



“Gheorghe Asachi” Technical University of Iasi, Romania



## COST-EFFECTIVE MASS PROPAGATION OF VIRGINIA FANPETALS (*Sida hermaphrodita* (L.) Rusby) FROM SEEDS

Erika Kurucz<sup>1\*</sup>, Gabriella Antal<sup>1</sup>, Fári Miklós Gábor<sup>1</sup>, József Popp<sup>2</sup>

<sup>1</sup>Department of Agricultural Botany, Plant Physiology and Plant Biotechnology, University of Debrecen, Hungary

<sup>2</sup>Faculty of Applied Economics and Rural Development, University of Debrecen, Hungary

### Abstract

The primary objective of this research was to develop safe, programmable and cost-effective technologies of plantlet production from the seeds of undomesticated American populations of Virginia fanpetals (*Sida hermaphrodita* RUSBY). During our seed priming treatment experiments, approximately 40% of the Virginia fanpetals seeds that were high-quality, infection-free, normally imbibing and germinating seeds. Our spring propagation tray experiments indicated that the spring large-scale tray plantlet production of Virginia fanpetals can be performed with using properly pre-treated and fractioned seeds and the phytotechnology that is characteristic of conventional, large air-space plastic tunnels that are used in white cabbage production. This phytotechnological method can be conducted in a simple and efficient way, making it possible to produce hardened, strong plantlets at an industrial scale, scheduled for planting in early spring (March). Our investigation showed that the combination of summer-autumn nurse-in-tray plantlet production technique and subsequent unprotected wintering of Virginia fanpetals with properly pre-treated and fractioned seeds is a promising new methods. There are no heating costs, and this phytotechnique can be easily and properly mechanized. Scheduled plantlets can be produced at an industrial scale by the time of early spring (March) plantlet planting. The digging up of the plantlets can be flexibly adjusted; the plantlets may even grow in the plantlet cases for an entire year. A comparative analysis of the costs of this procedure needs further research.

**Key words:** nurse-in-tray method, plantlet production, seed priming method, *Sida hermaphrodita* Rusby, Virginia fanpetals

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### 1. Introduction

Virginia fanpetals (*Sida hermaphrodita* Rusby) is a typical subshrub scattered in the area of Pennsylvania and Virginia in smaller natural populations, as well as in botanical gardens. Virginia fanpetals is classified as a highly endangered species in its original habitat (Spooner et al., 1985). The reason for the weak natural generative reproduction ability of this species is not yet known (Chudzik et al., 2010). The economic value of this species was first realized by Indians as a potential perennial fiber crop (Spooner et al., 1985). Due to similar considerations, Virginia fanpetals has been involved

in farming experiments in the Soviet Union (Medvedev, 1940) and later in Ukraine (Dmitrashko, 1970, 1972, 1973; Dmitrashko et al., 1971) since the middle of the 20th century. The large-scale production of this crop has not been launched yet due to economic, biological and agrotechnical reasons. Virginia fanpetals was first tested as biomass feedstock for energetic purposes 50 years ago in Poland (Borkowska and Styk, 2006; Szyszlak-Bargowicz et al, 2012). Dr. Zoltán Kováts, an ornamental plant breeder of the Research Institute for Fruit and Ornamental Plants, accidentally brought Virginia fanpetals to Hungary for the first time in the 1970s during a botanical garden seed exchange

\* Author to whom all correspondence should be addressed: E-mail: [era.kurucz@gmail.com](mailto:era.kurucz@gmail.com); Phone: +36 52 508 444/ 88486; Fax: +36 52 508 444/ 88486

(Kurucz et al., 2013). Later, the seed of Virginia fanpetals was brought also through commercial channels from Poland, and a smaller experimental biomass plantation was established under the name of 'Petemi'. The plant was enrolled under the name 'bársonymályva' in the list of energy crops that can receive Hungarian state subsidy (Decree no. 16/2010. II. 25 of the Ministry of Agriculture and Rural Development, herbaceous energy crops). Based on the experiments that have been performed to date, researchers list the following as the production values of growing Virginia fanpetals as a biomass target crop:

- high tolerance to extreme continental climate, especially winter-tolerant (tolerates snowless winters with temperatures below negative 20 °C) (Borkowska and Molas, 2011),
- tolerates summer drought (if yearly precipitation reaches 400-500 mm),
- no particular soil preference (Spooner et al., 1985),
- develops well in areas that are treated with wastewater and can absorb certain heavy metal pollutions from the soil (Borkowska and Wardzińska, 2003),
- biomass yield reaches or exceeds that of short rotation woody crops (10-15 absolutely dry ton ha<sup>-1</sup> year<sup>-1</sup>), (Wróblewska et al, 2009),
- can be used as a bee pasture plant (flowering takes place between June and the first chills at an intensity depending on the amount of rainfall),
- the moisture content of the stem decreases to less than 40% by the end of November; therefore, harvesting can begin at the beginning of winter, e.g., by chipping without artificial desiccation (Kasprzyk et al., 2014),
- and the C/N ratio and tissue structure of the stem potentially make it suitable for various energetic uses (Domínguez-Muñoz et al., 2010; Oleszek et al., 2013).

According to our previously unpublished research, Virginia fanpetals has several other important biological characteristics that were previously only slightly or not at all examined with scientific methods.

These characteristics must be given special attention in basic research. For example, based on our observations, the shrub nature of full-grown Virginia fanpetals is characterized by the following in a temperate climate:

Germination begins in March, when the soil is still cold and the spring weeds are not yet strong; therefore, this species becomes a weed suppressant by growing above the weeds (March – late June). The intensive phase of vegetative growth ends late June after the wet spring period. The generative phase starts takes place during the dry summer period. The intensity of growth during the generative phase (July – late October), i.e., flowering and the quantity of seeds, depends on the precipitation. During the dry period of the summer – often extreme – season, the leaves on the primary stem first turn

yellow – starting from the bottom – and then fall. Later, as a result of precipitation, axillary buds sprout, the stem comes into the leaf again, continuing to grow and bloom.

At the end of the vegetation period in October, a yet unknown internal molecular, physiological and histic transformation occurs at the basal part of the shoots – presumably in the former epicotyl hypocotyl zone. During this process, the part of the stem and the shoots above this zone dies, and the part of the stem below this zone continues to live and prepares for the winter. This process can most likely be characterized by the apoptosis of the cells in the affected tissue region, the exploration and understanding of which calls for basic research in conformity examining the above questions.

A large number of adventitious vegetative buds are differentiated in this zone at the end of winter and begin sprout by the spring. The anatomical and physiological descriptions of this process are still lacking.

## 2. Objectives

The size of the production area that was used for growing *Virginia fanpetals* has not been elucidated to date. According to Polish references, the production was only approximately a few hundred hectares in 2011 (Borkowska and Molas, 2008). Scientists agree that the more widespread production of Virginia fanpetals is limited by the following circumstances:

- The germination of the seed of this species, which occurs only during the early stage of domestication, is unpredictable, slow and difficult (5-10%, Dolinski, 2009).
- The mechanical sowing of seeds in the field can be solved technically, but a huge quantity of sowing seeds is needed (approximately 200,000 – 300,000 seeds per hectare) due to the weak germination ability (Krzaczek et al., 2006).
- The first three years of plantlet development of a population that was sown from seeds is relatively slow (Borkowska et al, 2006); therefore, cost-increasing weed control is necessary (Borkowska and Molas, 2008).
- There is no chemical weed control that would protect a population that was sown from seeds, and the herbicide sensitivity of the species is unknown in most cases (Borkowska and Molas, 2008).
- It is possible to propagate from plantlets (Kujawski et al., 1997); however, the necessary and sufficient large-scale seed biological and seed production technology is missing, unlike in field vegetables.
- The vegetative method of agamic propagation by root cuttings is also possible (Borkowska, 2007; Borkowska et al., 2009), but there is no special mechanical system or large-scale technology, unlike in *Salix sp.*, *Populus sp.* and *Eucalyptus sp.*

- The phytosanitary risks that are associated with large-scale vegetative propagation carried out on large surfaces are unknown, with special regard to the examination of the virological relationships of the species.

