoc analysis between multiple groups shows that the plant origin did not significantly affect the number of formed minitubers at the 7-d harvest interval in Sinora, Cleopatra, and Désirée while it caused significant changes in minituber number in Kennebec and Agria (Table 4). Kennebec and Agria plants of in vitro origin formed a significantly larger number of minitubers than plants originated from minitubers.

The minituber mass was also affected by factors cultivar and plant origin (Table 1). Significant two-way interaction of cultivar: plant origin indicated that the effect of plant origin differs between cultivars. The mass of formed minitubers was not significantly different in Désirée, Kennebec, and Agria plants of different origin, while Sinora and Cleopatra plants of minituber origin formed in average heavier tubers than plants of in vitro origin (Table 4). The heaviest minitubers were formed on Sinora and Cleopatra plants originating from tubers, 15.48 g and 12.28 g, respectively.

The yield was affected by the investigated factors of cultivar and plant origin (Table 1). Again, significant two-way interaction of cultivar: plant origin indicated that the effect of plant origin on potato yield differs between cultivars. Indeed, plant origin did not affect yield in Cleopatra and Agria, but significantly affected the yield in other three cultivars (Table 4); Sinora and Desiree in vitro-obtained plants had a lower yield per m^2 than minituber-propagated plants, while Kennebec plants of in vitro origin had higher yield than minituber-propagated plants. The highest yield of 10.50 kg m^{-2} had Kennebec plants of in vitro origin.

3.4. Effects of Temperature in the Aeroponic Module on Tuber Formation

In the course of experiment, it was also detected that temperatures during the pre-harvest period influenced the number or mass of collected minitubers at consequent harvest in some of the cultivars. A number of minitubers per plant or mass of minitubers collected at each harvest were correlated with a cumulative thermal time for preceding 7 days (pre-harvest period). For particular cultivars, early harvests where plants still did not produce tubers or late harvests where plants already completed their life cycle were not calculated.

Significant negative correlation was found between a number of minitubers collected per plant at harvest and a cumulative thermal time of pre-harvest period in cultivar Agria and Kennebec, regardless of plant origin (Figure 5A,B). In other cultivars, mostly moderate or week positive correlations between a number of minitubers collected per plant and pre-harvest cumulative thermal time were found (Figure 5C,E), which were insignificant at $p < 0.05$.

