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Fast and cost-effective production of transgenic potato microtubers of Gala and Milena varieties

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Summary

Potato microtubers play an important role in the technology of seed potato production, since they have great advantages in storage, transportation and mechanization due to their small size and weight. For research purposes, it is also often necessary to obtain microtubers, in particular when creating lines of transgenic potato plants. Unlike agricultural needs, research requires a small number of microtubers, but as fast as possible and with minimal financial costs. We propose a method for obtaining microtubers, which saves time for transferring *in vitro* studies to soil in 4-7 weeks compared to so called "high sucrose concentration" methods (high concentration of sucrose; reduced photoperiod). We designated the method as "2.5S" due to sucrose content (2.5%) in Murashige and Skoog medium which amount for each plant was reduced by approximately 3 times. That leads to a lack of nutrients and the rapid formation of microtubers. The proposed method was tested on two potato varieties and their transgenic lines; in case of one of the varieties, a reliable increase in the mass of microtubers by 1.6-3.6 times was recorded compared to the standard method of obtaining microtubers. The method is also cost-effective and does not require any additional labor costs from researchers.

Key words: potato microtubers, transgenic plants, *Solanum tuberosum*, sucrose, photoperiod

Introduction

The main purpose of microtubers production for research work is to solve the problem of transplanting plants from *in vitro* conditions to *in vivo* conditions. Since direct transfer of plantlets from tubes to soil, even under controlled greenhouse conditions, is sometimes unsuccessful and leads either to the death of plants or to the formation of weakened plants. Plants obtained from microtubers are free from such defects and, upon germination, develop into normal and healthy plants. In addition, rooting plantlets in soil requires a lot of labor, and the yield of such plants is on average 2 times less than that obtained from 'microtubers plants' (Hossain et al., 2017a).

There are many methods described in the literature for obtaining potato microtubers, since each variety requires selection of optimal conditions (Sivakumar et al., 2024). However, the general condition for inducing tuberization is a high sucrose content in the growth medium (Hossain et al., 2017b). The most commonly used concentration is 8% sucrose in MS medium (Sivakumar et al., 2024). Also, a number of researchers observed that the addition of certain phytohormones to a medium with a high sucrose concentration increase both the number and weight of microtubers formed (Sivakumar et al., 2024; Mohamed and Girgis, 2023). Most methods indicate phytohormones such as BAP and kinetin, either separately or together in concentrations from 0.2 to 10 mg/l. Less common are combinations of cytokines and auxins such as NAA or IAA (Sivakumar et al., 2024). In addition, some works described the effect of such exotic additives as coconut milk (Dwiati and Anggorowati, 2011), jasmonic acid (Samant et al., 2018), chloro choline chloride (Zakaria et al., 2008), or putrescine (Khorsandi et al., 2020). Nevertheless, all researchers indicate that the main factor in inducing tuber formation is precisely the high concentration of sucrose in the medium.

Most researchers also indicate a shortened photoperiod as one of the conditions for inducing microtubers formation (Sivakumar et al., 2024). The photoperiod duration varies from 8 hours of day light (Park et al., 2009) to complete darkness (Mohamed and Girgis, 2023). At the same time, there are studies where tuberization occurs under standard illumination and a 16-hour photoperiod (Lugovtsova et al., 2022). Dwiati and Anggorowati (2011) showed that dark period didn't have significant effect on the microtuberization of potato.

Thus, the procedure of microtubers production is not complicated, but it requires a long period of time necessary for the formation of microtubers. For different varieties, the time period is different, for example, *Lugovtsova* et al. showed that the formation of microtubers begins from the 3d week and ends by the 8th-9th (Lugovtsova et al., 2022). In other studies, the formation of microtubers took from 8 to 11 weeks (Dwiati and Anggorowati, 2011) or 10 weeks from the moment of planting on a high-sucrose medium (Hossain et al., 2017b). In addition, it is necessary to add at least three more weeks to the total period, during which the primary cultivation of plants occurs, which are then placed on media for microtubers formation. Thus, the average time for obtaining microtubers is about 3 months.

