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ASSESSING THE INFLUENCE OF 2,4-D AND BAP VARIATIONS ON *SOLANUM TUBEROSUM* 'BLUE CONGO' NODAL SEGMENTS

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Abstract

This study explores the effects of different concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6 benzylaminopurine (BAP) on the tissue culture of Solanum tuberosum cultivar 'Blue Congo'. Nodal segments derived from in vitro shoots were cultured on 9 variants of MS medium with varying concentrations of 2,4-D (0.5, 0.7, and 1.0 mg/L) and BAP (0.1, 0.2, and 0.5 mg/L). The explants were evaluated under different light conditions to determine their viability, shoot regeneration, and micro-tuber formation. Results indicated that light conditions significantly influenced explant viability and shoot growth, with optimal shoot regeneration observed in specific hormone combinations. Additionally, micro-tuber formation was highest in certain variants. Further studies will be published to evaluate the ability of the obtained callus to produce somatic embryos and, ultimately, to develop into plantlets.

Key words*: germplasm conservation, callus, in vitro, microtubers, tissue culture, potato*

INTRODUCTION

Potato (*Solanum tuberosum* L.) is cultivated worldwide and it is considered one of the most important staple food (Aksoy et al., 2021). This species was domesticated around Lake Titicaca, in southern Peru and northern Bolivia, between 7,000 and 10,000 years ago, originating from a species within the *Solanum brevicaule* complex. (Council et al., 1989; Spooner et al., 2005). Potatoes rank as the third most important food crop globally for human consumption, following rice and wheat (Shiwani et al., 2021).
A fundamental requirement for producing certified seed potatoes is that the planting stocks come from pathogen-tested materials grown *in vitro* (Westra et al., 2020), making plant tissue culture a standard method of certified potato propagation.
Mini-tubers are the primary material used for propagation to produce the first generation of seed potatoes in the field, as they are easier to plant and have a lower mortality rate than plantlets obtained in tissue culture (Westra et al., 2020). They do however have a dormancy issue, as they have a longer dormancy period compared to those grown in the field, leading to slower emergence (Westra et al., 2020). On the other hand, this prolonged dormancy is rather useful during *in vitro* conservation, as they can be easily stored in the fridge, even up to 210 days without loss of viability (Estrada et al., 1986). Micro-tuber dormancy can be reduced by pre-warming the mini-tubers or treating them with gibberellic acid (Westra et al., 2020).
The production of *in vitro* micro-tubers was first achieved more than 60 years ago, and factors inducing the *in vitro* tuberization are well-known and studied (Donnelly et al., 2003), the most important factor being the concentration of sucrose and growth regulators used in the medium. Abu Zeid et al., 2022 obtained a high percentage of *in vitro* potato tuberization using halved strength MS medium supplemented with 80-90 g/L sucrose, with 3.0 mg/L KIN and 1.0 mg/L PBZ (paclobutrazol) for 'Rosetta' and 'Victoria' cultivars.
Another growth regulator from the auxins group, 2,4-dichlorophenoxyacetic (2,4-D) aids the process of *in vitro* micro-tuber formation. When added to MS medium supplemented with 22.19 μM BAP and with 80 g/l sucrose, it has been observed to increase the formation of micro tubers from nodal segments of *Solanum*

tuberosum and *Solanum acaule* (Anjum, 1997), highlighting the synergic effect of both BAP and 2,4-D on the *in vitro* tuberization of potatoes. 2,4-D is an auxin that is mainly used for its ability to induce callus formation during the early stages of somatic embryogenesis. The role of 2,4D in somatic embryogenesis is well documented, as it is the most used auxin-type phytohormone for organogenic callus induction (Raghavan, 2004; Vondráková et al., 2011; García et al., 2019).

For callus induction, several types of explants have been used in potato tissue culture: leaves (Ghomari et al., 2013; Manisha & Nailwal, 2015; Kumlay & Ercisli, 2015; Singh et al., 2017; Kaur et al., 2018), internodes (Sharma & Millam, 2004; Ghomari et al., 2013; Manisha & Nailwal, 2015; Kaur et al., 2018) nodal segments (Anjum, 1997; Khatun et al., 2003; Kumlay & Ercisli, 2015), tuber segments (Khalafalla et al., 2010).

