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INFLUENCE OF CULTURE MEDIUM ON *IN VITRO* MICRO TUBER PRODUCTION IN *SOLANUM TUBEROSUM*

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Abstract

Potato (Solanum tuberosum) is highly susceptible to viral infections, which severely impact crop yields worldwide. In vitro microtuber production offers a valuable approach for producing disease-free planting material and conserving germplasm. This study evaluated the influence of culture medium composition - specifically varying sucrose concentrations and the presence of the cytokinin 6-Benzylaminopurine (BAP) - on microtuber induction in two blue-fleshed potato cultivars, 'Blue Congo' and 'Salad Blue'. Six medium variants were tested, combining three sucrose levels (3%, 4%, and 8%) with or without 0.5 mg/L BAP. Results showed that higher sucrose concentrations (8%) significantly enhanced microtuber formation frequency and number per explant, while BAP did not increase microtuber quantity but promoted larger and more elongated tubers. Both cultivars responded similarly across treatments, with media containing 8% sucrose and BAP yielding the most effective microtuber production within two months. These findings underscore the critical role of culture medium composition in optimizing in vitro microtuberization for efficient propagation and preservation of potato germplasm.

Key words*: germplasm conservation, growth regulators, micropropagation, potato, slow growth storage.*

INTRODUCTION

Potato (*Solanum tuberosum*) is a species extremely vulnerable to various infections, especially to viral ones. The increasing prevalence of insect vectors – as well as the limited effectiveness or absence of reliable seed-testing systems – greatly amplifies the impact of viral diseases in potato production. These viral infections can lead to yield reductions exceeding 50% of the crop's potential production (Harahagazwe et al., 2018; Kreuze et al., 2020). Although more than 50 different viruses are known to infect potato worldwide, only a small number of them are responsible for the major yield losses reported globally (Kreuze et al., 2020). Potato Virus Y (PVY) is the most damaging potato virus, causing up to 80% yield loss and it is transmitted by more than 50 aphid species, specially *Myzus persicae*, the highly efficient and globally widespread main vector (Robert & Bourdin, 2001; Kerlan, 2006; Quenouille et al., 2013). PVY also infects many other cultivated solanaceous crops and numerous weed species, broadening its impact on agriculture and horticulture (Kaliciak & Syller, 2009). Another major pathogen, Potato Leaf Roll Virus (PLVR), can reduce yields by up to 90% and is likewise spread primarily by *Myzus persicae*, though in a slower manner, due to its circulative transmission (Quenouille et al., 2013; Ștef et al., 2024). With the aphid populations expected to expand globally, the risk of infections with PVY, PLVR, and other aphid-borne viruses is projected to rise (Norse & Gommes, 2003; Jones, 2009; Cowan et al., 2023).

In potato seed production, micropropagation plays a vital role in maintaining genetic fidelity and ensuring the material is disease-free, especially regarding viral diseases. This process typically involves three main steps: (1). Generation of virus free stock material: achieved through virus elimination techniques, such as

meristem culture, thermotherapy, cryotherapy; (2). Multiplication through micropropagation techniques: once a stock of pathogen-free plantlets is established, they are multiplied under sterile laboratory conditions, using different types of culture media and growth regulators and/or obtaining microtubers on media with high concentrations of sucrose; (3). Cultivating the resulting microtubers as nuclear stock, in greenhouses, to obtain disease-free potato seed tubers (Naik & Karihaloo, 2007).