Next, it is necessary to take into account the need of the dormant period for microtubers. It is known that a period of dormancy is necessary for the germination of potato tubers (and microtubers). This is a physiological state in which shoots do not grow, even if the tuber is placed in ideal conditions for this (Sadawarti et al., 2016). The duration of the dormancy period of microtubers correlates with that of standard tubers of the same variety (Sadawarti et al., 2016). The difference in the duration of the dormancy period for different varieties can reach several months: from 1 to 7 (Leclerc et al., 1995). At the same time, differences in the storage of microtubers of the same variety can give a dormancy difference of 4-6 weeks (Karjadi and Waluyo, 2021). On average, in laboratory research, to simulate the dormancy period, microtubers are placed in a refrigerator at 4 °C for 3-4 months (Diémé et al., 2021).

That is, the total time for transferring plant from *in vitro* conditions to *in vivo* conditions takes on average half a year. In this paper, we propose a method for obtaining microtubers, which reduces the time for production of tubers ready for germination to 1.5-2 months.

We investigated the production of potato microtubers of Milena (Sedek, Russia) and Gala (NORIKA GmbH, Germany) varieties. For the study, we used both the original local varieties and transgenic lines of potatoes previously obtained in our laboratory (Nizkorodova

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et al., 2024). We compared the standard method of obtaining microtubers (Sivakumar et al., 2024) and the method we proposed (designated as "2.5S"): we took into account such parameters as the rate of microtubers formation, weight and number of microtubers, the need for a dormant period and viability of microtubers. The rate of formation of "2.5S" microtubers is approximately 3-4 weeks faster for both varieties. The number of microtubers varies within insignificant limits, but the weight for the "Gala" variety (the original variety and three transgenic lines) is significantly higher (by 1.6-3.6 times) for "2.5S" microtubers. As for dormant period, "2.5S" microtubers of "Milena" variety showed sprouting 3 months earlier than "8S" microtubers since they do not have a dormant period and germination begins immediately as they form in the cultivation tube. However, when planted in the soil, such microtubers showed 100% germination. "2.5S" microtubers of "Gala" variety also had no dormant period but they did not sprout in a tube although germinated in the soil immediately after transfer from cultivation tube.

Materials and methods

Micropropagation of plantlets

The plantlets were propagated using single-node cuttings. The explants were cultured in Kimble Culture Tubes ("DWK Life Sciences") with Magenta 2-way caps ("Merk") on MS medium (MS Basal Medium, "Sigma") containing 2 mg/l glycine, 100 mg/l myo-inositol, 0.5 mg/l nicotinic acid, 0.5 mg/l pyridoxine-HCl, 0.1 mg/l thiamine-HCl, 20 g/l sucrose and 0.6% agar. The pH was adjusted to 5.8-6 before adding the agar and autoclaving. The amount of medium in the tubes was 10-15 ml. The tubes were placed in a cultivation room at 23 °C and 16 h photoperiod. Sterile potatoes were micropropogated *in vitro* by subculturing the top shoots or stem segments including axillary buds every 4-5 weeks.

Production of potato microtubers using the "high sucrose concentration" method

Potato plants of both varieties were grown in Kimble Culture Tubes ("DWK Life Sciences") with Magenta 2-way caps ("Merk") on a standard cultivation medium (MS Basal Medium, "Sigma") containing 20 g/l sucrose and 0.7% agar, with a 16-hour photoperiod and a temperature of 23 °C. Three-week-old plants were aseptically transferred whole (with roots) to a medium for microtubers formation (MS Basal Medium containing 80 g/l sucrose, 0.2 mg/l BAP and 0.8% agar). Each plant was rooted with tweezers in 40 ml of medium in a culture jar with Magenta B-cap ("Merk"). The jars with plants were incubated in complete darkness at 21 °C for 6-8 weeks. Microtubers were collected under aseptic conditions in sterile tubes.

Production of potato microtubers using the proposed accelerated method ("2.5S")

Potato plants of both varieties were grown in Kimble Culture Tubes ("DWK Life Sciences") with Magenta 2-way caps ("Merk") on MS medium (MS Basal Medium, "Sigma") containing 25 g/l sucrose and 0.6% agar, with a 14-hour light day and a temperature of 23 °C. The amount of medium in the tubes was 3-4 times less than usually (3-4 ml instead of 13-15 ml). Microtubers formation began with depletion of the nutrient medium from about the 4^{th} week. Microtubers were collected under aseptic conditions in sterile tubes.