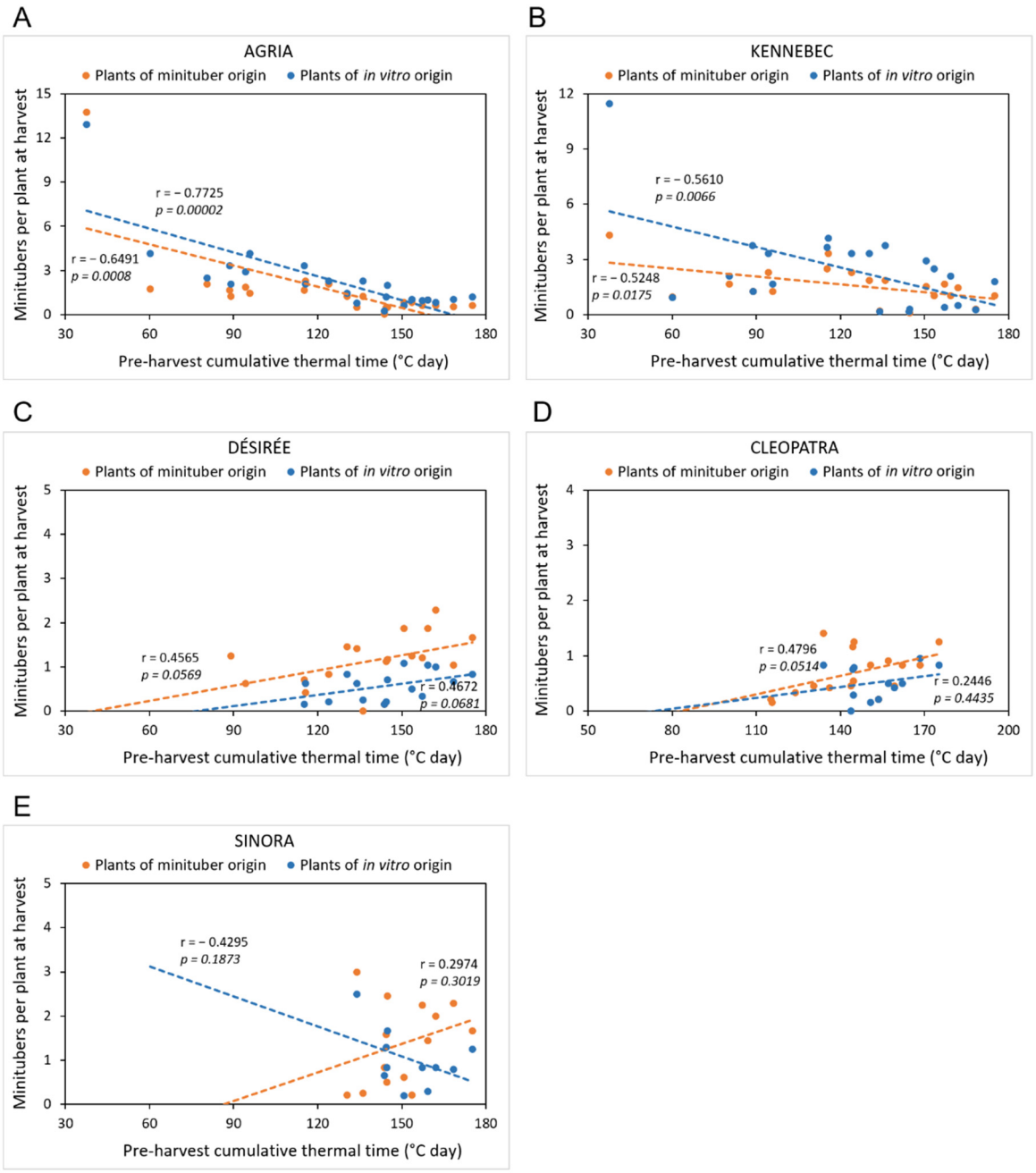

Figure 5. Effect of temperature during pre-harvest period on the number of formed and collected minitubers per potato plant at subsequent harvest. The mean number of minitubers collected per plant of (**A**) cv. Agria, (**B**) cv. Kennebec, (**C**) cv. Désirée, (**D**) cv. Cleopatra, and (**E**) cv. Sinora at each harvest was correlated with a cumulative thermal time for 7 days preceding harvest (pre-harvest cumulative thermal time). Pearson's correlation coefficient was calculated and a significant difference was determined at a confidence level of $p < 0.05$.

Significant positive correlation was found between a minituber mass per harvest and temperature during a pre-harvest period in cultivar Désirée, regardless of plant origin (Figure 6C). While insignificant, mostly very weak correlations were observed in other cultivars.

