



Article Mycorrhizal Inoculation Effect on Sweet Potato (*Ipomoea batatas* (L.) Lam) Seedlings

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Abstract: Sweet potato is an increasingly significant crop and its effective and sustainable cultivation has become important in temperate countries. The purpose of this pilot study was to investigate the effects of a mycorrhizal inoculum, Symbivit, and whether it could establish a symbiotic relationship with the seedlings of two sweet potato varieties (orange and purple). The effectiveness of the mycorrhizal inoculation with a sterilized substrate on the mycorrhizal parameters (F%, M%, m%, a%, A%) and physical parameters "[length of roots and shoots (cm), the fresh weight of shoots and roots (g) as well as the length of stem (cm)]" on the sweet potato seedlings has also been studied. Results show that the sterilization treatment with Symbivit in both varieties increased the frequency of mycorrhiza in the root system. For the intensity of the mycorrhizal colonization in the root fragments and the arbuscular abundance, there was a difference between the mycorrhizal inoculum and the sterilization treatment among the varieties. Overall, the preliminary results provided remarkable information about mycorrhizal inoculation, substrate sterilization on mycorrhizal development, as well as changes in the physical parameters between sweet potato seedlings. Our results could serve as a practical strategy for further research into adding significance to the effect of the beneficial soil microbes on sweet potatoes.

Keywords: mycorrhizal parameters; physical parameters; sterilization; sweet potato seedling; varieties

1. Introduction

Sweet potato (*Ipomoea batatas* (L.) Lam.) belongs to the Convolvulaceae family [1]. Currently, this plant is mostly grown in tropical and subtropical areas, with adequate water supply [2]. In 2019, 51, 992, 156 tons of sweet potato were produced in China [3]. Sweet potato contains many secondary metabolites, particularly antioxidant compounds including anthocyanins, carotenoids and vitamin C [4,5]. On the other hand, tubers also contain high amounts of minerals likes zinc, potassium, sodium, manganese, calcium, magnesium, and iron. They are also rich in carbohydrates and fibre [6].

Sweet potato can be grown in loam, clay loam and sandy loam soils. However, sandy loam with clay subsoil is one of the best for the growth of the sweet potato. Heavily clayed soil restricts root development, while sandy soil encourages the elongation of the root into the deep soil [7]. Worldwide, sweet potato has a lot of varieties and usually, countries have their own varieties. For instance, in the USA, more than 50 varieties are grown [8].

Mycorrhizal fungi and host plant symbiosis were important in the evolution of terrestrial plants and this relationship can be detected in more than 80% of vascular plant



Citation: ALHadidi, N.; Pap, Z.; Ladányi, M.; Szentpéteri, V.; Kappel, N. Mycorrhizal Inoculation Effect on Sweet Potato (*Ipomoea batatas* (L.) Lam) Seedlings. *Agronomy* **2021**, *11*, 2019. https://doi.org/10.3390/ agronomy11102019

Academic Editor: Samantha C. Karunarathna

Received: 1 August 2021 Accepted: 2 October 2021 Published: 8 October 2021

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Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). species [9]. The first fossils documenting the mutualistic association between plants and soil fungi known as arbuscular mycorrhiza (AM) with more than 400 million years old [10]. AM fungi belong to the phylum Glomeromycota [11] and it is a form of endomycorrhiza, which means that internal hyphae penetrate the root cells [12]. AM fungi are biostimulants and they occur everywhere in soil ecosystems [13]. Most essential nutrients that are important for the host plants such as phosphate, nitrogen, potassium and sulfate can be obtained by the fungal hyphae through different transporters [14].

One more beneficial function of AM symbiosis is that the arbuscular mycorrhizal fungi can increase the tolerance of the host plant for environmental stress such as drought, salinity and heavy metals and this leads to improved growth and development in unfavourable conditions [15]. Numerous mechanisms have been suggested to interpret the host plant protection effects of AM symbiosis from environmental stress, such as changes in plant hormones, the boost of the photosynthetic ratio and ROS scavenging [16].

One of the most destructive environmental stressors is drought, which limits crop growth and yield [17]. When a plant is exposed to drought stress, its membrane system becomes damaged and water dispersal becomes irregular [18]. In addition, the formation of reactive oxygen species such as superoxide radical (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) intensifies [19]. Reactive oxygen species are manufactured through the respiration of the mitochondria and during photosynthesis in the chloroplast [20].