- There is no practical experience using either in vitro biotechnology with Virginia fanpetals or the alternatives of breeding, polyploidization and hybridization between species.

- There is little to no reliable biological and genetic basic research, tool, or comparative pest management analysis that has been carried out in different ecological environments and that would provide a basis for breeding this species for practical purposes in an efficient and programmable way (Frabzaring et al., 2003).

If researchers discovered satisfactory solutions for these questions, Virginia fanpetals will have a promising and successful career as a biomass plant in areas with temperate continental climate. We believe that one of the main conditions of the future breeding of Virginia fanpetals will be the use of biotechnology. During the past 50 years, several new molecular breeding and other in vitro biotechnological tools and efficient methods have been developed and are available for researchers. The molecular genealogical tree of mallow genera and species has been prepared (García et al., 2009). In our opinion, the main practical objective is to develop a uniform, safe and most importantly cost-effective industrial-scale technology of propagation and plantlet production from the seeds of rootstock nurseries of undomesticated American populations. We are certain that further research will result in the economical and cost-effective propagation of this species by the off-season utilization of existing plantlet production plants in the summer and autumn without heating.

Furthermore, it is important to determine the proper biological-phytotechnical solution for the early spring establishment of the plantation instead of the unfavorable, dry late spring and summer seasons. This solution would allow the population to grow faster during the first year, and soil coverage would also be provided. The plantlets that were cost-effectively pre-cultivated and hardened during the previous year are the most suitable for this purpose.

### **3. Materials and methods**

#### *3.1. Seed production, harvesting, cleaning and pre-treatment for plantlet production*

The population of Virginia fanpetals that was involved in this experiment is registered as a potential new variety of mixed color, named *Sida hermaphrodita* Rusby 'Bicolor'. The seed was provided to us by Károly Ereky Foundation (Debrecen, Hungary). The 2011 and 2012 seed yield of the experimental population that was established in the Biomass Demonstration Garden called "Plants of the Future" of the University of Debrecen in 2009

was used as the propagation material of plantlet production. The seeds were manually harvested in the late autumn and early spring in both of the years. The seeds were cleaned manually. Following the collection and cleaning of the seeds, a two-step seed pre-treatment was performed. During the seed treatment procedure that was previously further developed by us (Kurucz and Fári, 2013a, 2013b), we first fractionated the seeds in distilled water based on their specific gravity and/or imbibing characteristics. The two-minute-long 80 °C hot water heat treatment was only performed on the sunken seed fraction. The experiment was performed using the seeds from 2011. The use value of the seeds in each crop year was examined during the subsequent steps.

The purpose of this examination was to determine what percentage of the settled initial material after fractioning (settled fraction) can be used as initial material for plantlet production. The approximate amount of initial material in the 2011 and 2013 seeds was 5 g. The seeds that were fractionated by soaking (settled and floating the seeds) were dried at room temperature for two days before evaluation. Due to the low amount of 2009 and 2012 seeds, the number of initial seeds was 200. In each case, the sampling was performed in at least three replications.

#### *3.2. Materials and methods of spring tray plantlet production*

The initial seed material of the experiment was treated two days before sowing. The spring plantlet production experiment was established at the Derecske Plantlet Production Site of KITE (Maize and Industrial Crop Production Union) on 28 March 2013. The production units that were used included 160 pieces of plastic 126-cell trays that were suitable for producing cabbage, paprika and tomato plantlets in soil blocks. The volume of the cells that were filled with medium was 60 cm<sup>3</sup>. A rooting soil mixture with a high nutrient content (3000 mg L<sup>-1</sup>) was used as planting medium (JÓ FÖLD XXL, Pax96 Kft, Kecskemét, Hungary). Manual sowing was performed while making sure that 2 seeds were placed in each cell, if possible. The reason for the double seed quantity (thereby, the necessity of manual sowing) was the uncertain germination rate, as this was the first time ever that the impact of the treatment was examined outside of a laboratory.

