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A Temporary Immersion System as a Tool for Lowering Planting Material Production Costs Using the Example of *Pennisetum* × *advena* 'Rubrum'

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Abstract: The aim of the study was to compare the variable costs of planting material production using the example of vitro cultures of *Pennisetum* × *advena* 'Rubrum'. In the study, temporary immersion system (TIS)- and agar-based methods were used in innovative workday organisation. The workday structure involved a six-hour passaging period followed by a two-hour break for medium preparation, autoclaving, and maintenance tasks. The TIS was found to be more cost-effective than the agar cultures, with lower labour costs and comparable growing expenses. The most expensive element of agar production was labour which was 43% of the costs. The second biggest cost was materials and reagents which represented 25%. In a TIS, production materials and reagents are the most expensive part of production (44%), while labour represents 24% of costs. A TIS offers a much faster multiplication of plants than agar cultures. Plants obtained in the multiplication phase are two times cheaper using a TIS. Rooting accounted for a significant portion of production costs in both methods. Overall, the TIS demonstrated superior efficiency and cost-effectiveness compared to agar cultures in producing *Pennisetum* × *advena* 'Rubrum' plants.

Keywords: temporary immersion system; bioreactor; production cost

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Citation: Pożoga, M.; Olewnicki, D.; Latocha, P. A Temporary Immersion System as a Tool for Lowering Planting Material Production Costs Using the Example of *Pennisetum* × *advena* 'Rubrum'. *Agriculture* **2024**, *14*, 1177. https://doi.org/10.3390/agriculture14071177

Academic Editor: Shanker Lal Kothari

Received: 13 June 2024
Revised: 15 July 2024
Accepted: 16 July 2024
Published: 18 July 2024

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1. Introduction

Modern agriculture requires a sustainable approach with a primary focus on reducing resource consumption and adopting modern methods of producing high-quality planting material that is disease free, pest resistant, and uniform in size. This facilitates effective control over plant growth and yield. Emerging solutions in the form of tissue culture techniques are increasingly addressing these challenges. Furthermore, in vitro production may address the declining agricultural labour resources, as noted by Girdziute et al. and Poosappan et al. [1,2]. Micropropagation is a technique used to propagate and cultivate plants under sterile and controlled conditions. It involves the aseptic culture of plant cells, tissues, or organs in an artificial nutrient medium, allowing for the regeneration of whole plants. This technique has various applications in plant science, agriculture, biotechnology, and especially horticulture. At the same time, the number of commercial laboratories involved in the production of horticultural plants is growing [3]. Reducing production costs in plant tissue culture laboratories is crucial for ensuring economic viability, expanding market reach, fostering competitiveness, and advancing research and development in the field of plant biotechnology. It aligns with the broader goals of making plant tissue culturing more accessible, affordable, and impactful on a global scale. This can be achieved by using plastic bags for growing instead of glass vessels, substituting agar with gellan gum, replacing laboratory-grade sucrose with food sugar, using photoautotrophic systems

and effective protocols, implementing proper workday organisation, or implementing a temporary immersion system (TIS) [3–6].

A TIS consists of two autoclavable vessels connected to each other and to the air pump with tubing. One vessel is for the medium and the second contains explants. Using air pressure, the medium from one vessel is transferred to the second vessel via the tubing and immerses the explants for a set amount of time. Then, the medium is transferred back to the first vessel by air pressure or by gravity, depending on the system used [4]. TISs can be adapted for various plant species and types of tissues. They are used in research laboratories for studying plant physiology, as well as in commercial settings for the large-scale propagation of plants [7–9]. One of the most commonly produced plants by tissue culture in agriculture for which TIS methods have been developed is the potato [10,11], as well as pineapple [12–14] and banana plants [13–17]. The use of a TIS in plant tissue culturing is a technique that involves the cyclic immersion of plant explants or tissues in a liquid nutrient medium using specialised bioreactors or culture vessels [18]. This cyclic immersion promotes efficient nutrient uptake by the plant tissues. During immersion, the explants absorb essential nutrients from the liquid medium, and during exposure, they exchange gases. The controlled immersion and drainage cycles contribute to the uniform growth and development of plant tissues [19,20]. This helps reduce variability in cultures and ensures consistent results which are much better than agar-based cultures. There are many reports of an increased number of leaves and roots and increased shoot lengths in comparison to plants from agar cultures [21]. Many temporary immersion systems are designed for automation, allowing for precise control of the immersion and exposure cycles. This automation reduces the need for manual intervention and ensures reproducibility in the tissue culture process [22].