Sweet potato can be grown in two ways: from tubers and shoots. Growing from tubers is done with seeds of 20–50 g sown to a depth of 3 cm, but this method results in a significantly lower yield. The widespread method of using cuttings is more preferable to propagate the plant so that to achieve higher yield and more favourable shape and size tubers [21].

Sweet potato can be propagated vegetatively by planting cuttings with sprouting tubers [22]. It is important to use first-class tubers in order to preserve varietal characteristics [23]. The first-class tubers must be in good shape, free from pathogens and pests and they should receive appropriate treatment before storage, which must be carried out separately from the other tubers [24].

To our knowledge, little is known in the literature concerning the influence of arbuscular mycorrhizal fungi on sweet potato varieties. Thus, our focus of in this pilot study is quite new as it highlights the differences of two sweet potato varieties (orange and purple) in various parallel tests such as the applied inoculation method. The main aims of the current study are to investigate if the mycorrhizal inoculum is effective in developing a symbiotic relationship with sweet potato (*Ipomoea batatas* (L.) Lam.) roots, which will affect the physiological and physical parameters of the plant; furthermore, to examine if the sterilization of the substrate has an influence on the mycorrhizal colonization.

2. Materials and Methods

2.1. Experiment Design

The experiment was set up in the spring of 2019 at Budai Campus of the Hungarian University of Agriculture and Life Sciences, at the experimental glasshouse of the Department of Vegetable and Mushroom Growing (47.28° N, 19. 04° E). Two sweet potato varieties were used: orange: "Norangel" and purple "Purple". The seedlings were grown in a plastic pot (1.5 L (11.5 cm × 12 cm × 15 cm)). For seedling production, non-treated ground/minced peat) was used as a growing medium from Latagro Basic Substrat KB2 type (white peat 100% (0–10 mm) with specification: pH value (H₂O) 6.4; soluble nutrients available to the plants: Nitrogen <7 mg/L; Phosphate <7.8 mg/L; Potassium oxid <40 mg/L). Mycorrhizal inoculation was performed with Symbivit (Symbiom Ltd. product from Sazava 170, 563 01 Lanskroun, Czech Republic, mycorrhizal, composition: five species of mycorrhizal fungi (*Claroideoglomus etunicatum, Claroideoglomus claroideum, Rhizophagus irregularis, Funneliformis geosporus, Funneliformis mosseae*), natural minerals, sapropel, extracts from sea organisms, natural keratin, humates and powdered biodegradable water-storing

polymer granules; the bioadditive part represents 110 g in each Kg of product) [25]. For 1 L of the substrate, 15 g of Symbivit was mixed.

The experiment was made with three treatments for each variety in three replications; in each treatment, 15 seedlings were used with a total of 45 seedlings. In each pot, one seedling was used (applied). Table 1 shows the code of the treatments. There was no additional nutrient replenishment during seedling cultivation. For the inoculation experiment, half of the peat moss was sterilized to detect if the sterilization has any influence on the mycorrhizal inoculation efficiency. Peat moss was sterilized at the mushroom lab in the Department of Vegetable and Mushroom Growing by a trade Raypa steam sterilizer at a temperature of 121 °C for 20 min. Temperature and humidity were measured during the experiment by Voltcraft co. DL-181THP data logger. The maximum temperature was 33.12 °C, the minimum was 16.14 °C. Maximum relative humidity was 88.44%. As a control, the soil of the experimental farm was used in which previous experiments had already shown mycorrhizal colonization (pH (KCl) 7.45; NO₂+NO₃-N (mg/kg) 71.6; P₂O₅ (mg/kg) 577; K₂O (mg/kg) 166).

Table 1. Treatments used during the experiment per variety.

Substrate	Number	Treatment Code	Treatment Description
Non-Sterilized Peat Moss	1	(L+SYM)	Non sterilized peat moss with mycorrhizal inoculum
Sterilized Peat Moss	2	(L+SYM).S	Sterilized peat moss with mycorrhizal inoculum
Soil from experimental farm (Control)	3	F	Soil from experimental farm

The roots were sampled on the 8th week after transplanting. In addition, several physical parameters were measured manually by ruler: the length of roots and shoots (cm), the length of the stem(cm) as well as the fresh weight of shoots and roots (g) of the seedlings for both varieties were measured by electronic balance. Ink based staining was carried out using the method suggested by Phillips and Hayman (1970) [26].