Statistical analysis

To analyze the reliability of differences between groups (by microtubers weight) due to their small size and independent distribution, the nonparametric Mann-Whitney U test was used (Iuliano and Franzese, 2019). After calculating the Mann-Whitney statistic (z), significance levels (p) were determined for the obtained values using the normal distribution function of Microsoft Excel. The significance level was taken to be 5% ($p \leq 0.05$); i.e. at $p \leq 0.05$, the samples were considered to be reliably different.

Germination of microtubers in soil

After a dormant period (8 weeks at 4 °C), the microtubers were placed in pots with universal soil (Borresursy, Russia) and germinated at a 16-hour daylight period and a temperature of 23 °C. Some microtubers of both varieties, obtained using the "2.5S" method, were planted without the dormant period immediately after cutting.

Results

Microtubers production

For the experiment, we used two early-ripening potato varieties – Gala, a German-bred variety (NORIKA GMBH), and Milena, a Russian-bred variety (Sedek agrofirm). In addition to the original plants of the varieties (wild type), we also used a number of transgenic potato lines obtained in our laboratory earlier (Nizkorodova et al., 2024). We used transgenic lines for clarity, since the main application of the described method, as we see, is the acceleration of the process of obtaining modified potato plants.

The process of obtaining ready-to-germinate microtubers for both varieties using the high-sucrose method typically required approximately 5-6 months. Microtubers of both varieties began to form approximately on the 5^{th} week after transferring the plants to culture jars with the MS8S medium. After 8 weeks on the MS8S medium, more than 90% of the plants formed microtubers; Fig. 1 shows the plants before harvesting the microtubers. The harvested microtubers were weighed and placed in closed tubes in a refrigerator at 4 °C.

After that, we started to obtain microtubers using a modified "low-sucrose" method. According to our observations, a slightly higher concentration of sucrose in the medium compared to the micropropagation medium (25 instead of 20 g/l) accelerates the rooting of plants. The tubers began to form on the 4^{th} week after planting single internodes. Microtubers of Gala variety were fully formed by the 6^{th} week of plant cultivation; Fig. 2 shows plants of this variety before harvesting the microtubers. Microtubers of Milena variety were fully formed and began to sprout immediately by the 5^{th} week of cultivation (Fig. 3).

Fig. 1: Microtubers of different transgenic lines and WT of Milena variety (upper part) and Gala variety (lower part) after 8 weeks of cultivation on MS8S in darkness. WT plants are in the 2^{nd} jar in the row both for Milena and Gala varieties, all the other plants are transgenic.

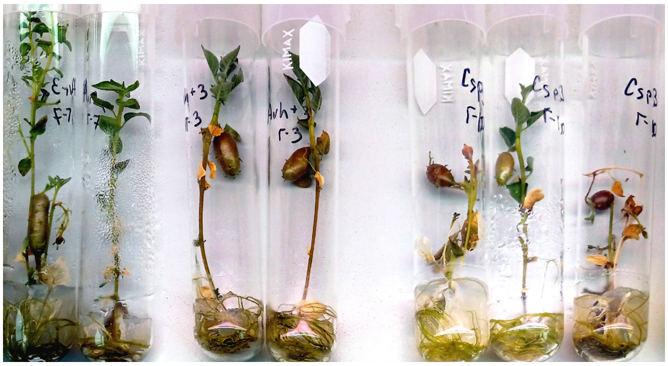

Fig. 2: Microtubers of different transgenic lines of Gala variety after 5 weeks of cultivation on MS2.5S.

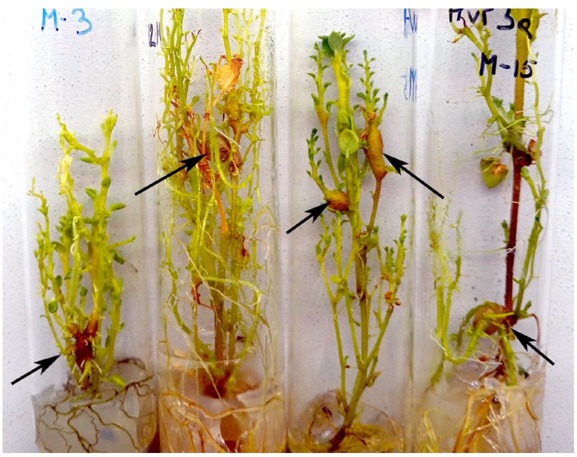

Fig. 3: Microtubers of different transgenic lines of Milena variety after 4 weeks of cultivation on MS2.5S. Sprouting microtubers are shown with arrows.