The Murashige & Skoog (1962) is regarded as the most widely used medium for potato micropropagation, and it is frequently applied without the inclusion of plant growth regulators (Naik & Karihaloo, 2007). While the standard MS vitamin mixture is typically retained, adjustments may be made – such as increasing the thiamine concentration or incorporating additional vitamins, such as biotin (0.5 mg/L) – to further improve growth performance (Abdullateef et al., 2009). In most cases, culture medium is solidified using agar or gelrite; however several studies have reported improved growth performance when liquid media are used instead (Del Avila et al., 1996; Jiménez et al., 1999; Abdullateef et al., 2009). A common approach in potato micropropagation involves first establishing a sufficient number of cultures on a semi-solid medium, followed by transferring them to a liquid medium for large-scale multiplication (Naik & Karihaloo, 2007; Abdullateef et al., 2009) found that nodal segments of various potato cultivars ('Ackersegen', 'Blaue Schweden', and 'Frühnudel') produced more and taller shoots, with a greater number of axillary buds when grown in liquid media with very low BAP concentrations in temporary immersion systems, compared with those maintained on semi-solid media. These improvements are likely due to more efficient nutrient absorption and enhanced gas exchange, which reduces ethylene buildup within the culture vessels (Etienne & Berthouly, 2002; McAlister et al., 2005). Potato is a species known for its sensitivity to ethylene when cultivated in in vitro conditions, especially in cuture vessels that do not allow gas exchange (Hussey & Stacey, 1981; Zobayed, 2001; Jackson et al., 1991; Sarkar et al., 1999). Growth regulators may be included in the medium for multiplication, though typically at low concentrations (Abdullateef et al., 2009) The crucial role of elevated sucrose levels in promoting *in vitro* microtuber formation is widely documented by several authors (Mohamed Ali & Esmail, 2011; Cioloca et al., 2023; Develi & Miler, 2023). Sucrose levels used for microtuber induction typically range from 3% to 8% (Yu et al., 2000; Dhital & Lim, 2012; Develi & Miler, 2023), and concentrations as high as 9% have also proven effective in this process (Gudeva et al., 2016) Cytokinins – including 6-Benzylaminopurine (BAP) and kinetin (KIN) – also play a major part in this process, as reported by numerous studies (Pelacho & Mingo-Castel, 1991; Dhital & Lim, 2012; Abu Zeid et al., 2022). In *Solanum* species, kinetin had been shown to promote microtuber formation and to act synergistically with other cytokinins such as BAP (Anjum & Villiers, 1997).

For the *in vitro* microtuberization experiments presented in this paper, the cultivars 'Blue Congo' and 'Salad Blue', both characterized by their blue-fleshed tubers, were used. Blue pigmented potato cultivars are relatively uncommon and regarded more as a gourmet speciality, enjoying notable popularity in high-end restaurants. Nonetheless, farmers are often reluctant to cultivate them due to their considerably lower yields, compared to white- and red-fleshed cultivars, as well as their high susceptibility to diseases during tuber propagation (Witbooi et al., 2021; Bvenura et al., 2022). The intense blue pigmentation of this varieties results from the presence of anthocyanins – malvidin and petunidin, along with delphinidin, peonidin, pelargonidin, and cyanidin (Han et al., 2006; Kita et al., 2013). Moreover, studies on blue-fleshed potato cultivars have shown that they contain higher levels of phenolic acids, flavonoids, and coumarins, compared to white - and yellow - fleshed cultivars (Murniece et al., 2013; Ru et al., 2019; Saar-Reismaa et al., 2020).

'Blue Congo', also referred to by various names including 'Blauer Schwede', 'Blaue Schweden', 'Idaho Blue', 'Purple Congo', and 'Blå Kongo', is a moderately late-maturing heritage potato variety un uncertain origin, believed to have emerged either in Europe or in the Americas. Yield data indicate that this cultivar demonstrates stable performance under a wide

range of environmental conditions (www.spk.fi; www.heritagepotato.ca). Tubers typically have a round-oval to long-oval shape. This cultivar is known to be susceptible to *Streptomyces scabies* (Dees et al., 2013) and has a moderate resistance to *Phytophthora infestans* (Pazderů & Hamouz, 2017). 'Salad Blue' is a potato cultivar that is distinguished by its high resistance to the golden nematode *Globodera rostochiensis* (The European Cultivated Potato Database - www.europotato.org/) and for its striking blue skin and flesh. Originating from Scotland, it is an early-maturing and hardy variety, known for its drought tolerance and ability to maintain good yields under adverse environmental conditions. The tubers are typically oval in shape, featuring blue to purple skin and purple flesh accented with white streaks (Pęksa et al., 2013; Witbooi et al., 2020; Nagy et al., 2023).

MATERIALS AND METHODS

Plant material consisted of *in vitro* cultivated shoots of 'Salad Blue' (Figure 1) and 'Blue Congo' varieties. The shoots were subcultured every 3-4 months on MS medium (Murashige & Skoog, 1962) with an increased concentration in vitamins, as used in the formulation described by Gamborg et al. (1968).

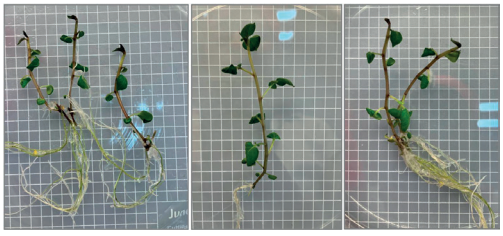

Figure 1. *In vitro*-obtained shoots of 'Salad Blue' cultivar used for *in vitro* tuberization

For the *in vitro* production of microtubers, six culture media variants were prepared, each containing different combinations of sucrose and 6-Benzylaminopurine (BAP), as detailed in the table below (Table 1). The mineral composition followed the formulation of (Murashige & Skoog, 1962), with a modified concentration of thiamine HCl (1 mg/L). All stock solutions used in the media for this experiment were prepared separately for each reagent. Macroelements were formulated at ten times their final working concentration, while microelements were prepared at 1,000-fold strength, with the exception of the sodium molybdate dihydrate ($NaMoO4 \cdot 2H_2O$), prepared at 1,690 mg/L, and FeNaEDTA, prepared at 7,340 mg/L.