2.2. Determination of Colonization

Colonization was determined according to the method of Trouvelot et al. [27]. Based on the equation proposed by Trouvelot et al., all parameters of colonization were calculated and expressed as a percentage by using Mycocalc software (developed by Biro Zsombor: frequency calculations were performed using a Windows forms application written in C # and developed to facilitate the process, based on the equations of Trouvelo et al. (1986)). (F%: Frequency of mycorrhiza in the root system, M%: Intensity of the mycorrhizal colonization in the root system, m%: Intensity of the mycorrhizal colonization in the root fragments, a%: Arbuscular abundance in mycorrhizal parts of roots fragments, A%: Arbuscular abundance in the root system. Slides were prepared to check the hyphal and the arbuscular development by Zeiss Axio CAM Hr3 microscope camera.

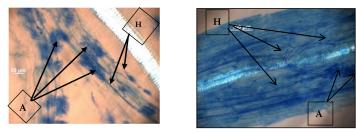
2.3. Statistical Analysis

Data analysis was carried out with IBM SPSS 25 software (Version 25.0. Armonk, NY: IBM Corp [28]. The fresh weight of shoots and roots (g), as well as the root and stem lengths, were analyzed by two-way MANOVA model with factors variety (orange and purple) and treatment levels (L+SYM).S, F, L+SYM). The normality of the residuals was tested by Shapiro–Wilk method (K (74) > 0.95; p > 0.05). Having an overall significant MANOVA result, we ran follow-up univariate ANOVA tests with Bonferroni's correction. In some cases, the homogeneity of variances was slightly violated (Levene's 0.05 > p > 0.02), so pairwise comparisons of the treatments were performed by the Games–Howell post hoc test.

3. Results

3.1. Proof of Symbiotic Relation Establishment

(Figure 1I,II) indicate that mycorrhizal inoculum (Symbivit) in a sterilized peat moss established a symbiotic relationship with sweet potato seedlings for both varieties, orange and purple. In the case of control seedlings, we could observe hyphal and arbuscular development in as well.



I: Treatment (L+SYM).S

II: Treatment control (F)

Figure 1. (**I**): Sweet potato seedlings treated with mycorrhizal inoculum in a sterilized form with the hyphal (H) and the arbuscular development (A). (**II**): Dense arbuscular formation in control sweet potato seedlings. Arbuscular mycorrhiza could also develop in both varieties when they were treated with soil from the experimental and research farm (F). The hyphal development (H) and the arbuscular establishment (A) can also be observed.

3.2. Mycorrhizal Parameters

According to Table 2, the highest frequency of mycorrhizal fungi in the root system (F%) in purple variety was found in (L+SYM).S, while the control treatment F had the lowest frequency of mycorrhizal colonization. In the orange variety, the highest frequency of mycorrhizal colonization was recorded in (L+SYM).S and the lowest one was found in treatment F. The sterilization treatment with Symbivit in both varieties orange and purple increased the frequency of colonization in the root system.

Table 2. Mycorrhizal parameters (F%, A%, a%, M% and m%) within different treatments and varieties.

Variety	Treatment	F%	M%	m%	a%	A%
purple	L+SYM	20.22	11.49	58.07	86.82	9.97
	(L+SYM).S	84.86	42.73	50.29	5.38	2.40
	F	4.52	1.25	36.50	no arbuscule	no arbuscule
orange	L+SYM	21.63	0.32	1.47	no arbuscule	no arbuscule
	(L+SYM).S	67.19	21.44	32.13	35.28	7.59
	F	15.89	2.82	18.67	no arbuscule	no arbuscule

F%: Frequency of mycorrhiza in the root system, M%: Intensity of the mycorrhizal colonization in the root system, m%: Intensity of the mycorrhizal colonization in the root fragments, a%: Arbuscular abundance in mycorrhizal parts in root fragments, A%: Arbuscular abundance in the root system.

We measured the high intensity of the mycorrhizal colonization in the root system (M%) of purple variety in (L+SYM).S treatment compared to the treatments (L+SYM) with medium and control F with low mycorrhizal intensity (Table 2). In the orange variety, the sterilization treatment (L+SYM).S had also the highest mycorrhizal intensity but the L+SYM without sterilization had the lowest intensity of the mycorrhizal colonization in the root fragments (m%).

