Influence of planting density of micro-plants on the number of potato mini-tubers

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Abstract. For the first time in the conditions of the Volga-Vyatka region of the Russian Federation, we determined the parameters of planting micro-plants for the output of potato mini-tubers. The study has shown that to increase the yield of mini-tubers of potatoes, it is necessary to develop an optimal method for planting micro-plants that is most suitable for each variety. Some potato varieties, such as Reggae and Samba, produce more mini-tubers when the planting density increases. Whereas Bellarosa potato plants will produce more mini-tubers if the nutrition area for each plant is increased by reducing planting density. In potato varieties Reggie and Samba, by increasing the density of planting, the yield of mini-tubers increases. For the Bellarosa variety, a decrease in the planting density of micro-plants is required.

1. Introduction
Seed production is an important pillar of food security in any country [1]. Potato is of particular importance in human consumption [2]. Potato seed multiplication scheme is one of the longest and takes an average of five years. During this period, tubers accumulate viral and bacterial infection leading later to depressed yield [3].

To achieve excellence, seed farms run curtailed seed production schemes [4]. However, to fully satisfy agricultural producers with quality potato planting stock, there should be a massive increase in the breeder seed yield, including mini-tubers [5].

A different way to increase the number of potato mini-tubers yield included inoculating micro-plants [6]. Mini-tuber yield per plant as well as a weight of each greenhouse-grown tuber was increased by inoculating the mycorrhizal fungus *Glomus fistulosum* together with the B1 (*Bacillus subtilis*) bacterial isolate. However, it was found that the best effect of inoculation was achieved if a suitable mixture was provided for each particular variety.

It has been found that the most suitable for potato micro-plants is a substrate based on perlite and peat moss in a 1:1 ratio. This substrate has optimal porosity and pH level for potato micro-plants [7]. Tuber size and their number was directly linked to potato foliage development and stolon formation [8].

India is a country with constantly changing climatic conditions, where potato micro-plants can be planted at different times to produce mini-tubers. It was shown that micro-plants planted during the onset of southwest monsoon over the Indian mainland, with its long-term rainfall, produced more mini-tubers per unit land over the autumn planting. The observations also showed that tuber yield and size
depended on a potato variety [9]. Correlation between the number of tubers and varietal features was studied in [10]. To meet the needs of agricultural producers in high-quality potato seed, the production of mini-tubers should be increased. This can be done by selecting varieties that have a high tuber-forming ability and have good economically valuable traits [11]. When growing potato varieties Kufri Chandramukhi and Kufri Surya with different planting densities (30 × 10, 20 × 10, 15 × 10, and 10 × 10 cm), plants planted according to the 10 × 10 cm scheme significantly exceeded other options in terms of the number of tubers, giving 259.74 tubers per square meter of area. On average, plants planted at 10 × 10 cm gave a twofold advantage in the number of tubers [12].

The purpose of our study was to estimate the number of mini-tubers of potatoes against the background of the planting method when obtaining good-quality potato planting material.

2. Methods and materials
To attain the goal set we correlated the yield of potato mini-tubers against leaf area and examined the yield of mini-tubers at different growing spaces for potato plants.

The study was made in the laboratory for potatoes propagation research of Chuvash State Agricultural Academy (Cheboksary, Russia). The experiments were carried out in the spring of 2019. Reggae and Samba, domestically bred potato varieties, designated as study objects, were compared with the Bellarosa chosen as a standard variety.

Reggae variety was kindly supplied by Kazan Scientific Center of the Russian Academy of Sciences” (Kazan, Russia). The variety is early table variety (vegetation period – 65-75 days). The tuber is oblong-oval and yellow in color, while the flesh is light yellow. This variety is quite resistant to potato cancer, wrinkled striped mosaics and leaf curling. Then, it is moderately susceptible to late blight of tops and tubers and affected by the golden potato cyst nematode.

Samba variety was taken from the Kazan Scientific Center of the Russian Academy of Sciences (Kazan, Russia). The variety is medium early table variety with vegetation period of 65-80 days. The tuber is oval-round and yellow in color, while the pulp is light yellow. This variety is quite resistant to potato cancer, wrinkled striped mosaics and leaf curling. Then, it is affected by late blight of tops and tubers, and is susceptible to golden cyst nematode.