The advantages of temporary immersion systems include increased efficiency, reduced labour requirements, and improved growth rates compared to traditional agar-based tissue culture methods. TISs enable better control over nutrient utilisation and resource consumption. This optimisation can result in a reduced wastage of media, nutrients, and other resources, leading to cost savings [23].

These systems are especially advantageous for the large-scale propagation of plants in a controlled and sterile environment [16,23–25]. Automated systems can handle a larger number of cultures simultaneously, increasing the overall throughput of the tissue culture process. This is particularly valuable for high-throughput applications, such as the large-scale propagation of plants for commercial purposes. A TIS enables the expansion of operations while maintaining the quality of the produced plants [26–28]. Automation can be integrated with other technologies such as robotics, sensors, and imaging systems to further enhance the efficiency and capabilities of plant tissue culture processes.

TISs offer flexibility in production scheduling, allowing for better a alignment with market demands. This flexibility can prevent overproduction and unnecessary costs associated with maintaining excess inventory. TISs are designed to maximise space utilisation, allowing for more efficient use of the available laboratory or production space. This can be particularly advantageous in settings where space is a limiting factor.

It is important to note that while a TIS can contribute to cost savings, the initial investment in acquiring and setting up these systems should be considered. The magnitude of savings will depend on factors like the size of the operation, the frequency of production cycles, and the degree of automation implemented. In many cases, the long-term benefits of improved efficiency, consistent production, and reduced labour costs can outweigh the initial investment, making a TIS a cost-effective solution for plant tissue culture.

While TISs offer numerous advantages, it is crucial to consider the specific requirements of the plant species and the objectives of the tissue culture project. TISs are not universally suitable for all plant types. Factors like cost, complexity, and scale should be considered when choosing the most appropriate tissue culture method. By optimising resource use and minimising waste, TIS contributes to more environmentally sustainable plant tissue culture practices. This is particularly relevant in large-scale production settings.

2. Materials and Methods

2.1. Experimental Model

This study compared explants excised from in vitro cultures of *Pennisetum* × *advena* 'Rubrum' as a continuation of previous research on agar-based and TIS cultures from the most efficient workday organisation proposed by Pożoga and Olewnicki (2023) [6]. This workday organisation started with six-hour passaging and was followed by a two hour on break, preparing medium for the next day, autoclaving, and removing contaminated cultures from the growth room. Passage consisted of transferring all plants from the container or TIS on a paper plate, cutting off leaves approximately 1.50–2.00 cm above the clump base, dividing plants into single explants, and transferring them to a new container or TIS. Rooting clumps were divided into single plants and placed in an agar medium. At the end of each work hour, the person not involved in passaging collected several containers and TISs with transferred plants. The experiment was repeated three times. Each time there were ten containers or TISs with ten explants. Plants obtained from agar cultures and TISs were comparable in size and quality after acclimatisation. Data including the number of plants in clumps obtained from agar cultures and TIS (19.5 and 36.2, respectively) and rooting percentage (84%) were acquired from our previous study [5].

2.2. Culture Conditions

The culture medium used was Murashige and Skoog [29] (MS) medium with added vitamins: 2 mg /L Glycine, 100 mg/L *Myo*-inositol, 0.5 mg/L Pyridoxine, 0.5 mg/L Nicotinic acid, and 0.1 mg/L Thiamine, supplemented with 1 mg/L 6-benzyl amino purine (BAP), 2% sucrose, and 7 g/L plant agar, except for the TIS. The rooting medium for plants from both agar culture and TIS was MS medium with vitamins, supplemented with 0.5 mg/L indole-3-butyric acid (IBA), 0.5 mg/L naphthalene acetic acid (NAA), 2% sucrose, and 7 g/L plant agar. The photoperiod was set to 12 h of daylight followed by 12 h of darkness, with lighting provided by cool-white fluorescent tubes (3100 lm). The temperature was maintained at a constant 23 °C both day and night. The subculture period was eight weeks for agar cultures and four weeks for TIS. Agar cultures were carried out in 350 mL single-use plastic containers, each containing ten explants. The TIS, which was reusable, consisted of two 1.8 L jars. (Figure 1). The immersion frequency was 1 min/1 h. Rooting took 3 weeks.