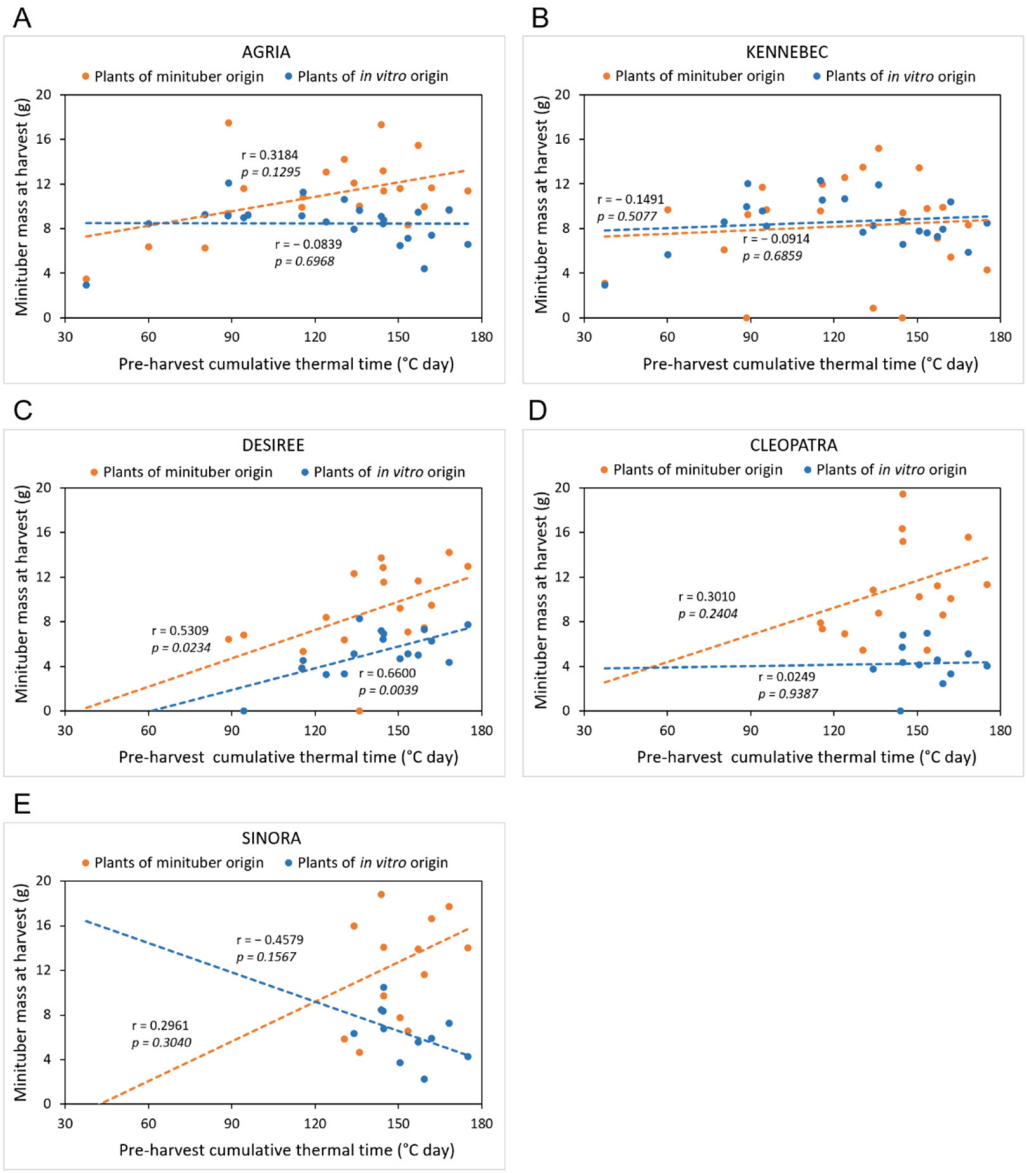

Figure 6. Effect of temperature during the pre-harvest period on the mass of formed and collected potato minitubers at subsequent harvest. The mean number of minitubers collected from (**A**) cv. Agria, (**B**) cv. Kennebec, (**C**) cv. Désirée, (**D**) cv. Cleopatra, and (**E**) cv. Sinora at each harvest was correlated with a cumulative thermal time for 7 days preceding harvest (pre-harvest cumulative thermal time). Pearson's correlation coefficient was calculated and a significant difference was determined at a confidence level of $p < 0.05$.