The crucial step during the process of somatic embryo formation appears to be the trans-differentiation of the pro-embryogenic masses into SE, which begins with the removal of auxin from the culture medium and is marked by the formation of globular embryos (Sharma et al., 2008; JayaSree et al., 2001). The medium used after the callus induction can be either hormone-free or various cytokinins might be added to aid the process (JayaSree et al., 2001; Sharma et al., 2008). Sharma et al. (2008) were able to obtain somatic embryos in potato cultivar 'Desire' by culturing internodes for 2 weeks on a medium with 5 µM 2,4-D and then sub-cultivating the induced callus on a hormone-free medium. Although 2,4-D alone should be able to produce embryogenic callus mass, growth regulators from the cytokinins group might be supplemented to the medium. JayaSree et al. (2001), observed that when applied alone, 2,4-D produces a white callus that is friable, without meristematic centers, whereas the addition of BA generated a nodular callus that was further able to sustain the development and germination of embryos in other species. Callus induced on medium with 2,4-D was successfully used for generating shoots when cultivated on medium with TDZ at a concentration of 5 mg/L, with better results compared to the mediums supplemented with BA (Khalafalla et al., 2010). Abu Zeid et al. (2022) obtained high percentages of callus formation using MS medium supplemented with 1.5 and 2 mg/L 2,4-D in combination with 0.5 mg/L KIN from leaf explants of 'Rosetta' and 'Victoria' cultivars. JayaSree et al. (2001) used for the induction and maturation of embryos medium supplemented with 22.8 µM zeatin 10 µM BA and obtained complete plantlets by placing the embryos in the cotyledonary stage on a hormone-free medium. Low concentrations of zeatin (for example, 9.1 µM), can develop the embryos into the globular stage, while, at higher concentrations (over 13 µM), zeatin was able to generate.

Regarding the type of explant used for callus induction, Manisha & Nailwal (2015) observed leaf explants to be more efficient compared to internodal segments. Explants exercised from stems are more potent for generating organogenic callus (Ghomari et al., 2013). The composition of the culture medium in relation to the type of explant is also an important factor to be taken into account.

At the same time, if present in high concentrations or for a prolonged time, 2,4-D can be responsible for causing several abnormalities in somatic embryos, mainly by disrupting the endogenous auxin balance and the auxin polar transportation interfering with the embryo apical-basal polarity (García et al., 2019).

Some of these abnormalities can be both genetic and epigenetic, for example, DNA methylation and mutations and, if present in high concentrations, they can disturb the typical genetic and physiological functions within cells and might obstruct the normal development of the embryo (Loschiavo et al., 1989; Cruz et al., 1990; Tokuji & Masuda, 1996; Gaj, 2004; Leljak-Levanić et al., 2004; Vondráková et al., 2011) or causing somaclonal variations. For example, albinism in *Agave anguslifolia* has been associated with the environments present in tissue culture, where the absence of chlorophyll pigments was linked to increased levels of DNA methylation (Duarte-Aké et al., 2016).

This study aims to examine the response of *Solanum tuberosum* 'Blue Congo' (purple variety) explants to different combinations of 2,4-D and BAP concentrations using nodal segments of *in vitro*-derived shoots, cultivated under light conditions or in the absence of light.

MATERIALS AND METHODS

To assess the influence of combinations of 2,4-Dichlorophenoxyacetic acid (2,4-D) and 6-Benzylaminopurine (BAP) and light conditions on the response of potato nodal segments of *Solanum tuberosum,* 'Blue Congo', 9 variants of medium were prepared, which are detailed in Table 1. The culture medium for this experiment was prepared according to the recipe described by Murashige & Skoog (1962) with the concentration of thiamine HCl modified to 1 mg/L, solidified with 3% agar, and with different concentrations of 2,4-D (0.5, 0.7, 1.0 mg/L) and BAP (0.1, 0.2, 0.3, 0.5 mg/L). Hormones were added in the concentrations presented in Table 1, from stock solutions prepared at concentrations of 100 mg/L and stored in the refrigerator.