The arbuscular abundance in the mycorrhizal parts (a%) showed that in the purple variety notably higher percentage was recorded in L+SYM than in (L+SYM). S. However, in F treatment, no arbuscules were found, although, in the orange variety, arbuscules were recorded only in L+SYM.S treatment while in L+SYM and F treatment no arbuscules were found.

As for the arbuscular abundance in the root system (A%), in the purple variety, the highest arbuscular abundance was recorded in L+SYM followed by L+SYM.S while in F treatment no arbuscules were found. For the orange variety, arbuscules were found only in L+SYM.S treatment while in L+SYM and in the control treatment no arbuscules were detected.

3.3. Physical Parameters of Sweet Potato Transplants

The two-way MANOVA resulted in significant variety and treatment effects (Wilk's lambda = 0.48, p < 0.001; Wilk's lambda = 0.18, p < 0.001, respectively) with significant interaction (Wilk's lambda = 0.64, p < 0.05). The follow-up univariate ANOVA was significant in cases of fresh weight of shoots and the length of the stem both for variety and treatment (F (1;38 > 6.48; p < 0.05) and in case of the fresh weight of the total roots for the treatment effect (F (4;38) = 7.90; p < 0.01) as well as for their interaction in case of fresh weight of shoots and the length of stem (F (4;38) > 4.88; p < 0.01). The variety effect was not significant in cases of the fresh weight of the total roots (F (4;38) < 3.15; p > 0.08) and the treatment effect was insignificant in case of the length of the roots (F (4;38) = 3.13; p = 0.06). The means and standard deviations of the four physical parameters together with the post hoc tests results are summarized in Table 3.

Table 3. Means and standard deviations of the four physical parameters fresh weight of shoots (g), fresh weight of total roots (g), length of roots (cm) and length of stem (cm) together with the post hoc tests results (Games–Howell's; p < 0.05). The different letters are for significantly different groups (lower case: comparison of treatments for fixed varieties—read vertically), upper case: comparison of varieties for fixed treatments—read horizontally).

Variety	Orange					Purple			
Parameters	Treatment	Mean	Std. Dev	Comparison of Treatments	Comparison of Varieties	Mean	Std. Dev	Comparison of Treatments	Comparison of Varieties
FW of ` shoots (g)	(L+SYM).S	4.76	0.88	а	А	3.80	2.99	a ¹	A2
	F	2.83	1.73	а	А	9.42	3.04	b ¹	B2
	L+SYM	11.43	3.85	b	А	15.81	4.32	c ¹	A2
roots (g) F	(L+SYM).S	4.89	1.14	b	А	3.88	2.38	a ¹	A2
	F	1.09	0.89	а	А	3.44	1.87	a ¹	B2
	L+SYM	4.79	1.87	b	А	5.89	2.97	a ¹	A2
Length of roots (cm)	(L+SYM).S	35.52	6.26	а	А	36.13	13.58	a ¹	A2
	F	23.17	8.69	а	А	32.74	6.54	a ¹	B2
	L+SYM	25.90	7.44	a	А	30.83	8.30	a ¹	A2
Length of stem (cm)	(L+SYM).S	35.56	12.89	a	В	14.69	5.80	a ¹	A2
	F	20.00	11.21	а	А	29.37	7.83	b ¹	A2
	L+SYM	75.32	36.27	b	А	47.69	14.72	c ¹	A2

¹ Comparison of treatments for fixed varieties—read vertically; 2 comparison of varieties for fixed treatments—read horizontally.

The highest mean of shoot fresh weight for orange sweet potato seedlings was found in L+SYM treatment (11.43 g). There was no significant difference between treatments (L+SYM). S and F, so we concluded, that the mycorrhizal inoculum could increase shoot weight by the time, while sterilization had no increasing effect on shoot weight (g) in case of treated orange sweet potato seedlings (Table 3). The highest fresh shoot weight in purple sweet potato seedlings was under the treatment L+SYM with a mean of (15.81 g) whilst (L+SYM). S treatment resulted in the lowest mean (3.80 g), i.e., the mycorrhizal inoculation also increased the fresh weight of shoot in the purple sweet potato seedlings, whereas the sterilization had no effect on the fresh weight of shoot (Table 3).