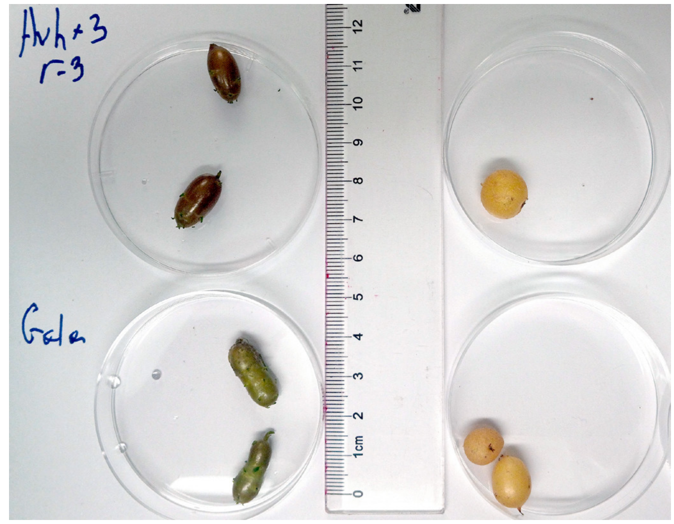

Fig. 4: Microtubers of Gala variety obtained on MS2.5S medium (left part) and on MS8S (right part). The microtubers of WT (lower part) and transgenic line (upper part) are shown.

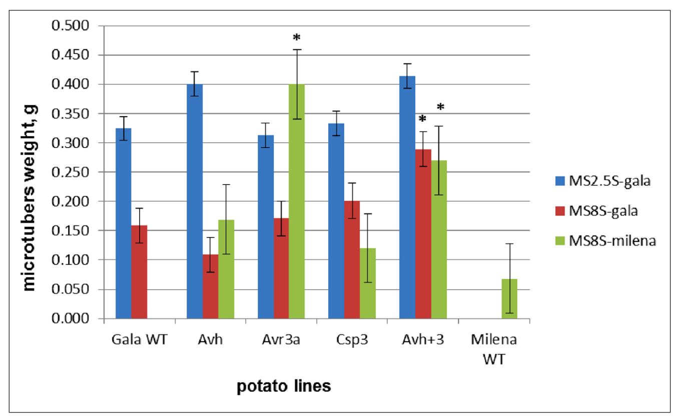

Fig. 5: Representation of the average weight of microtubers of different transgenic lines and the original variety (Gala WT, Milena WT) obtained on MS2.5S and MS8S media. Asterisk indicates transgenic lines which microtubers have significant difference with those of wild type plants.

Microtubers' characteristics

The number of tubers obtained on MS8S and MS2.5S varied slightly between lines (Tab. S1, Supplementary) and was not statistically significant. At the same time the difference in appearance between the microtubers obtained on the high-sucrose MS8S medium and the low-sucrose MS2.5S medium was significant. No difference was found between the wild type and transgenic lines of the same variety. Fig. 4 shows the Gala variety microtubers of MS2.5S and MS8S – the difference was both in the color of the microtubers and in their shape. In fact, all the microtubers (including the Milena variety) obtained on MS8S were round, very close to a ball-shape and light yellow or beige in color, with almost invisible eyes. While the microtubers obtained on MS2.5S were elongated, spindle-shaped and dark in color – from greenish-brown to red-brown with clearly visible, protruding eyes. The shape of Milena variety microtubers, obtained on MS2.5S, was also spindle-shaped and color – brown of different shades (Fig. 3).

The difference in color certainly reflects the difference in the lighting conditions by microtubers production – the light color reflects the complete absence of light when obtaining tubers on MS8S. The shape of the tubers in this case most likely depends on the availability of free space – for plants on MS8S, the volume of the culture jars (200 ml) exceeded the volume of the tubes of plants on MS2.5S (55 ml) by almost 4 times.