Table 1. The composition of medium variants used for the nodal segments

No.	Medium variant	Concentration of growth regulators (mg/L)	
		2,4-D	BAP
1.	DB 1	0.5	0.1
2.	DB 2	0.5	0.2
3.	DB 3	0.5	0.5
4.	DB 4	0.7	0.1
5.	DB 5	0.7	0.2
6.	DB 6	0.7	0.5
7.	DB 7	1.0	0.1
8.	DB 8	1.0	0.2
9.	DB 9	1.0	0.5

After the addition of hormones, the pH of the solutions was adjusted to 5.75 and autoclaved at 121°C and 1.1 bar atmospheric pressure for 20 minutes.
After sterilizing the medium, while still in liquid form, it was distributed in sterile petri plates inside the laminar vertical airflow hood.

Inoculation of explants on the medium
The biological material used in this experiment was represented by *in vitro*-grown shoots of *Solanum tuberosum* 'Blue Congo' (Figure 1). Shoots were cultivated on MS medium for 30 days from *in vitro* cultures, maintained in the Plant Micropropagation Laboratory of the Research Center for Studies of Food Quality and Agricultural Products from the University of Agronomic Sciences and Veterinary Medicine of Bucharest.

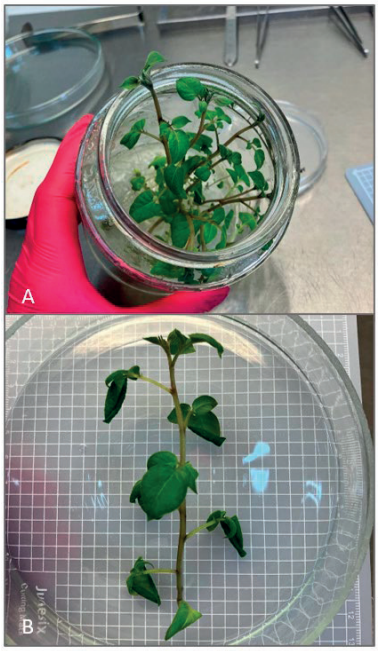

Figure 1. *In vitro* obtained shoots of *Solanum tuberosum* 'Blue Congo', used as a source for the nodal segments

The shoots were cut into nodal segments of 3- 4 mm in length, and, after the leaves and leaf petiole (Figure 2A), they were placed horizontally in the culture medium prepared in sterile Petri plates., in number of 10 explants per plate (Figure 2B).

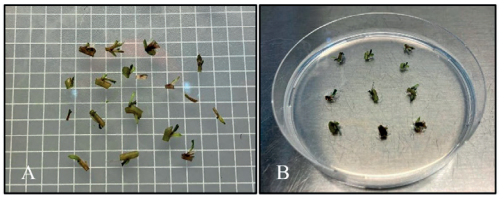

Figure 2. Nodal segments obtained from the *in vitro*-grown shoots (A); Nodal segments placed in the growing medium (B)

Explants were cultured either in dark conditions, at 22°C, or in the growing room at a temperature of 22-25°C, with a light intensity of 5023 lx, and a photoperiod of 16 hours of light followed by 8 hours of darkness. The light source was

represented by a combination of white, blue, and red light-emitting diodes (LEDs).

Microscopical observations and measurements
Pictures regarding the microscopical observations and measurements were made using The Leica Application Suite (V4) using Leica S8AP0 stereomicroscope and the Leica DFC925 connected to it.

Statistical Analysis
The statistical analysis was conducted using The Real Statistics Resource Pack (https://real-statistics.com/) for Excel 2019. Due to the unequal sample size across the different variants, the Kruskal-Wallis test was employed for the analysis of variance instead of ANOVA.

RESULTS AND DISCUSSIONS

Viability of explants cultured on medium with combinations of 2,4-D and BAP
We refer to the viability of explants as explants that were both able to generate shoots and/ or callus mass and had cells that were still viable after 40 days of culture. No data is available for the explants cultivated on variant DB7 (1.0 mg/L 2,4-D and 0.1 mg/L BAP), as all plates were contaminated and thus, they were removed from the experiment.