4. Discussion

The main problems in the current, conventional production of seed potatoes are the low reproduction rate and variable size of minitubers which can be overcome by utilization of aeroponics. In the present study, we investigated the production of sizeable minitubers (tuber length $\geq$ 2 cm) using five potato cultivars—Agria, Cleopatra, Désirée, Kennebec, and Sinora—cultivated in an aeroponic system at a planting density of 24 plants per m^2, and at relatively short 7 day-harvest intervals. Acclimated microplants and plants developed from sprouted minitubers were used as the starting plant material.

The dynamics of minituber formation varied between the five investigated cultivars. Cleopatra's and Sinora's plants of both in vitro and minituber origin first started with tuberization and first reached the final number of minitubers (Figure 4A,B). This was related to the fast maturation of Sinora and Cleopatra plants. Both cultivars are characterized as early maturing plants with a vegetation period of 85–100 days in open field production in Serbia [21], and it seems that this developmental trait was unaffected by aeroponic cultivation, although the plants' vegetation in aeroponics was slightly prolonged in Sinora (109–120 DAT) and more evidently in Cleopatra (116–145 DAT). Plants of Agria and Kennebec did not form minitubers before the second and third harvest, respectively, and afterwards they steadily tuberized during the growing season (200 DAT), which ended because of low temperatures in the aeroponic module during the last week of November (4.2–6.8 °C average daily temperatures). Consequently, fast plant maturation occurred during the last harvest interval, and the largest number of minutubers per plant were collected at the final XXIV harvest. Kennebec and Agria are characterized as medium-late to late cultivars with a vegetative cycle of 120–135 days [25] that was significantly prolonged by aeroponic cultivation. Interestingly, the late maturing Désirée (~130 days) showed dynamics similar to early maturing potato cultivars (Figure 4A,B) and also prolonged vegetation in aeroponics, spanning 151 DAT for plants of in vitro origin and 165 DAT for plants of minituber origin. All these findings confirm that the vegetation period of the plants can be prolonged by aeroponic growing in different potato genotypes. According to Otazú [9], aeroponic cultivation prolongs the potato plants' vegetation period by one to two months. Mateus-Rodriguez et al. [26] reported a significant increase in the duration of a vegetative cycle for 10 investigated potato genotypes grown in aeroponics compared to the length expected under field conditions, with the genotypes Venturana and Serranita taking up to 291.3 days to senescence. Tierno et al. [27] reported that plants in the aeroponic system showed increased growth and their vegetative cycle was prolonged 12–36% compared to the plants cultivated in greenhouse beds. The type of planting material also affected the dynamics of minituber formation in the investigated cultivars. Sinora, Cleopatra, and Désirée's plants of in vitro origin reached the final number of minitubers and the vines started yellowing much earlier than the vines from plants of minituber origin. This was most likely related to the more vigorous growth of plants obtained from minitubers.

Regarding minituber fresh mass, the highest masses of tubers were recorded at the first several harvests in Sinora, Cleopatra, and Désirée and during the second half of the cultivation period in Agria and Kennebec (Figure 4C,D). A significant decline in Agria and Kennebec minituber mass at the last XXIV harvest (Figure 4C,D) resulted from accelerated plant maturation due to low temperatures in the aeroponic facility when a large number of lighter minitubers was formed per plant.

The results of our study revealed a strong influence of the type of planting material (plant origin) and genotype (cultivar) on the total number of minitubers produced by plant, minituber mass, and total yield of potato grown in aeroponics (Tables 1 and 4). Muthoni et al. [13] also reported a significant effect of the type of planting material and cultivar on the aeroponic production of minitubers. The authors investigated two cultivars (Asante and Tigoni) and three types of planting material (microplants, stem cuttings, and minitubers) and reported that cv. Tigoni and plants of in vitro origin produced the largest number of minitubers in two successive growing periods. In our study, Agria and Kennebec plants obtained from in vitro culture produced a significantly larger number of minitubers and had better total yield than plants of other cultivars and origin; the total number of minitubers per plant was 54.5 and 53.8 in Agria and Kennebec, respectively. The plants of minituber origin also produced a relatively large number of tubers: 39.1 and 28.6 in Agria and Kennebec, respectively. Our previous study reported that in vitro obtained plants of Agria and Kennebec produced 18.9 and 19.1 minitubers, respectively, while Agria and Kennebec plants of minituber origin produced 19.9 and 14.7 minitubers, respectively, when grown in the same facility in 2018 [14]. The major differences between the two growing years were the transplanting time and harvest interval. In 2020, plants were transferred to the aeroponic