Table 1. Composition of the culture media used for obtaining *in vitro* microtubers

No.	Composition of culture medium	Culture medium variants					
		T1	T2	T3	T4	T5	T6
1.	6-Benzylaminopurine (BAP) (mg/L)	0	0.5	0	0.5	0	0.5
2.	Sucrose (g/L)	30	30	40	40	80	80
3.	Agar (g/L)	7	7	7	7	7	7

Vitamins and 6-Benzylaminopurine (BAP) were prepared as stock solutions at 100 mg/L. The medium was dispensed into 750-ml glass jars, with approximately 100 ml in each jar, and then covered with lids with filters, to allow air exchange and prevent contamination. BAP was sterilized together with the rest of the components, in the autoclave, for 121°C for 20 minutes.

RESULTS AND DISCUSSIONS

Percentage of explants producing microtubers

The most effective media for inducing microtuber formation were those containing higher sucrose concentrations and supplemented with cytokinin, specifically media variants T5 and T6, which achieved 100% micro tuber formation across all shoots in both 'Salad Blue' and 'Blue Congo' cultivars (Figure 2). On these media variants, tubers developed within two months of culture (Figure 2). Media containing 3% sucrose not only exhibited the lowest tuberization rates, but also required a significantly longer initiation period; in the case of variant T1, microtuber formation occurred only after 4 months.

Similar findings have been reported by other researchers, highlighting that the carbon source and growth regulators are the primarily factors influencing *in vitro* potato tuberization (Momena et al., 2014). Studies by Gudeva et al. (2016) have shown that increasing the concentration of sucrose up to 90 g/L strongly enhanced microtuber formation.

However, plant growth regulators also played a crucial role, as the addition of 6-Benzylaminopurine (BAP) and 1-Naphthaleneacetic acid (NAA) to a medium

containing only 60 g/L sucrose resulted in over 85% microtuberization in the case of 'AgriaSR' cultivar. Hoque, (2010) reported strong tuberization responses in segments grown with 6% sucrose and kinetin. Conversely, Gautam et al. (2021) found better results on hormone-free medium - producing more microtubers per explant, with greater average weight and diameter - compared to a medium supplemented with 10 mg/L BAP. Microtuber formation can occur at lower sucrose concentrations (30-40 g/L), but the frequency and tuber size are notably reduced.

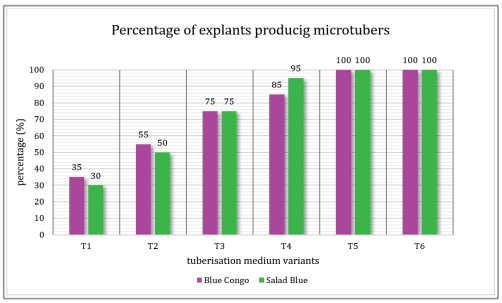

Figure 2. Percentage of explants that produced microtubers across the six different micro tuberization media formulations

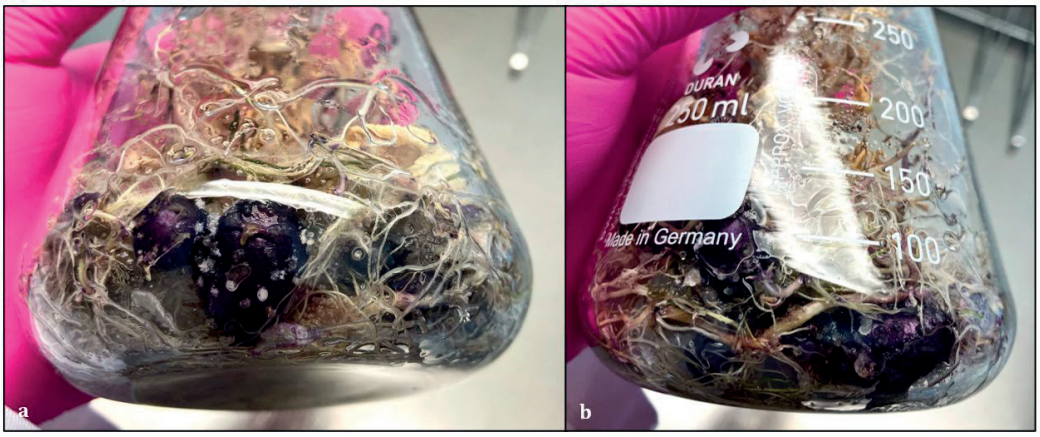

Figure 3. a,b. Microtubers obtained on tuberization media with 80 g/L sucrose and 6-Benzylaminopurine (BAP)