Figure 3. Influence of light conditions on explant viability (A); Influence of culture medium on explant viability (B);. Explant viability (%) after 40 days of culture for all experimental variants (C)

Explants cultured under light conditions had an overall higher viability, of 97.56%, compared to the one cultured in the absence of light, which had a viability of only 83.29% Regarding the influence of the composition of the culture medium, high percentages of 100% were

recorded on DB5, DB6, and DB8, under both light conditions. Variants DB1, DB3, and DB 9 recorded 100% viability, but only when cultured under light, highlighting the importance of this factor, as only 1 variant, DB2, recorded higher viability when cultured in the absence of light.

Average number of shoots and shoot length of explants cultured on medium with combinations of 2,4-D and BAP

The highest values regarding the average number of shoots regenerated from each explant were recorded on variants DB2 and DB9 (1.53 shoots/explant), followed by DB1 with 1.41 shoots and DB6 with 1.33 shoots/ explant, all cultivated under light conditions.

All variants recorded higher values for this parameter under light conditions, except for variants DB3, DB4, and DB5, where the light conditions did not influence the capacity of shoot growth, the values being either equal (1.0 shoots/ explant in DB3 and 1.05 shoots/ explant in DB4) or very similar (DB5, with 1.20 shoots/ explant in darkness and 1.18 shoots/explant under light). Between the medium variants, Kruskall-Wallis recorded no significant differences between the explants grown under light or dark conditions.

Concerning the average shoot length of the explants, all measured values were higher for the shoots grown without light (Figure 4).

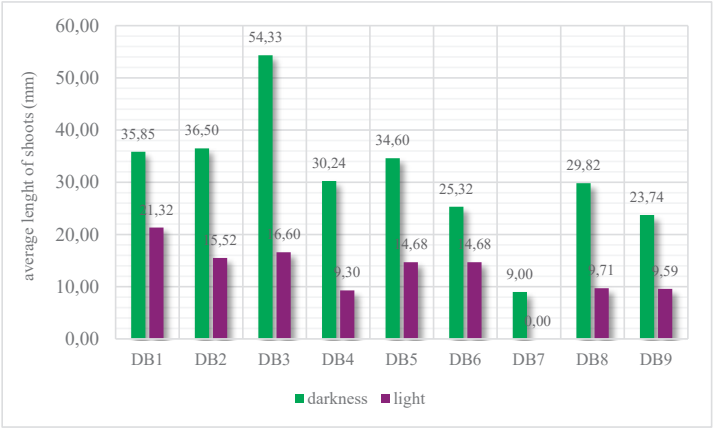

Figure 4. The average length of the shoots regenerated from the nodal segments, after 40 days of culture

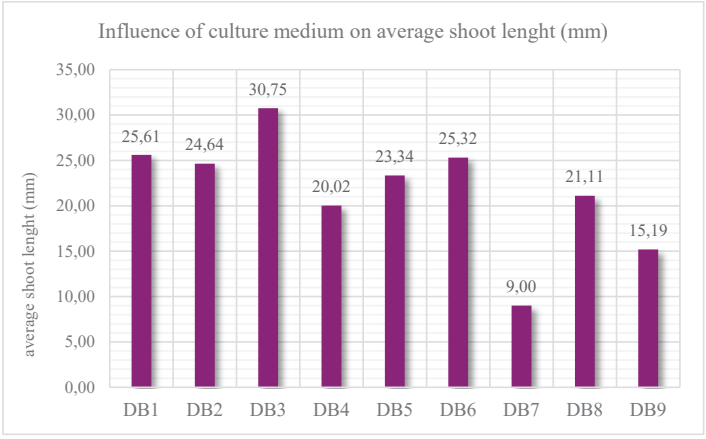

Figure 5. The overall influence of growth regulators in the culture medium on the average length of shoots regenerated from the nodal segments, after 40 days of culture