Bellarosa variety was supplied by the Europlant Pflanzenzucht GmbH (Germany). The variety is early table variety with vegetation period of 55-70 days. The tuber is oval-rounded, with small eyes, with a red skin and light yellow flesh. It is resistant to potato cancer and golden potato cyst nematode.

We provided a two-factor experiment. Potato micro-plants were planted in 5.6 L plastic pots 200 x 200 x 250 mm in size. Experimental design included the options: Bellarosa (control) (2 plants per pot), Reggae (2 plants per pot), Samba (2 plants per pot), Bellarosa (control) (3 plants per pot), Reggae (3 plants per pot), Samba (3 plants per pot), Bellarosa (control) (4 plants per pot), Reggae (4 plants per pot), and Samba (4 plants per pot). Each variant consisted of 300 plant pots. The experiment was repeated four times. The plants were planted in greenhouses 22 m long and 6 m wide. The experiment was conducted in four greenhouses. The options in the greenhouse were placed randomly. For mini-tuber growing, the medium was high-moor peat with low decomposition degree. This peat had standard pH of the growing medium (5.5). The substrate for growing mini – plants was riding peat of a low degree of decomposition. The substrate had the pH of 5.5 normalized by lime materials (dolomite and limestone flour). In addition, peat was initially enriched with the main nutrients, mg/L: NH4NO3*CaCO3 – 150; P2O5 – 150; K2O – 250; Mg – 30; Ca – 120. The organic matter content was about 80%. The substrate had a highly porous structure, which allowed the root system of potato micro-plants to grow freely and build up mini-tubers. Peat did not contain pathogens or weed seeds. Before filling the pots, peat was sieved through a sieve with 20 x 20 mm mesh size.

The prepared peat was supplemented with a complex mineral fertilizer "Nitroammofoska" (Uralchem, Russia). It contained three main components as NH4H2PO4, NH4NO3, and KCl, which are necessary for the normal growth and development of plants at different stages nitrogen, phosphorus and potassium. Nitrogen, phosphates and potassium were divided equally here, at 16% each. Overall, the total share of active ingredients was 48%. Fertilizers were well mixed with the medium. During planting,
the pots were filled with peat 2/3 high of the total volume. Later, as potato plants grew, peat was added until planting pot was full.

Potato micro-plants were planted on 4 June. Before planting potato micro-plants, the soil in the pots was moistened with a drip irrigation system. Plants were removed from test tubes using anatomical tweezers and immediately planted in pots with a substrate. The nutrient medium in which the plants were located was not washed off the root system. When planted in pots, micro-plants were buried to the second internode. On hot days, plants were watered daily, on cloudy days – every other day. Greenhouse air temperature was maintained at 25 °C. To ensure automatic adjustment of the main parameters (temperature, humidity and soil), a microclimate system is installed in greenhouses. Soil moisture was regulated by a drip irrigation system, and air humidity was maintained by a sprinkler system. Irrigation was carried out from a 10 m³ container. Water intake for irrigation was carried out from the well. The irrigation rate varied depending on the soil humidity, which was maintained at 65-70%.

As potato plants were growing and developing, leaf surface area was measured and recorded for control and experimental plants. The punching/cutting out method was used, where the weight of a wet leaf of the selected plants is measured, cutouts are made, weighted, and the leaf area of each plant is identified. An average sample of plants was taken, the leaves were quickly cut off, and their weight was calculated. Then, from the central part of the leaf blade of each leaf of the plants with a drill with a diameter of 1.1 cm, several punches were knocked out, combined and their weighing weight was set, with an accuracy of 0.01 g (423i-1SRU balance, Sartorius Entris, Germany, accuracy ± g). The die-cuts were cut in the part of the leaf that is characterized by an average density (relative to the leaf plate as a whole). The leaf area was determined by the formula (1):

\[ SI = \frac{M_l \cdot S_v}{M_v}, \]

where, \( SI \) – area of plant leaves, cm\(^2\); \( M_l \) – mass of raw leaves, g; \( M_v \) – mass of raw die-cuts, g; \( S_v \) – area of die-cuts, cm\(^2\).

Linear correlation method and MC Excel software were used to identify the relationship between the studied features.