The highest weight of roots was detected in orange sweet potato seedlings with the highest mean in (L+SYM). S treatment (4.89 g), while the lowest mean was found in control treatment (F) with a mean of (1.09 g). This means, that mycorrhizal inoculation and the sterilization had a high effect on the weight of roots in orange sweet potato seedlings (Table 3).

For the weight of roots of purple sweet potato seedlings, the highest mean value was in the L+SYM treatment (5.89 g) while the lowest mean value was in F treatment with a mean (3.44 g). This is because the mycorrhizal inoculation had a stimulating effect on the fresh roots weight of purple sweet potato seedlings (Table 3).

The highest length of roots in orange sweet potato variety was measured in seedlings treated by Symbivit in sterilized substrate (peat moss) (L+SYM). The S treatment resulted in a mean value of (35.52 cm). However, there was no significant difference between the mean values when comparing the three levels of treated orange sweet potato seedlings. Therefore, mycorrhizal inoculation with sterilization had no effect on the length of the roots in the orange sweet potato seedlings (Table 3).

The highest mean of the root length (36.13 cm) was measured in the purple sweet potato seedlings propagated in a sterilized Latagro peat moss with Symbivit (L+SYM).S treatment. However, differences between the treated seedlings were not significant (Table 3).

The highest mean of stem length in orange sweet potato seedlings was measured in L+SYM treatment (75.32 cm) while the lowest mean value was in F treatment (20.00 cm). Accordingly, mycorrhizal inoculation could increase the length of the stems in the orange sweet potato seedlings. On the other hand, sterilization had no increasing effect on the length of the stems in orange sweet potato seedlings (Table 3).

In the purple sweet potato seedlings, the highest length of stem was measured in L+SYM (47.69 cm) while the lowest mean value was in treatment (L+SYM). S (14.69 cm). The mycorrhizal inoculation resulted in a positive influence on the length of roots. However, the sterilization had no stimulating effect on the length of roots in purple sweet potato seedlings.

4. Discussion

Sweet potato originates from Central America and it has been cultivated widely in tropical and subtropical countries like Malaysia, Indonesia, China, USA and Japan [29,30]. In Malaysia, for instance, they have been cultivated for a long time and have become one of the country's major vegetable crops due to them being an inexpensive source for energy, carotene, ascorbic acid, niacin, riboflavin, thiamine and minerals [31]. Recently, temperate countries like Hungary have started its cultivation [32]. The increasing demands for sweet potato have also encouraged growers and led to an increase in the cultivation area of sweet potato around Hungary [5]; however, little is known about its cultivation in this climate. During its cultivation technology seed root selection involves the process of selecting, curing and storing sweet potato roots for the production of slips or also known as vine cuttings [33]. This is important to ensure high quality slips which will lead to the establishment and production of high-yielding sweet potato crops [24], which can be differentiated through their flesh color. The most common ones are like potato's flesh color, with a range from white to yellow [30].

The present study showed that the used mycorrhizal inoculum could establish a symbiotic relationship with the treated and control sweet potato seedlings. The confirmation of the establishment can be shown by the calculated mycorrhizal parameters (Table 2). Gai et al. also showed that several mycorrhizal fungi species are able to colonize sweet potato to various degrees [34]. More than 90% of plant species are able to establish symbiotic relationship with arbuscular mycorrhizal fungi, which indicates their common abundance [35]. This is also visible in our findings as sweet potato grown on control soil also showed signs of colonization. Arbuscular mycorrhizal fungi have effectively inoculated cotton, tomato, pepper, horse bean, garlic, soybean, cucumber, melon, watermelon, maize and eggplant plants [36]. In order to ensure the colonization, a few circumstances must be met, which are the use of a variety of arbuscular mycorrhizal fungi species simultaneously, with a high amount of infective propagules, the absence of pathogens and pests, the availability of beneficial bacterial additives and the use of dry solid inoculums [37]. These requirements were observable in our results as well, as colonization was the highest when the commercial

inoculant with several AMF species and controlled propagule number was used, while the growth medium was sterilized and was free of pests and pathogens.