The explants cultured under no light recorded the highest values, with 54.33 mm on DB3 (with 0.5 mg/L 2,4-D and 0.5 mg/L BAP) and lowest on DB 7, with only 9.00 mm. The growth of the shoots decreased as the concentration of 2,4 increased in the medium, as the highest values were obtained on the variants with only 0.5 mg/L BAP (DB1, DB2, and DB3), and the lowest values were recorded on the variants with 1.0 mg/L 2,4-D (DB7, DB8, and DB9). The explants cultivated under light followed the same decreasing trend, the lowest values being obtained on the variants with higher concentrations of 2,4-D. However, these shoots cultivated under light tended to grow more in width leaning to create tuberous tissue than to grow in length (Figure .6).

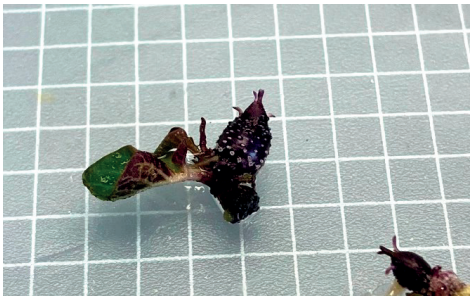

Figure 6. Explant on variant DB3, grown under light

Influence of combinations of 2,4-D and BAP on micro tuber formation

The highest capacity to produce micro tubers was observed on variant DB8 (with 1.0 mg/L 2,4-D and 0.2 mg/L BAP), in which 71.43% of the explants could generate micro tubers, as shown in Figure 7.

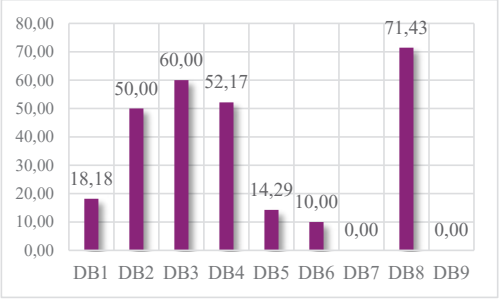

Figure 7. Influence of medium composition on the capacity of explants to produce micro tubers (%)

Following this value, explants on variant DB3 (equal amounts 2,4-D and BAP: 0.5 mg/L) recorded 60.00% and explants on DB2 and DB4 recorded similar values, of 50.00% and 52.17%, respectively. Two variants with higher concentrations of 2,4-D recorded 0 % capacity to produce micro tubers (DB7 and DB9) and the lowest values were obtained on DB6 (10%) and DB5 (14.29%).

Shoots cultivated in the dark produced between one and a maximum of 3 micro tubers/ explant (on DB2). The culture medium that produced the highest number of micro tubers was DB2, with an average value of 1.67 micro tubers/ explant, as shown in Figure 8.

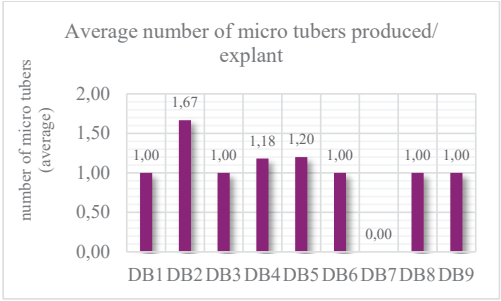

Figure 8. Influence of medium composition on the average number of micro tubers produced from each explant

The statistically significant differences between the medium variants were verified by applying the Kruskall-Wallis test. Dunn's post-hoc test confirmed statistically significant differences between variant DB2 and all other variants, except for DB5, case in which there are no significant differences. Other significant differences were revealed between DB4 and DB8.

On average, the length of the micro tubers measured 3.02 mm and the width 2.45 mm, and the Kruskal-Wallis test pointed out significant differences between the variants, both in terms of length and width.

The values for the length of the micro tubers ranged between 0.5 mm (the lowest, on DB8) and 6.1 mm (the highest, on DB2). The highest average lengths were recorded on variants DB2, with 3.94 mm in length, and DB6 with 3.80 mm in length (Figure 8), but with no significant differences between those two variants, according to Dunn's test. Lowest values were

observed on DB4 (1.88 mm) and DB5 and DB8 (2.38 mm and 2.52 mm, respectively), as presented in Figure 9 A.