Figure 1. TIS used in the experiment consisted of two 1.8 L jars connected with tubing.

2.3. Variable Costs Comparison

Variable costs, such as reagents and containers, labour costs, electricity consumption, and plant growth in a 'growth room' were considered. All prices are presented as net prices. Most of the essential reagents were purchased from Duchefa Biochemie (BH Haarlem, The Netherlands). Wholesale prices for 100 L of MS medium are USD 55.60 per 100 L, plant agar costs USD 2132.04 per 25 kg, and BAP costs USD 122.67. As a source of carbon, sugar was used as a cheaper alternative to highly purified sucrose, with the cost of 1 kg of 'Diamant' sucrose being USD 1.23. Wholesale prices are listed in Table 1. When costs were low, the prices were presented in thousandths and marked with an asterisk (*). Fixed costs, including the laboratory building and equipment, were not considered in this study due to differences between laboratory equipment and the lack of comparability. The 350 mL containers used in the experiment cost USD 0.08. TIS costs USD 4.44. It was assumed that a single TIS would be used 60 times until tubing and sealing should be replaced. So, for further calculations TIS price was divided by several uses which gave USD 0.07 plus 20% overhead for autoclaving, connecting and washing. The total cost of TIS was USD 0.09.

Table 1. Wholesale prices and costs of reagents used in research.

Ingredients for Medium Preparation	Wholesale Prices of Ingredients (in USD)	Ingredients for 1 L of Medium Preparation	Costs of Ingredients for 1 L of Medium Preparation (in USD)
MS medium Duchefa 100 L	55.60	MS medium Duchefa (1 L)	0.56
Sucrose (food sugar) 1 kg (1000 g)	1.23	Sucrose (20 g)	0.02
Agar Ducheffa 25 kg (25,000 g)	2132.04	agar Ducheffa (7 g)	0.60
BAP Ducheffa 25 g (25,000 mg)	122.67	BAP Ducheffa (1 mg)	0.005 *
IBA Ducheffa 25 g (25,000 mg)	75.64	IBA Ducheffa (0.5 mg)	0.002 *
NAA Ducheffa 100 g (100,000 mg)	27.75	NAA Ducheffa (0.5 mg)	0.001 *
Total cost per 1 litre of agar-based multiplication medium			1.19
Total cost per 1 litre of TIS medium			0.59
Total cost per 1 litre of rooting medium			1.18

*—Prices were exceptionally presented in the thousandth part when costs were low.

Each 350 mL container was filled with 0.083 L of medium, meaning 1 L of medium is enough to fill 12 containers. The TIS was filled with 400 mL of medium, so 1 L of medium was sufficient for 2.5 TIS. The cost of one container with medium was calculated by dividing the total cost of 1 L of medium by the number of containers/TIS it could fill, and then adding the price of a single container/TIS.

The labour cost per man-hour was USD 5.43. The power consumption of the laminar flow chamber was 49 W (0.049 kW), and the electricity cost was USD 0.26 per kilowatt hour. To calculate the cost of labour required to produce one container with plants, we divided the man-hour salary by the number of containers produced within one hour. The cost of electricity consumption necessary to produce one container with plants was calculated by multiplying the electricity consumption (of the laminar flow chamber) by the cost of 1 kilowatt hour and then dividing by the average number of containers or TIS produced during one hour.

The total cost of labour and electricity for the production of a single plant is the cost of producing one container with plants, divided by the number of explants in the container. The "growing room" was equipped with racks containing 5 shelves. Each shelf measured 140 × 60 cm, providing an area of 0.84 m². Each shelf was illuminated by one 36 W (0.036 kW) cool-white fluorescent tube. Each shelf can accommodate either 55 containers of 350 mL each, with 10 explants per container, or 30 TIS bioreactor units with plants and

an additional 30 TIS bioreactor units underneath. The cost of growing plants in agar and rooting was calculated using the formula proposed by Pożoga et al. (2019) [30]:

$$PGC = (\frac{EC*PH*xW}{NC}*kWh)/N$$

where:

PGC—plant growth cost;
EC—hourly electricity consumption per one shelf;
PH—photoperiod length;
xW—number of weeks, where W is 7 days;
NC—number of containers on one shelf;
kWh—cost of 1 kWh;
N—number of explants.