The trays were placed into a so-called germination chamber for two days at 28 °C and 99% relative humidity. On the third day, the trays were moved to one of the plastic tunnels, which serve the purpose of early cabbage cultivation; therefore, the plantlet production took place in the same air space and under the same physical circumstances as the early head cabbage. Due to stem elongation resulting from high plant density and the appearance of *Rhizoctonia* spp., the single-shoot stem of the plantlets had to be cut back on two occasions. Surveying was performed twice in 2013, on 30 April

and 17 May before cutting back the green stems to a height of 5-10 cm. During the calculation of the germination rate, the double seed quantity was considered. In each case, 20 trays were used for sampling. The sampling of plants was performed during the second surveying.

3.3. Materials and methods of nurse-in-tray plantlet production

Single-space (30 x 60 cm) propagation trays were used during the summer plantlet production experiment (29 June 2013) (Fig. 1). We established space in the trays for 30, 40 and 50 seeds. Each seed density had four replications, and two seeds were sown in one space.

The 1:1 mixture of low-nutrient content field soil and commercially available medium-nutrient content soil was used in a quantity of 20 liters per tray. A thin layer of pine bark mulch was spread under the soil mixture. In addition, a 60 x 60 cm piece of foil was placed between the soil mix and the

tray to remove the soil mixture that was interwoven with roots in one unit at the time of planting (Fig. 2).

During the experiment, the germination rate of the seeds was also examined in the time that passed since the treatment. Therefore, two treatments were added to the experiment during which some of the seeds were subjected to the two-step seed treatment 30 days before sowing, while the rest of the seeds were treated one day before sowing.

The germination rate of the trays was surveyed three weeks after sowing, considering the double-sown seeds. Following germination, the plantlets were grown in an easy-to-assemble field space that was shaded from above and from the side and covered with agrotextile. After three weeks, the plantlets were planted into specially prepared 10-cm-deep, 1.5 m x 1.5 m field plantlet cases where they remained for wintering. The cases were established by filling with soil, but it was also important to establish a horizontal surface. The plants were irrigated with the necessary amount of water once a week after planting.