It should also be noted that there were no differences in the appearance of microtubers from transgenic and non-transgenic plants of the same variety obtained under the same conditions. In terms of the average weight of microtubers obtained on MS8S, statistically significant differences were shown for two transgenic lines (Fig. 5). Microtubers of the 'Avh+3' line of the Milena variety were almost 4 times heavier than those of the wild type, and those of the Gala variety were 1.8 times heavier. Microtubers of the 'Avr3a' line of the Milena variety were 5.9 times heavier than those of the wild type. At the same time, for microtubers obtained on MS2.5S, there was no significant difference in weight between the transgenic and original plants.

Comparative analysis of the weight of Milena microtubers obtained by two different methods was not possible, since MS2.5S microtubers sprouted immediately as they formed. At the same time, analysis of the weight of Gala microtubers (Tab. 1) showed a statistically significant difference between the microtubers obtained by different methods. On average, the microtubers obtained on MS2.5S were larger and heavier than those obtained on MS8S: the original plants (wild type) – twice as large, transgenic lines – from 1.6 to 3.7 times. Only one transgenic line 'Avh+3' did not show statistically significant differences in microtubers weight.

Tab. 1: Weight of microtubers of different lines of 'Gala' variety

Tubers production method	Tubers characteristics	Potato line				
		WT	Avh	Avr3a	Csp3	Avh+3
tubers on MS2.5S	mean weight*, g	0.32±0.07	0.40±0.06	0.31±0.02	0.33±0.03	0.414±0.04
	relation of transgenic line to WT, %	100.0%	123.3%	96.5%	102.8%	127.6%
	z		-0.704	-0.634	-0.545	-0.623
	p		0.241	0.263	0.293	0.267
tubers on MS8S	mean weight*, g	0.16±0.04	0.11±0.06	0.17±0.07	0.20±0.07	0.29±0.04
	relation of transgenic line to WT, %	100.0%	68.2%	107.1%	126.1%	**181.3%**
	z		-0.617	0.159	-1.133	-1.933
	p		0.269	0.563	0.129	0.027
comparison of methods by tubers weight	relation of tubers "MS2,5S" to tubers "MS8S", %	**203.4%**	**367.8%**	**183.3%**	**165.8%**	143.3%
	z	-2.317	-2.345	-1.659	-2.236	-0.731
	p	0.010	0.010	0.049	0.013	0.232

z – Mann-Whitney statistic which corresponds to shown p-values. Values which difference is statistically significant ($p \leq 0.05$) are shown in bold.
* – weights of microtubers obtained on MS2.5S were measured after harvesting on the 6th week of microtubers production; weights of those obtained on MS8S – on the 9th week of microtuber production.

Microtubers germination in soil

It is also worth noting the differences in the germination of microtubers obtained by different methods. The most rapid sprouting was observed for Milena microtubers obtained on MS2.5S. These microtubers were placed in pots with soil immediately after cutting, placing them in the soil so that the shoots formed *in vitro* were above the soil. On average, on the 10th-15th day after planting in the soil, strong green shoots appeared from additional eyes of the microtubers, and the initial shoots gradually died off (Fig. 6). The germination rate of such microtubers was 100%. A number of microtubers with sprouts were placed under aseptic conditions in sterile 2-ml microtubes and stored in a refrigerator for 2 months. That was done in order to germinate simultaneously all kinds of microtubers for comparative analysis.

Fig. 6: Germination of microtubers of the Milena variety obtained on MS2.5S (wild type). Day 10 after planting in soil (left), day 15 (right). The arrows indicate the initial shoots that appeared during the germination of the tubers *in vitro*.

Some microtubers of Gala variety obtained on MS2.3S were also planted in soil immediately after cutting. We decided that judging by the appearance of the microtubers (well developed and protruding eyes) they most likely did not need a dormancy period. On the days 20-23 all planted tubers germinated, so germination rate was also 100%. However, most part of produced microtubers was stored in the refrigerator for 1.5 to 2 months. Thus, the microtubers obtained on MS2.5S were simply stored until the dormancy period of the microtubers obtained on MS8S expired. After the microtubers obtained on MS8S had passed the dormancy period (3 months), all tubers were planted in pots with soil.

On the third day after planting in the soil, the microtubers of the Gala variety, obtained on MS2.5S, began to sprout. On the 8th day, the microtubers of the Milena variety, obtained on MS2.5S, began to sprout. Just as in the case of the microtubers of this variety, which were planted immediately after cutting, new shoots began to sprout and the old ones, sprouted *in vitro*, gradually died off. Microtubers of the Gala and Milena varieties, both transgenic and wild type, showed 100% germination – by the 18th day all planted microtubers obtained on MS2.5S had sprouted (Fig. 7).