3. Results and discussion
The results show that the more micro-plants were per pot, the larger total leaf surface area of potatoes was. For instance, the largest leaf surface area per one pot for the Bellarosa cultivar, which is a standard cultivar, was 0.3474 m\(^2\) where four plants were planted in one 5.6 L pot (table 1).

Table 1 shows that Samba showed a similar trend: the more plants there were in the pot, the larger the leaf surface area. For Reggae variety, this figure was different from other varieties. It had the largest leaf surface area per 1 pot – 0.23 m\(^2\) (table 1), where two micro-plants were planted in a pot.

There is a positive correlation between potted plants and the leaf area created by plants that grow in the same pot in Bellarosa and Samba: correlation coefficient \( R^2 \) is 0.847 and 0.89, respectively (figure 1a). The Reggie variety also shows a positive relationship (\( R^2 = 0.559 \), figure 1a). A close relationship is observed between the indicators "leaf area per plant" and "number of plants in a pot" (\( R^2 = 0.836...0.998 \), figure 1b). Bellarosa cultivar shows direct correlation between leaf surface area of each micro-plant and the yield of mini-tubers. Reggae potatoes show inverse correlation – the smaller the leaf surface area of a plant is, the more mini-tuber sit produces. Samba variety showed no correlation between mini-tubers yield and leaf surface area. Speaking of mini-tuber yield produced by each plant rather than by a unit land, two micro-plants per pot produced higher yield of tubers in all potato varieties.
Table 1. Potato plant leaf surface area, m².

| Cultivar | Plants per pot, pcs. | Sl, m² per a pot | Sl, m² per a plant |
|----------|----------------------|-----------------|-------------------|
| Bellarosa | 2                    | 0.2997          | 0.1498            |
|          | 3                    | 0.3060          | 0.1020            |
|          | 4                    | 0.3474          | 0.0868            |
|          | 2                    | 0.2294          | 0.1149            |
| Reggae   | 3                    | 0.1516          | 0.0505            |
|          | 4                    | 0.1681          | 0.0420            |
|          | 2                    | 0.2061          | 0.1030            |
| Samba    | 3                    | 0.2614          | 0.0871            |
|          | 4                    | 0.2749          | 0.0687            |

Figure 1. Leaf surface area in a pot (a) and per a plant variety (b).

Therefore, the study of the leaf surface area formed by each plant showed inverse correlation – the less plants are in the pot, the larger leaf surface area of each plant is. This correlation is observed for all studied varieties. This suggests that the larger potato growing space is, the larger leaf surface area it develops.

The potato mini-tubers were harvested and counted on the 22nd of September after the foliage had completely died out, when a dense skin had formed. The biggest number of Bellarosa potato mini-tubers was produces by two micro-plants per a pot – 5.7 pcs. Samba and Reggae potato mini-tubers yielded the most in pots with four plants – 7.1 and 9.9 pcs, respectively (table 2).

Table 2. Average yield of mini-tubers.

| Cultivar | Plants per pot, pcs |
|----------|---------------------|
|          | 2  | 3  | 4  |
| Bellarosa | 5.7 | 5.5 | 5.4 |
| Reggae   | 6.5 | 8.2 | 9.9 |
| Samba    | 7.0 | 6.8 | 7.1 |

It was observed that plant growing space had effect on the mini-tuber yield. Bellarosa cultivar showed that its smaller growing area does not much affect the yield of mini-tubers, which is the case for Samba variety as well. Plant growing area has markedly affected the yield of mini-tubers in Reggae variety. Studies of a number of authors show the influence of the planting density of micro-plants potato on the number of mini-tubers. Researchers at the Central Potato Research Station, Kufri (Shimla) found that a higher stocking density of micro-plants potato (20 × 10 cm) resulted in more mini-tubers than a lower
stocking density (30 × 10) [12]. At the Central Potato Research Station (Shillong), two varieties of potatoes, Kufri Himson and Kufri Girdhari, were planted with a planting density of 20 × 10 cm and 15 × 10 cm. More tubers formed on closely planted plants (15 × 10 cm) [13].

The Volga-Vyatka region is the main region for the production of seed potatoes in Russia. Most seed farms grow micro-plants potato in pots. To obtain the maximum number of Reggae potato mini-tubers, plant 3 or 4 micro-plants per pot. The Samba potato variety is more plastic; however, to save space, you can plant 4 plants in a pot.