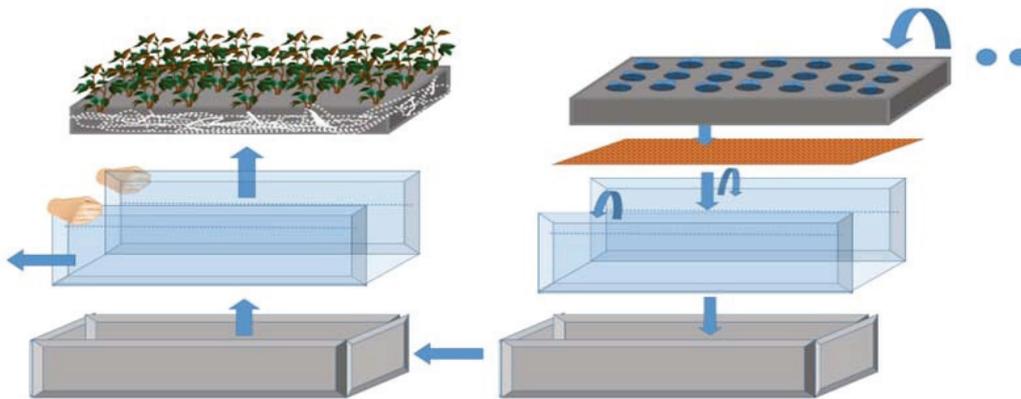


Fig. 1. Diagram of nurse-in-tray plantlet production

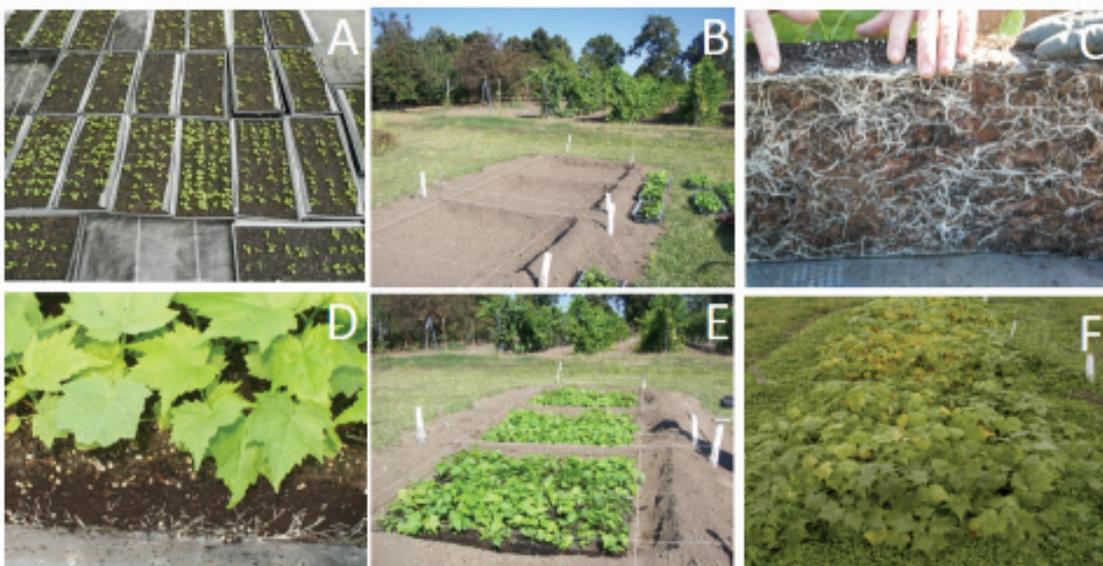


Fig. 2. Process of the nurse-in-tray plantlet production of *Sida hermaphrodita* Rusby. A: 1 week after sowing (05/08/2013). B: 1.5 m x 1.2 m experimental planting trenches (29/08/2013). C and D: soil mixture interwoven with the four-week-old plantlets, removed from the nurse-in-tray (30/08/2013). E: plants from trays of different plant density immediately after planting (30/08/2013). F: four-month-old population (10/12/2013)

### 3.4. Materials and methods of the wintering of the nurse-in-tray plantlets

The wintering of the nurse-in-tray plantlets was performed under field conditions. The four-week-old plants were planted in the Biomass Demonstration Garden called “Plants of the Future” of the University of Debrecen on 30 August 2013.

Using the foil, the soil blocks that were densely interwoven with roots were placed in a 1.5 m × 1.2 m planting trench in one unit, planting the trays of different densities in separate trenches (Fig. 2).

The planting of the plantlets was surveyed on two occasions, at the time of planting (30 August 2013) and on 21 October 2013. The physical dimensions (fresh weight, shoots and leaves, dry matter content, and number of leaves) of the plants were also measured 10 December 2013.

### 3.5. Materials and methods of the wintering of tray plantlets

Of the 160 plantlet trays (approximately 9500 plants altogether), 77 trays were commercialized as experimental material in different locations in Hungary, while the remaining 83 trays (approximately 10,300 plants) were used as material for wintering.

The wintering of large-scale soil block plantlets was performed in unheated plastic tunnels. The cell trays were surrounded from the side with 5 cm thick polystyrene (“Hungarocell”) trays and were covered with shading foil from above during the winter.

### 3.6. Statistical analysis

Descriptive statistical methods (sum, mean, standard deviation) and a one-way ANOVA were used to determine the impact of treatments. The data were evaluated with PSPP. The significant differences between each treatment were determined with Tukey’s test at the 5% probability level.