Fig. 7: Germination of microtubers, day 18 after planting in soil. Left row: tubers of Gala variety from MS2.5S – transgenic line in the first pot, wild type in the rest pots. Middle row (microtubers from MS8S): Milena WT – first in line pot, Gala WT – last in line pot, transgenic line of Gala – in the middle. Right row: tubers of Milena variety from MS2.5S – transgenic line in the first in line pot, wild type in the rest pots.

As for the microtubers obtained on MS8S, their germination for both varieties began on the 16th day (Fig. 7) and ended by the 25th; the germination rate was 81% (Tab. S2, Supplementary). The microtubers obtained on MS2.5S showed a difference in sprouting dynamics depending on the mass of the microtubers – larger ones germinated faster, smaller ones slower. In Fig. 7, this difference is clearly visible in the Gala microtubers (left row) – wild-type microtubers were planted in the second (two small microtubers) and third pots (one large); the first pot in the row was planted with transgenic line microtubers (one small and one large). But the smaller microtubers of both varieties obtained on MS8S did not germinate at all.

Discussion

The analysis of literature that preceded our work showed that researchers consider a high concentration of sucrose in the nutrient medium (Sivakumar et al., 2024) to be the main condition for the formation of microtubers. However, a number of authors (Mohamed and Girgis, 2023; Ranalli, 2007) have shown that the process of microtuberization also occurs with low sucrose content in the medium (3-4%). However, the process itself takes twice as long, and the microtubers are 2-3 times smaller.

Upon further analysis of the literature, it was observed that microtubers were initially produced using a medium containing 2% sucrose (Barker, 1953), and there we also found the source of the shortened photoperiod. As it turned out, microtubers were initially obtained from the eyes of ordinary tubers, which were germinated in sterile conditions in the medium in the dark, and then left in the dark until microtubers appeared, although microtubers were formed when they were transferred to the light as well (Barker, 1953). Thus, neither the sucrose concentration nor the photoperiod, are the main conditions for the induction of microtubers formation.

Since microtubers are complete analogues of ordinary field potato tubers (Ranalli, 2007; Hossain et al., 2017a), we turned our attention to the process of tuber formation *in vivo*. Further analysis of the literature showed that tubers can form on any part of the potato stem when the plant is placed in soil deficiency conditions (Thijn, 1959). It was also shown that plants attacked by *Rhizoctonia solani* Kühn are spontaneously capable of forming tubers on the axils of the lower leaves, and when grown on stones at the end of the growing season – on flower trusses (Thijn, 1959). The formation of tubers on these parts of plants is very similar to the formation of microtubers, which also form on the upper parts of plants. This led us to the hypothesis that perhaps the trigger mechanism for microtubers formation, as in the described cases with tuber formation, is stress. After all, high concentration of sucrose as well as shortened photoperiod, are certainly stressful conditions for plants.

With this hypothesis as a basis, we decided to try to reduce the time required for microtuberization. We reasoned as follows: most likely, not all types of stress will induce microtubers formation, but nutritional stress (high sucrose concentration) has already been shown to lead to microtuberization. Slow formation of microtubers using high-sucrose methods is most likely due to the fact that it takes a long time for stolons (stems) to form on such a medium (Wazir, 2015). If one takes an already formed plant and places it under nutritional stress, then microtubers should form faster. On the other hand, nutritional stress is not only an excess of sucrose, but also an extreme lack of nutrients, as Thijn (Thijn, 1959) showed.

Having come to this conclusion, we created the proposed scheme for obtaining microtubers – plants in the form of single-node segments are planted on a standard nutrient medium under standard temperature conditions and with slightly less illumination than usual. In our opinion, 14 hours of daylight should correspond to the end of summer in the field. But several times less nutrient medium is added; we took 3-4 times less. Thus, the plant develops normally, forms a powerful stem and root system, and by about the 4^{th} week it faces gradually approaching hunger, since the nutrient medium by this time is almost completely processed by the plant. From this moment, the plant begins to actively form 1-2 fairly large microtubers and this process occurs within 1-2 weeks. Thus, the time saved on microtuberization is on average 2-3 weeks, if we consider the standard time for microtubers formation to be 8 weeks.