Arbuscular mycorrhizal fungi can promote plant growth directly and indirectly. It can directly promote the growth of the root system through the modulation of the phytohormones of the host, which leads to its indirect growth promotion through the increased availability of some immobile nutrients through the increased root zone [38–40]. Besides immobile nutrient, mycorrhiza can also help accumulate nitrogen from its different forms like nitrate (NO3⁻), ammonium (NH₄⁺) and amino acids using their extraradical hyphae [41]. Some studies proved the positive effect of arbuscular mycorrhizal fungi towards absorption of phosphorus, nitrogen, potassium, magnesium, copper, zinc, calcium, iron cadmium and nickel [14,42]. Arbuscular mycorrhizal symbiosis can affect biochemical and physiological processes such as protection towards oxidative damage, improved water usage efficiency, weight of shoots, improved gas exchange rate, and enhanced osmotic regulation [43,44]. It is obvious from our results of fresh shoots and root weights that mycorrhizal inoculation could enhance the physical parameters of sweet potato seedlings.

Several other studies have already shown the positive effect of AMF on physical parameters. In maize, it was proven that Glomus intraradices has the ability to promote shoot and root dry weight [45]. Kakabouki et al. [46] examined the effect *Rhizophagus irregularis* on cannabis seedlings and found significantly increased root length, stem dry weight, and improvement in survival rate and phosphorous content. In another study it is also confirmed that Arbuscular mycorrhizal fungi increased sweet potato shoot and root fresh weights for PROC 65-3 (white-fleshed) and Tainung 57 (orange-fleshed) sweet potato varieties [47]. In addition, in the study of Sakha and Jefwa [48], two sweet potato varieties, Kemb-10 and Bungoma, were examined with and without AMF inoculation focusing on physical parameters, namely on the number of branches, vine length and yield. They found that mycorrhizal inoculation improved the yield and growth [49]. The yield of storage roots is positively correlated to the vegetative characteristics and the correlation with the number of leaves per plants is significant [50]. In terms of the length of roots, we found no positive effect of the mycorrhizal inoculation while there was an increasing in the length of stem. The effect of the sterilization treatment was low; the reason behind this might be that the microbial population in autoclaved growth medium was lower than in the non-autoclaved growth media. This is in accordance with the results of Köhl et al. [51].

Nevertheless, the defined impact of AMF on plant growth and development is not stable due to the complex relation between the AMF, the inoculation method and the environmental conditions [52]. It was visible in our results that although the highest colonization rates were found in sweet potato grown on sterilized peat, the treatment was not performing better in terms of the physical parameters when compared to non-sterilized inoculated treatments. Moreover, this can be assured by other studies finding that plants with high rates of mycorrhizal colonization can be obtained on peat-based substrate but that under these conditions, plants may not consistently benefit in growth from the mycorrhizal symbiosis [53]. Likewise, the interaction amongst arbuscular mycorrhizal inoculum seemed to have an equalizing effect on the varieties, as the significant differences during control found that the fresh weights of shoot and root, and root length were missing during both mycorrhizal treatments.

5. Conclusions

In this pilot study, we examined the ability of the mycorrhizal inoculum product Symbivit to establish an association with two varieties of sweet potato seedlings, orange and purple. The results showed that the symbiotic relationship was successfully developed; we could observe this by the scoring of the mycorrhizal colonization in stained roots under microscope. We could detect different mycorrhizal developmental structures such as hyphae and arbuscules, especially in the seedlings that were treated with Symbivit in sterilized peat moss. According to our observations, substrate sterilization may influence the mycorrhizal colonization development during the plant growth. The mycorrhizal inoculation with a sterilized substrate had a different effect among the varieties on the physical parameters of the seedlings. This study suggested that inoculation of sweet potato seedlings with AMF can be functional, which presumably improves plant growth, nutrient uptake, and significantly improves sweet potato yield.

Author Contributions: Conceptualization and methodology Z.P.; writing–original draft preparation N.A., M.L. and V.S.; validation, analysis, and visualization, N.A. and M.L.; writing—review and editing, N.K.; revision N.K. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry for Innovation and Technology within the framework of the Thematic Excellence Program 2020—Institutional Excellence Subprogram (TKP2020-IKA-12) for research on plant breeding and plant protection. In addition, funding was received from the "ÚNKP-20-3-II" New National Excellence Program of the Ministry of Human Capacities.

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 authors.

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

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