## 4. Results and discussion

### 4.1. Increasing the practical use value of the seeds

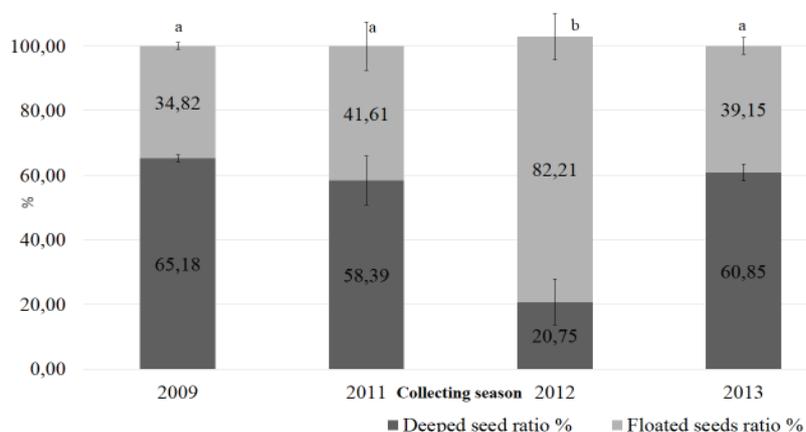
Fig. 3 shows the proportion of the two seed fractions that were established as a result of settling in the case of sowing seeds of different age. This proportion can be considered to be the use value of the seed lots because only the sunken and imbibed seeds, which have a higher specific gravity, are used for plantlet production. According to our previously research the majority of seeds in 2012 were not imbibed, most likely due to adverse storage conditions and secondarily occurring seed infections (Kurucz and Fári, 2013a).

There is no significant difference between the use values of the seeds from other crop years. Altogether, it was concluded that approximately 35-40 % of the Virginia fanpetals seeds that were collected during the four crop years were high quality, infection-free, normally imbibing and germinating seeds.

### 4.2. Tray plantlet production suitable for spring planting in heatable plastic tunnels

The germination rate exceeded 50% (56.22%), even in the case of basal heating under large-scale, double-sown circumstances. The proportion of double sprouted seeds per cell is low and was reduced to a minimal value by 2014. The fullness of the trays was rather favorable in the case of the 90-day-old plantlets (84.72%) and was greater than 60% even after wintering (65.95%).

This experiment validated our previous hypothesis that the large-scale spring tray plantlet production of Virginia fanpetals can be performed using properly pre-treated and fractioned seeds and the phytotechnology that is characteristic of conventional, large air space plastic tunnels that are used in brassica production. A comparative analysis of the costs of this procedure calls for further research.



**Fig. 3.** Fraction proportion of seeds of various years after settling (the different letters represent the significant differences determined with Tukey’s test)

#### 4.3. Results of the summer-autumn field nurse-in-tray plantlet production

The soil of the plantlets that were planted on 30 August 2013 was weedless until winter because the soil mixture that was used for sowing did not contain any weed seeds. Fig. 4 shows the sharp difference between the efficiency of the seed treatment when performed at different times. The germination rate of the freshly treated seeds was greater than 70% when the plant density was 40 plants per tray. For the treatments that were marked with 1, the seed treatment was performed 30 days before sowing, and the germination rate was between 24.33% and 30.8%. The proportion of double-sprouted seeds can still be considered low (less than 20%).

It is worth mentioning that the most efficient plant density was 40 plant propagation boxes in both of the treatment times. This experiment confirmed our previous hypothesis that the summer-autumn nurse-in-tray plantlet production of Virginia fanpetals can be performed with properly pre-treated and fractioned seeds. A comparative analysis of the costs of this procedure calls for further research.

#### 4.4. Wintering of tray plantlets that are suitable for early spring planting in unheated plastic tunnels

It is a positive result that 72% of plantlets in the trays survived and grew until March 2014, despite the unheated conditions. The proportion of double plantlets further decreased and did not reach 10% by the end of March 2014. This experiment confirmed our hypothesis that the wintering of tray plantlets of Virginia fanpetals can most likely be developed into an economical and safe new method. This method does not call for extra investment as it can be performed in an already existing, under-utilized plastic tunnel, and there are no heating costs.