The air pump for TIS support had a capacity of 22 W (0.022 kW). The air pump for TIS worked for 2 min to set pressure on each jar. During the day it worked for 96 min.

$$PGC=(\frac{(EC*PH*xW)+(AP*AW*xW)}{NTIS}*kWh)/N$$

where:

PGC—plant growth cost;
EC—hourly electricity consumption per one shelf;
PH—photoperiod length;
xW—number of weeks, where W is 7 days;
AP—air pump consumption;
AW—air pump working time during one day;
NTIS—number of TIS on one shelf;
kWh—cost of 1 kWh;
N—number of explants.

Other costs, such as preparation time for the medium, transportation of containers from the laminar chamber room to the growing room, and power consumption for autoclaving, air conditioning, water distillation, and pH meter usage, were considered as economic overheads. These overheads added 20% to the total cost of producing one container with ten explants. Rooting costs were similarly calculated. Additionally, the final cost was adjusted to account for the percentage of unrooted shoots.

The gross margin of variable costs in this research was calculated based on the value of production, following the method described by Elum et al. (2016) [31]. The gross margin was calculated using the following formula:

$$GM = (TR - TVC)/TR * 100\%$$

where:

GM—gross margin expressed as a percentage;
TR—total revenue in USD;
TVC—total variable costs in USD.

The Gross Margin model is an excellent tool for conducting the initial assessment of production. According to market research, the price of a single plant sold in agar is USD 0.32.

3. Results

The average number of containers of plants obtained from the agar cultures each day was 107.6 and 17.6 per hour. The TIS proved to be the most productive method. In the TIS, the average daily number of containers of plants obtained was 191.1 and 31.5 per hour, while the average number of containers of plants obtained daily from the agar rooting was 138.8 and 22.8 per hour. The average number of containers/TIS obtained at specific

hours is presented in Table 2. In each system, the fourth hour was the most efficient for passaging. The efficiency of fourth hour of the workday with agar cultures was 44.4% higher than the first hour of work. For the TIS and agar rooting system, it was 35.0% and 39.2% more, respectively.

Table 2. Average number of containers/TIS with 10 plants inside produced at specific hour [±SD].

Work Hour	Agar Cultures	TIS	Agar Rooting
1	14.4 ± 0.7	27.1 ± 2.1	18.9 ± 0.8
2	15.9 ± 1.5	28.8 ± 2.7	20.4 ± 1.4
3	17.1 ± 1.0	30.1 ± 2.2	22.0 ± 1.4
4	20.8 ± 0.7	36.6 ± 1.8	26.3 ± 1.1
5	19.3 ± 1.4	34.2 ± 1.0	25.5 ± 1.1
6	18.1 ± 1.0	32.3 ± 2.2	23.7 ± 1.5

The share of individual costs of *Pennisetum* × *advena* 'Rubrum' showed differences between the agar cultures and TIS. The agar cultures had the highest labour cost, which constituted USD 0.309. The TIS had a much lower labour cost of USD 0.172. The highest cost in this type of production is the cost of materials and reagents, which amounted to USD 0.326 per unit. The cost of growing in both cases was similar, at USD 0.114 and USD 0.113 for the agar cultures and TIS, respectively. The total cost of single-plant production in an agar culture was USD 0.004. The TIS offered production at half the cost, at USD 0.002. Rooting is an expensive part of this production because the cost of rooting a single plant is USD 0.055. Taking the rooting percentage into account, the total cost of rooting one plant from the agar cultures and TIS is USD 0.068 and USD 0.067, respectively (Table 3).

Table 3. Costs of *Pennisetum* × *advena* 'Rubrum' production using agar cultures and TIS [in USD].

Tissue Culture Type	Materials and Reagents	Labour	Cost of Growing	Economic Overhead	The Total Cost of a Single Container with 10 Explants	The Total Cost of a Single Plant
Agar cultures	0.179	0.309	0.114	0.120	0.723	0.004
TIS	0.326	0.172	0.113	0.122	0.734	0.002
Agar rooting	0.178	0.238	0.043	0.092	0.551	0.055
Single-rooted agar plant						0.068
Single rooted TIS plant						0.067

Taking into account the cost structure of using agar cultures, the largest share is labour costs (43%). Next, with a large share constituting 25% of all costs, are the costs of materials and reagents. However, growth costs and other overhead costs account for 16% each (Figure 2A).