Width values were measured in the range between 0.30 mm, on DB4 and 3.80 mm on DB6. Values are mostly proportionate with the length one, except for DB2. The highest ones were obtained on variant DB6 (3.78 mm), followed by DB3, with 3.17 mm and, with no significant differences between them, and lowest recorded were on variant DB4, with 1.48 mm, and DB2 with 2.10 mm (Figure 9 B).

Visually, the shape of the tubers appears to be mostly isodiametric, and width values are similar or slightly lower than the length, except for variant DB2, which had average dimensions of 3.94 mm in length x 2.10 mm in width, with the tubers appearing rather elongated.

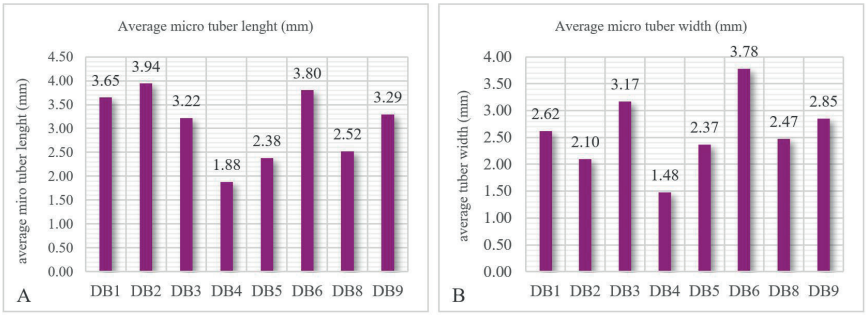

Figure 9. Influence of culture medium on the average micro tuber length (mm) (A); Influence of culture medium in the average micro tuber length (mm) (B)

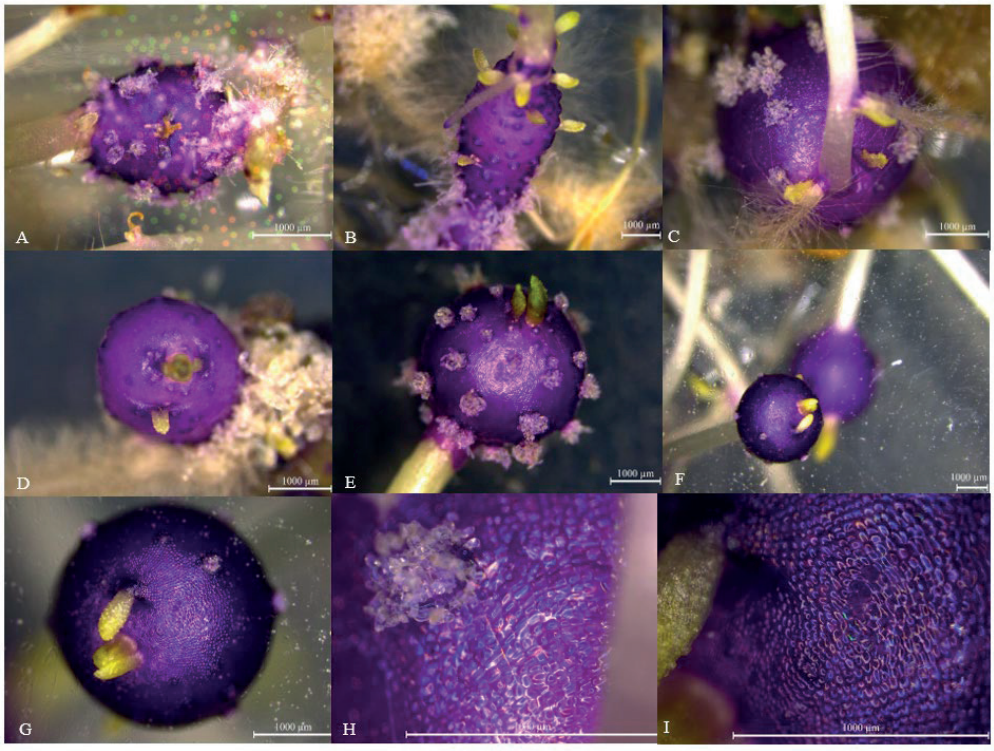

Figure 10. Micro tubers obtained on culture medium with 2,4-D and BAP: A: DB1, magn. 25X; B: DB2, magn. 12.5X; C: DB3, magn. 20X; D: DB5, magn. 20X; E: DB4, magn. 16X; F:DB9, magn. 10X; G: DB9, magn. 25X; H and I: tuber surface of DB3 and DB9, respectively, mang. 80X