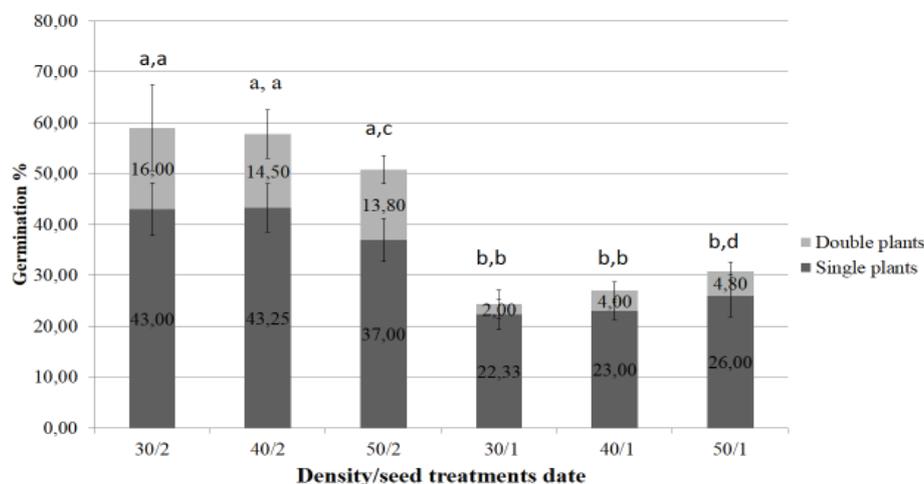
This phytotechnological method can be carried out in a simple and efficient way, making it possible to produce hardened, strong plantlets at an industrial scale and that are scheduled for planting in early spring (March). A comparative analysis of the costs of this procedure calls for further research.

#### 4.5. Uncovered wintering of field nurse-in-tray plantlets that are suitable for early spring planting

The growth of spring field weeds started only on the sides of the cases, and no weeds grew from the bottom, similarly to the weed growth during the autumn. During the winter, 5-10 cm snow covered the cases on two occasions, and winter precipitation fell onto the wintering plantlets without any external protection.

The survival rate after wintering was higher in the plots with smaller spacing, also considering the fact that the trays in the second treatment had a higher germination rate, resulting in a higher plant density. Based on the data, the individuals of the more densely sown trays had a higher plant mortality rate (55.45% and 43.48%) than that of the less densely sown individuals (29.19% - 41.99%).

Considering all of the indexes, the moderately dense sowing (200-220 plantlets per m<sup>2</sup> or 40 plantlets per propagation tray) of freshly treated seeds was the most successful method. With this procedure, the amount of propagation material that is needed to plant one hectare (10,000 plantlets per hectare) can be produced on nearly 70 m<sup>2</sup> (68.2 m<sup>2</sup>), in contrast to previously treated seeds, which require an area is larger than 100 m<sup>2</sup> (158.18 ± 25.34; 139.35 ± 37.19; 104.07 ± 10.87 m<sup>2</sup>). This experiment verified our previous hypothesis that the summer-autumn nurse-in-tray plantlet production and unprotected wintering of Virginia fanpetals with properly pre-treated and fractioned seeds can be developed into an economical and safe new method if further research is conducted.



**Fig. 4.** Effectiveness of nurse-in-tray plantlet production in three spacings from seeds treated at two different dates. The different letters show the significant differences with Tukey's test. Letters on the left: impact between the two treatment dates. Letters on the right: differences between the different spacings

The investment cost of the method is minimal. There are no heating costs, and this phytotechnique can be easily and properly mechanized. Plantlet production can be performed near large-scale plots. With this method, it is possible to produce plantlets with hardened and strong roots at an industrial scale and that are scheduled for planting in early spring (March). The digging of plantlets can be flexibly adjusted; the plantlets may even grow in the plantlet cases for a whole year. A comparative analysis of the costs of this procedure calls for further research.

## 5. Conclusions

During a four year investigations we observed that the imbibing characteristics of the Virginia fanpetals seeds did not exhibit any significant correlation with the storage period. In the next step, two plantlet production technologies were tested in different locations and at different times after applying a seed treatment priming technique that was developed by us in former investigations.

Regarding the spring large-scale plastic tunnel plantlet production, marketable plantlets were successfully produced in two months for planting in May. We also developed a combination of summer-autumn nurse-in-tray plantlet production technique and subsequent unprotected wintering of Virginia fanpetals with properly pre-treated and fractioned seeds. The nurse-in-tray plantlet production is a novel method by means it is possible to produce entirely hardened plantlets scheduled for industrial-scale planting in early spring (March).

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