In TIS production, materials and reagents are the most expensive part of production (44%). Next, with a large share constituting 24% of all costs, is labour. The costs of growth costs and economic overhead account for 15% and 17%, respectively (Figure 2B).

The TIS has an extremely fast production process compared to agar cultures. In the fourth month of production using the TIS, 474,379 plants were obtained, while using agar cultures, only 195 could be produced starting from 10 plants (Table 4).

Table 4. Number of *Pennisetum* x *advena* 'Rubrum' plants obtained in the following months of production.

Specification	Months				
	1	2	3	4	5
Number of plants in agar cultures	10	10	195	195	3802
Number of plants in TIS	10	362	13,104	474,379	17,172,530

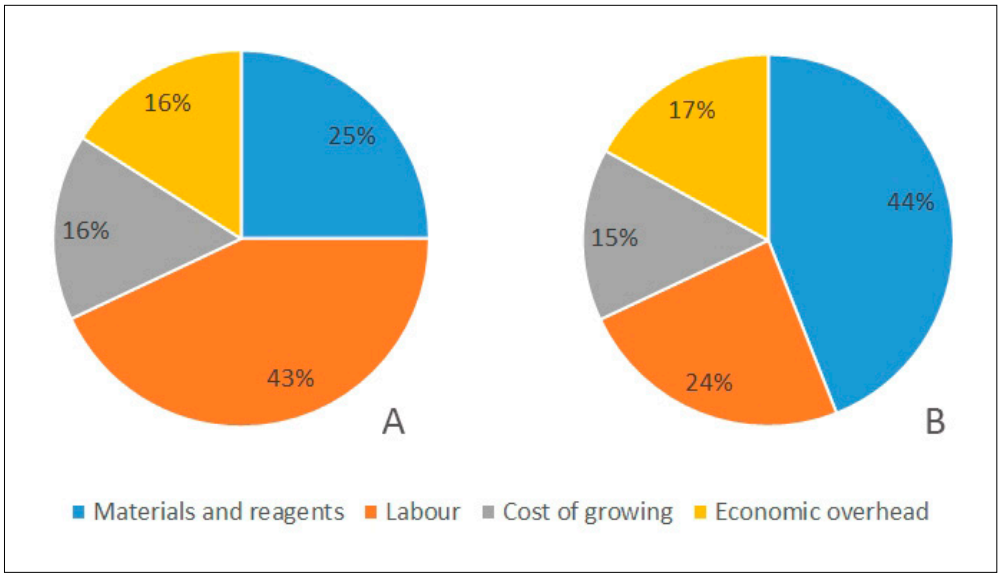


Figure 2. Share of costs of *Pennisetum* × *advena* 'Rubrum' production using TIS (**A**) versus agar cultures (**B**).

The gross margin of *Pennisetum* × *advena* 'Rubrum' plants obtained from agar cultures or a TIS is 79%. A high gross margin indicates a good profitability of producing this plant. Assuming fixed production costs are included, it can be inferred that his production could be very appealing to investors.

4. Discussion

The use of bioreactors presents significant opportunities. Various types of liquid medium bioreactors have been developed, such as stirred-tank bioreactors, cone balloon-type airlift bioreactors, rotating-drum bioreactors, nutrient mist bioreactors, radial flow bioreactors, and wave bioreactors. Among these, the temporary immersion system (TIS), which includes the SETIS and RITA systems, has emerged as the most widely adopted choice [32–34]. TIS plant production can address growing labour costs and lack of employees. It offers much faster and cheaper plant multiplication with comparable or even better quality than agar cultures [35,36]. Gas exchange is a merit of this system, as it stimulates growth and makes the plants easier to acclimate. Some systems lead to total gas exchange by forced ventilation, while others use indirect ventilation by moving the liquid medium during the immersion process [37,38].