4. Conclusion
The studies show that the leaf surface area of the studied varieties has a varying effect on the yield of mini-tubers: for Bellarosa cultivar, the yield of mini-tubers is greater if the leaf surface area is bigger, Reggae potatoes show inverse correlation, while Sambo variety is little sensitive to leaf surface area changes. To maximize the yield of mini-tubers of Bellarosa and Sambo cultivars, the number of micro-plants in a pot shall be 2 to 4 since the growing area does not affect their yield. For Reggae variety, it is best to plant 3 or 4 micro-plants in a pot.

Thus, to increase the yield of potato mini-tubers from a unit land, the best planting method of micro-plants for each particular variety shall be developed. To boost the yield of Bellarosa potato mini-tubers, growing space of each plant shall be increased, for Reggae variety, it shall rather be smaller. Samba potato plants yielded more mini-tubers with four plants per pot. With increasing plant density in the conditions of the Volga-Vyatka region of Russia, the yield of mini-tubers of Reggae potatoes increases, and the Bellarosa variety decreases. The Samba potato variety is more plastic – the method of planting does not significantly affect the number of tubers.

References
[1] Rana R K, Pandit A, Pandey N K and P.C. Meena P C 2012 Sustaining potato revolution: demand of seed-potato in Gujarat (India). Indian J. Agr. Res. 3 (46) 242
[2] Tokbergenova Z A, Babayev S A, Togayeva D U, Kudusbekova D Z and Zagurskii A V 2017 Efficiency of microtubers application in the production of original potato seeds. Online Journal of Biological Sciences 4 (17) 316 DOI:10.3844/ojbsci.2017.316.322
[3] Gong H, Igiraneza C and Dusengemungu L 2019 Major in vitro techniques for potato virus elimination and post eradication detection methods: a review. Am. J. Potate Res. 4 (96) 379 doi: 10.1007/s12230-019-09720-z
[4] Ayalew T 2014 Analysis of seed potato (solanum tuberosum l.) systems with special focus in ethiopia: review. Asian Journal of Agricultural Research 3 (8) 122 doi: 10.3922/agar.2014
[5] Kononov O D, Popova L A and Golovina L N 2007 Science based seed potato production development in the Archangelsk region Potato production and innovative technologies ed A J Haverkort and B V Anisimov (Wageningen) pp194-211 doi: 10.3920/978-90-8686-608-3
[6] Vosatka M and Gryndler M 2000 Response of micropropagated potatoes transplanted to peat media to post-vitro inoculation with arbuscular mycorrhizal fungi and soil bacteria. Appl. Soil. Ecol. 2 (15) 145 doi: 10.1016/S0929-1393(00)00090-1
[7] Hajiaghaei K M, Rahimi C A and Hosseinniya H 2019 Effects of different growing media on yield and growth parameters of potato minitubers (Solanum Tuberosum L.). Commun. Soil Sci. Plan. 50 (15) 1838 doi: 10.1080/001030624.2019.1648487
[8] Struik P C 2007 Above-ground and below-ground plant development. Potato Biology and Biotechnology: Advances and Perspectives 2007 219 doi: 10.1016/B978-044451018-1/50053-1
[9] Sharma A K, Buckseth T and Singh R K 2018 Effect of seasons on subsequent production behavior of small size potato mini-tubers. Agr. Res. 7 (2) 245 doi: 10.1007/s40003-018-0325-7
[10] Sharma A K, Kumar V and Pandey K K 2016 Breeder seed potato production through conventional and hi-tech systems - A comparative study in high hills of North Western India.
Potato Journal 43 (1) 45

[11] Birch P R J, et al. 2012 Crops that feed the world 8: potato: are the trends of increased global production sustainable? Food Secur. 4 (4) 477 doi: 10.1007/s12571-012-0220-1

[12] Kaur R P, Minhas J S, Singh S, Singh A K and Singh R K 2019 High density planting of potato (Solanum tuberosum) minitubers for increased seed productivity. Indian J. Agr. Sci. 89 (6) 989

[13] Srivastava A K, Yadav S K, Diengdoh L C, Rai R and Bag T K 2016 Effect of plant density on mini-tuber production potential of potato varieties through micro-plants under net-house in North Eastern Himalayan region. Journal of Applied Horticulture 18 (1) 61