system 45 days earlier, and the harvest intervals were shortened from 14 to 7 days. A longer growing period and more frequent harvests in 2020 most likely caused a 2.4-fold increase in the number of minitubers produced by Agria and Kennebec. This is in accordance with findings that frequent harvests promote the initiation of novel tubers and enlargement of uncropped tubers for future collecting [28]. Farran and Mingo-Castel [16] induced an increase in the number of formed minitubers and minituber mass in cv. Zorba by reducing the harvest interval from 14 to 7 days during the aeroponic cultivation at a density of 60 plants m^{-2}. Interestingly, shortening of the harvest interval did not promote tuberization in Cleopatra, whose which plants of in vitro and minituber origin (Table 4) produced 2.5- and 1.2-fold less minitubers in 2020 than in 2018, respectively [14]. Taken together, the start and length of the growing season, as well as the harvesting frequency, have to be optimized for each potato cultivar/genotype grown in a particular aeroponic facility.

In the course of the experiment, we also detected that temperatures in the aeroponic module might have influenced the tuberization of some of the cultivars. A higher average daily temperature during the pre-harvest periods, represented by the cumulative thermal time (Figure 5), might be responsible for a reduced number of formed and collected minitubers in Agria and Kennebec at harvests in the first half of the cultivation period. This is in concordance with the findings that even moderately elevated temperature reduces tuberization in potatoes [29,30]. The estimated optimal temperature for maximum rates of tuber induction is 14 °C, tuber initiation 22 °C, and tuber bulking 14–22 °C [31]. Interestingly, a significant positive correlation was found between a minituber mass at harvest and cumulative thermal time during a pre-harvest period in cultivar Désirée (Figure 6). However, cultivar Désirée, as well as Sinora and Cleopatra, were characterized by a significantly shorter vegetative cycle compared to Agria and Kennebec, and the effect of lower average daily temperatures (lower pre-harvest cumulative thermal times) on the number of formed minitubers and minituber mass during the second half of the cultivation period was impossible to investigate.

5. Conclusions

The results of our study signify the specific requirements of particular potato cultivars/genotypes regarding aeroponic cultivation. An earlier transfer to the aeroponic system, short harvest intervals and use of acclimated microplants as a starting material promoted minituber production and increased the yield of Agria and Kennebec, but not the other investigated cultivars. Our future research will be focused on the further optimization of conditions for aeroponic production of potato minitubers, including approaches to regulate or mitigate the effects of elevated temperature in the aeroponic module.

Author Contributions: Conceptualization, Z.B.; methodology, Z.B. and J.O.; validation, J.R., D.P. and I.M.; formal analysis, Z.B., I.M.; investigation, Z.B., J.O., D.P., J.R. and I.M.; resources, J.R. and D.P.; data curation, Z.B. and J.O.; writing—original draft preparation, Z.B.; writing—review and editing, I.M., J.O., D.P. and J.R.; visualization, J.R. and I.M.; supervision, I.M.; project administration, Z.B. and I.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Education, Science, and Technological Development of the Republic of Serbia, contract numbers 451-03-68/2022-14/200116 and 451-03-68/2022-14/200007.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Acknowledgments: We would like to thank RZ Plant & Potato Research Center, Guča, Serbia, for kindly allowing us to use their aeroponic facility.

Conflicts of Interest: The authors declare no conflict of interest.

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