According to Mirzabe et al. (2022) [18] and Pożoga and Olewnicki (2023) [6], plant tissue culture production has some limitations due to human workforce costs. In developed countries, labour can reach 60–80% of costs [39], while in developing ones, it can reach about 40% [40]. This study shows that the use of a TIS efficiently reduces this cost. We proved that the use of a TIS can reduce the share of labour in the variable costs of producing *Pennisetum* × *advena* 'Rubrum' from 43% to 23%. This is especially important in the post-COVID-19 pandemic era when increasing inflation has led to higher salary expenditures in many countries. Additionally, proper workday organisation plays an important role in work efficiency. Transplanting should not take more than 4–6 h, as 8 h of transplanting results in lower worker effectiveness [4,6].

The multiplication of *Pennisetum* × *advena* 'Rubrum' in a TIS is two times cheaper than in agar cultures. In a TIS, two times more plants can be obtained than in agar cultures. Additionally, the production time is much shorter. After four months of production using agar cultures, only 195 plants can be produced starting from 10. In contrast, using a TIS for the same period can yield 474,379 plants, which is a spectacular result. Similar results have been achieved by other researchers. Harris and Mason (1983) [41] achieved seven times more plants of *Vitis vinifera* in a TIS compared to agar cultures. Tiesserat and Vandercook

(1985) [42] obtained four times more plants of *Potinera* using a TIS. These results suggest even greater opportunities to reduce production costs than those shown in our study. Reduced costs translate into improved financial results for companies. Consequently, companies can invest increased income in expanding their enterprises or enhance their competitiveness by lowering the price of their plants.

The rooting phase has proven to be a particularly expensive part of production for *Pennisetum* × *advena* 'Rubrum'. Rooting costs USD 0.055, whereas multiplication costs USD 0.004 in agar culture and USD 0.002 in a TIS. The rooting phase should be a focus for improvement, especially in TISs, which offer greater potential for cost reduction. Spinoso-Castillo et al. (2024) [43] successfully rooted sugarcane in a TIS. Lopez et al. (2018) [44] achieved in vitro regeneration from leaf explants, shoot multiplication, and rooting of *Thapsia garganica*. Another strategy could involve rooting during the acclimatisation phase. The absence of roots during transplantation to soil provides a significant advantage due to ease of planting. Erst et al. (2019) [45] compared in vitro rooting and acclimatisation with the ex agar rooting and acclimatisation of *Rhododendron ledebourii* and *Vaccinium uliginosum* demonstrating successful results with ex agar rooting and acclimatisation showing similar or only slightly worse effects. Successful ex agar rooting and acclimatisation were also presented by Wojtania et al. (2020) [46] for blue honeysuckle (*Lonicera caerulea* var. *kamtschatica*). Piao et al. (2003) [10] and Septi et al. (2021) [11] have shown that a TIS is also a valuable option for potato microtuber production. This technique not only induces a higher number of tubers per plant compared to a solid medium but also increases tuber size and weight. This opens new opportunities for commercial potato seed laboratories, as these tubers can be stored and directly transplanted without an acclimatisation step.

5. Conclusions

The temporary immersion system (TIS) offers a significantly faster plant throughput, averaging 191.1 containers of plants per day and 31.5 containers per hour, compared to agar cultures which handle 107.6 containers per day and 17.6 containers per hour.

A TIS is also more cost-effective, providing seedlings that are 50% cheaper with production costs at USD 0.002 per plant, whereas agar cultures cost USD 0.004 per plant. Rooting is the most expensive phase of plant production. Agar cultures have the highest labour costs, accounting for 43% of the variable production costs. In contrast, a TIS has lower labour costs, constituting only 24% of the variable costs, with the highest expense being materials and reagents, which make up 44% of the total cost.

The gross margin for *Pennisetum* × *advena* 'Rubrum' plants produced via either agar cultures or TIS is 79%, indicating a strong profitability.

Author Contributions: Conceptualization, M.P. and D.O.; methodology, M.P. and D.O.; validation, M.P., D.O. and P.L.; formal analysis, M.P.; investigation, M.P.; resources, Plant Research Laboratories; data curation, M.P.; writing—original draft preparation, M.P.; writing—review and editing, M.P. and P.L.; visualisation, M.P.; supervision, D.O. and P.L.; project administration, M.P. and D.O. All authors have read and agreed to the published version of the manuscript.

Funding: The research was supported by Warsaw University of Life Science.

Data Availability Statement: Data are contained within the article.

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

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