Explants cultivated under light conditions did not produce micro tubers, but rather their main shoots grew in width and created tuberous tissue around them (Figure 11).

Figure 11. Tuberous shoot regenerated from nodal segment on variant DB2

Regarding the capacity to produce callus, all explants generated callus, either cultivated under light or in the absence of light, but statistical data and observations will make the subject of another paper.

Compact and dark callus developed on both ends of the nodal segment, from the internodal tissue, as can be observed at the end of the explants in Figure 6 and Figure 11. On shoots and nodal segments, friable calli emerged, with high multiplying capacity and undifferentiated, elongated cells. Depending on the light conditions and age, they appeared to be either white, with no pigments, light purple to purple on explants cultivated on both light conditions or pale green with chlorophyll pigments on the explants exposed to light (Figure 12).

As an overview, two types of callus groups were observed: compact and dark colored and friable, high multiplying white/light purple.

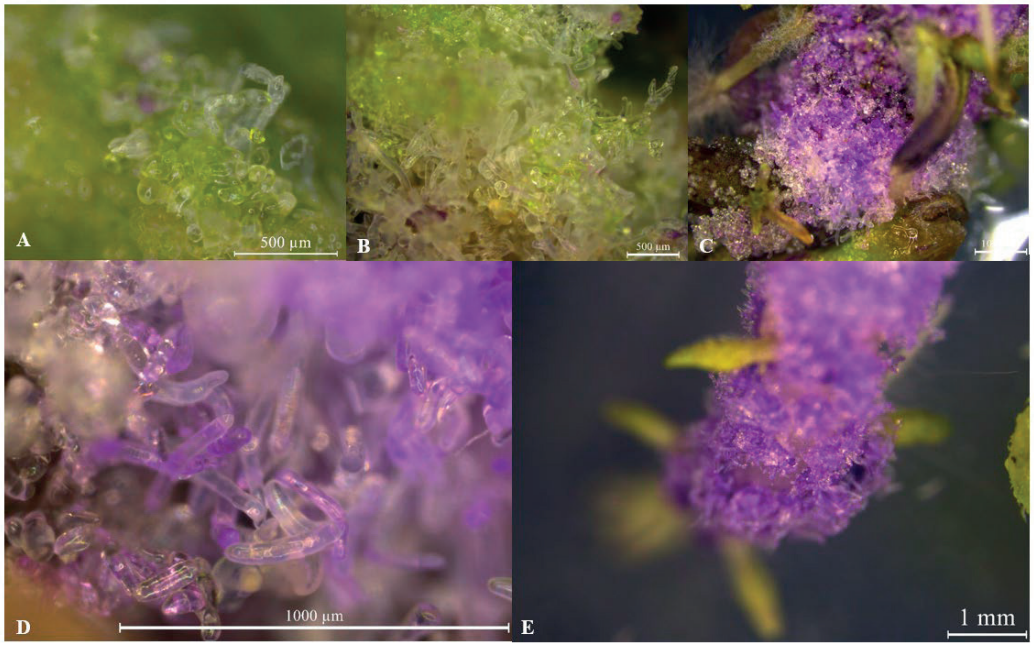

Figure 12. Friable callus obtained on culture medium with 2,4-D and BAP. A: DB2 (light); B: DB4 (light); C: DB2 (light); D: DB4 (darkness) and E: DB6 (darkness)

CONCLUSIONS

The combinations of 2,4-D and BAP and light conditions (cultivated under light or in the absence of it depend greatly on the type of tissue that needs to be obtained. The higher number of shoots were obtained on DB2, with 0.5 mg/L 2,4-D and 0.2 mg/L BAP, but the overall highest values in terms of shoot length were recorded on variant DB3, with equal concentrations of 2,4-D and BAP (0.5 mg/L).