Dessoky et al. (2016) achieved over 50% tuberisation and an average of 2.9 microtubers per shoot using standard sucrose levels (3%), combined with 7 mg/L BAP and 0.2 mg/L KIN. Borna et al. (2019) succesfully induced microtubers in the 'Diamant' variety by culturing microcuttings on media with high plant growth regulator levels and normal sucrose. Conversely, Abu Zeid et al. (2022) found that excessively high sucrose concentrations (120-160 g/L) negatively affect *in vitro* tuber production.

In our study, the percentage of tuberization was comparable between the two cultivars, showing no statistically significant differences.

Average microtuber count per explant

The number of microtubers produced per explant was strongly affected by sucrose concentration, while the influence of BAP was comparatively minor. The highest results were achieved on media with elevated sucrose levels - specifically variants T5 and T6 – which produced averages pf 4.13 and 4.08 microtubers/explant, with no statistically significant differences between them. Microtuber production did not vary notably between cultivars; both 'Blue Congo' and 'Salad Blue' exhibited similar responses. The mean value of 4.25 microtubers/explant, was recorded for 'Salad Blue' in the cytokinin-supplemented medium containing 8% sucrose, followed closely by 'Blue Congo' cultured on a hormone-free medium with the same sucrose concentration, averaging 4.20 microtubers/explant (Figure 4).

In contrast, the lowest averages were observed in 'Blue Congo', with 1.60 microtubers/explant on variant T1 (standard sucrose, no growth regulators) and 1.70 on variant T2 (standard

sucrose with BAP). Media containing 40 g/L sucrose resulted in slightly better outcomes – 1.88 microtubers/ explant on T3 and 2.15 in T4 – roughly half the number obtained on the media with 80 g/L sucrose

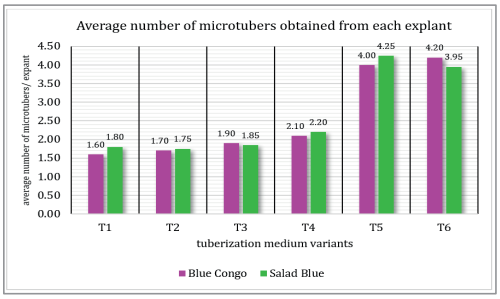

Figure 4. Average number of microtubers obtained per explant

Comparable findings have been reported by Develi & Miler (2023), who achieved between 1.0 and 4.25 microtubers per explant in 'Aruba' and 'Oberon' cultivars, using only media with 4% sucrose and no growth regulators, under varied lighting conditions. Simillarily, (Mohamed Ali & Esmail, 2011) observed that single-node explants from several potato cultivars produced between 0.48 and 0.70 microtubers/ explant on medium containing 6% under different illumination sources.

Overall, BAP did not show a significant influence on thee quantitative aspect of microtuber production. Gudeva et al. (2016) reported averages ranging from 0.61 to 3.2 microtubers/ explant on media with 3-9% sucrose, combined with elevated levels of BAP and NAA. Simillarily, Develi & Miler (2023) achieved up to 4.25 microtubers per explant in 'Aruba' cultivar grown on medium supplemented only with 4% sucrose.

Average microtuber growth

Although BAP appeared to have a minimal impact on the number of microtubers produced from each explant, subsequent parameters revealed notable differences among the media variants, depending on the presence or absence of BAP. Scheirer-Ray-Hare (SRH) test indicated significant variation in both microtuber length p = 5.84124E-30) and width (p = 1.59171E-29). No considerable differences were observed between the two studied cultivars. Regarding the microtuber length, the highest values were obtained on variant T6, averaging 16.97 mm, followed by T5, with an average of 13 mm. Dunn's tst indicate a significant difference between these two variants, underscoring the role of cytokinins in microtuber development (Figure 6.a). The smallest tubers appeared under conditions of low sucrose and in the absence of plant growth regulators, with the lowest means recorded in T1 (7.13 mm) and T3 (9.67 mm). Intermediate lenghts were seen in the shoots cultivated with lower sucrose but supplemented with BAP: 10.18 mm for T2 and 11.56 for T4, with no statistically significant differences between these two variants.