The next factor that reduces the time required for transferring plants from *in vitro* to *in vivo* conditions is the dormancy period of the microtubers obtained through the method we proposed. The duration of the dormancy period of microtubers depends mainly on two factors – the genotype and environmental conditions during formation and storage (Sadawarti et al., 2016). It is known that the main conditions that determine the duration of the dormancy period of tubers are temperature, illumination and humidity (Di et al., 2024; Sadawarti et al., 2016). When storing microtubers at higher temperatures and lower humidity, the dormancy period decreases. The so-called “heat sprouting” is known, when immediate termination of tuber dormancy occurs at very high (>35 °C) field temperatures (Suttle, 2007). Increased illumination is used to induce germination of tubers and microtubers; when microtubers were exposed to 8-h photoperiods instead of being held in complete darkness dormancy duration in microtubers was significantly reduced (Suttle, 2007). Another important factor affecting the duration of the microtubers dormancy period is their size – the larger the size, the shorter the dormancy period (Park et al., 2009).

Microtubers obtained by the method proposed in this work meet the conditions for inducing rapid germination. Firstly, microtubers are obtained under normal illumination; secondly, the size of the obtained microtubers is higher than average; thirdly, since the effect of light occurs during the microtuberization, the temperature factor also begins to play a role at the same time. Apparently, for the combination of these reasons, the dormant period of microtubers is completely absent.

In the case of Gala variety, the microtubers can be planted directly into the soil or stored in the refrigerator for later use. For Milena variety, it is a little more complicated, since they begin to germinate immediately as they form in the test tube. However, such microtubers have 100% germination rate and can also be stored in the refrigerator in tightly sealed test tubes. Thus, the microtubers of this variety are less convenient for storage and transportation. Since we assume the use of this method mainly for laboratory research, this is not critical.

Conclusion

The presented method of microtubers production could be highly valuable for research teams working on developing new potato lines. The simplified scheme of work involves directly transferring the regenerant of the transgenic plant (or somaclonal variant) from the Petri dish into a test tube containing MS 2.5S medium, where it develops normally for 3-4 weeks. During this time, it is analyzed for the presence of the required feature (1-2 leaves are used for DNA analysis or RNA profile); additionally, it can be micropropogated. If the analysis shows the desired result, then in 1-2 weeks, as the nutrient medium is depleted, microtubers are formed, which are either placed in the refrigerator (rest period), or immediately planted in the soil.

Thus, further study of the obtained line in soil is postponed for a maximum of a month, and not three or four, as with other methods of microtubers production. It should also be taken into account that there is no need to prepare separate media for obtaining microtubers, unnecessary costs for cytokines and reducing labor costs associated with transferring plants to new media.

It is important to highlight that for Gala variety, the weight of microtubers obtained through the proposed method was 1.6-3.6 times higher than the weight of microtubers produced via the standard method. Larger microtubers have a greater germination potential, which can significantly enhance the effectiveness of the proposed method.

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Conflict of interest
No potential conflict of interest was reported by the authors.

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Supplementary material

Tab. S1: Total number of microtubers produced during the experiment

Potato lines	Number of Gala microtubers on MS2,5S	Number of Gala microtubers on MS8S	Number of Milena microtubers on MS2,5S	Number of Milena microtubers on MS8S
WT	11	9	8	7
Avh	6	4	5	5
Avr3a	10	7	8	7
Csp3	3	5	3	3
Avh+3	5	5	19	24

Note: the number of plants of each line is 4 for each media; the total number of microtubers obtained in the experiment for all plants of each line is given. For the Milena variety (MS2.5S), only microtubers which size exceeded 5 mm and which were cut for planting in the soil were taken into account. 'WT' means non-transgenic initial plants (wild type).

Tab. S2: Germination of microtubers in soil

Potato lines	Microtubers germination rate, %			
	Gala microtubers on MS2,5S [1)]	Gala microtubers on MS8S [2)]	Milena microtubers on MS2,5S [1)]	Milena microtubers on MS8S [2)]
WT	100	78	100	86
Avh	100	75	100	80
Avr3a	100	86	100	86
Csp3	100	80	100	100
Avh+3	100	60	100	79

Note: 'WT' means non-transgenic initial plants (wild type).
[1)] germination ended by the 18th day after planting microtubers in soil
[2)] germination ended by the 25th day after planting microtubers in soil