Microtubers exhibited a narrower width, indicating they tend to be more elongated than round or isodiametric in shape (Figure 5)

Figure 5. Microtubers obtained on different tuberization medium variants: a. T6; b. T4 and c. T1

The pattern for this parameter mirrored previous observations, with the greatest measurements recorded on variant T6, averaging 13.57 mm across both cultivars, and variant T5, averaging 11.79 mm, showing a significant difference between them.

Smaller widths were observed in variants T1 (6.47 mm) and T3 (8.00) (Figure 6.b).

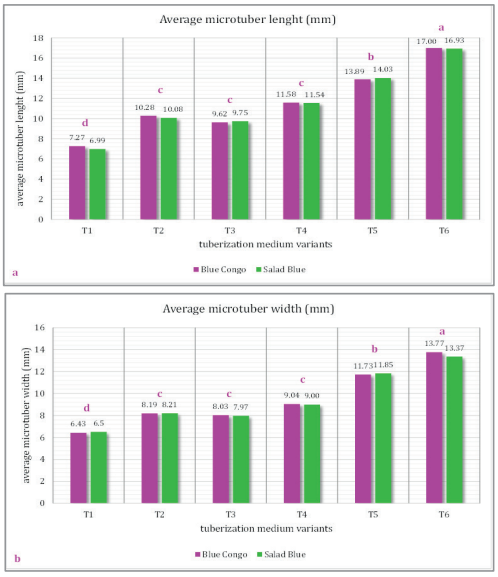

Figure 6. a. Average microtuber length (mm) on the six tuberization medium variants and b. Average microtuber width (mm) on the six tuberization medium variants

Findings indicate that both the levels of sucrose and the presence of cytokinin play crucial roles in microtuber development. Yu et al. (2000) reported a significant decline in microtuber growth rates when sucrose concentration was reduced from 8% to 4% in bioreactor cultures. Regarding the effect of plant growth regulators on tuber growth Gautam et al. (2021) found that explants grown without hormones but with 8% sucrose produced larger microtubers compared to those cultured on media with high BAP concentrations (10 mg/L).

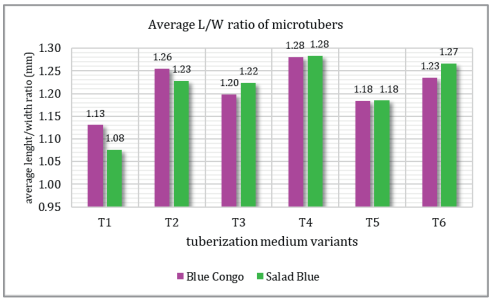

Figure 7. Average length/width ratio of microtubers obtained on the tuberisation media

Similarly, Gudeva et al. (2016) observed smaller microtubers – averaging 5 to 5.5 mm in length depending on the variety – when using media enriched with high levels of BAP and NAA, combined with 60-90 g/L sucrose. Interestingly, the shape of the microtubers was affected by the presence of growth regulators. Analysis pf the length-to-width ratio across all measured tubers revealed significant differences between various media formulations. Microtubers grown on media containing BAP tended to be more elongated (Figure 7), with the greatest length-to-width ratio observed in the T4 medium variant. These findings indicate that both sucrose concertation and the presence of cytokinins play crucial roles in microtuber development.

CONCLUSIONS

Microtubers are favoured over tissue-cultured plantlets, because they are easier to plant, have lower mortality rates, and do not require an acclimatization phase. However, they present a drawback with their longer dormancy period compared to field-grown tubers leading to a delayed sprouting (Westra et al., 2020). On the other hand, this prolonged dormancy is beneficial for in vitro conservation (Estrada et al., 1986)

The study demonstrated that the composition of the culture medium significantly influences in vitro microtuber production in *Solanum tuberosum*. Higher sucrose concentrations (8%) notably enhanced both the frequency of microtuber formation and the average number of microtubers per explant, compared to lower sucrose levels (3% or 4%). The addition of the cytokinin 6-Benzylaminopurine (BAP) did not significantly increase the number of microtubers but positively affected microtuber size and shape, producing larger and more elongated tubers. Both Blue Congo and Salad Blue cultivars responded similarly to the tested media, indicating no major cultivar-specific differences in microtuber production. Overall, media with high sucrose combined with BAP (T5 and T6 variants) were the most effective for rapid and abundant microtuber induction, supporting their use for disease-free potato propagation and germplasm conservation

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