Concerning the capacity to produce micro tubers, variant DB8, with 1.0 mg/L 2,4-D and 0.2 mg/L BAP showed a high rate of 71.43% explants that generated tubers, but the variants that were more suitable for tuber growth were observed to be DB2 (0.5 mg/L 2,4-D and 0.2 mg/L BAP), DB6 (0.7 mg/L 2,4-D and 0.5 mg/L BAP) and DB3 (0.5 mg/L 2,4-D and 0.5 mg/L BAP). With regards to the callus that was induced, further studies will be published to assess the capacity of these tissues to generate somatic embryos and future plantlets.

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REFERENCES

Anjum, M. A. (1997). Induction of microtubers in vitro from stem segments of *Solanum tuberosum* L., *S. commersonii* Dun. and *S. acaule* Bitt. *Scientia Horticulturae*. 70. 231-235.

Abu Zeid, I. M., Soliman, H. I. A., & Metwali, E. M. R. (2022). *In vitro* evaluation of some high yield potato (*Solanum tuberosum* L.) cultivars under imposition of salinity at the cellular and organ levels. *Saudi Journal of Biological Sciences*, *29*(4), 2541–2551. https://doi.org/10.1016/j.sjbs.2021.12.040

Aksoy, E., Demirel, U., Bakhsh, A., Zia, M. A. B., Naeem, M., Saeed, F., Çalışkan, S., & Çalışkan, M. E. (2021). Recent Advances in Potato (*Solanum tuberosum* L.) Breeding. In J. M. Al-Khayri, S. M. Jain, & D. V. Johnson (Eds.), *Advances in Plant Breeding Strategies: Vegetable Crops: Volume 8: Bulbs, Roots and Tubers* (pp. 409–487). Springer International Publishing. https://doi.org/10.1007/978-3-030-66965-2_10

Council, N. R., Affairs, P. G., Development, B. S. T. I., & Innovation, A. H. P. A. C. T. (1989). *Lost Crops of the Incas: Little-Known Plants of the Andes with Promise for Worldwide Cultivation*. National Academies Press. https://books.google.ro/books?id=_P3rwAEACAAJ

Cruz, G. S., Canhoto, J. M., & Abreu, M. A. V. (1990). Somatic embryogenesis and plant regeneration from zygotic embryos of *Feijoa sellowiana* berg. *Plant Science*, *66*(2), 263–270. https://doi.org/10.1016/0168-9452(90)90212-7

Donnelly, D. J., Coleman, W. K., & Coleman, S. E. (2003). Potato microtuber production and performance: A review. *American Journal of Potato Research*, *80*(2), 103–115. https://doi.org/10.1007/BF02870209

Duarte-Aké, F., Castillo-Castro, E., Pool, F. B., Espadas, F., Santamaría, J. M., Robert, M. L., & De-la-Peña, C. (2016). Physiological differences and changes in global DNA methylation levels in *Agave angustifolia* Haw. Albino variant somaclones during the micropropagation process. *Plant Cell Reports*, *35*(12), 2489–2502. https://doi.org/10.1007/s00299-016-2049-0

Estrada, R., Tovar, P., & Dodds, J. H. (1986). Induction of in vitro tubers in a broad range of potato genotypes. *Plant Cell, Tissue and Organ Culture*, *7*(1), 3–10. https://doi.org/10.1007/BF00043915

Gaj, M. D. (2004). Factors Influencing Somatic Embryogenesis Induction and Plant Regeneration with Particular Reference to *Arabidopsis thaliana* (L.) Heynh. *Plant Growth Regulation*, *43*(1), 27–47. https://doi.org/10.1023/B:GROW.0000038275.29262.fb

García, C., Almeida, A.-A., Costa, M., Britto, D., Valle, R., Royaert, S., & Marelli, J.-P. (2019). Abnormalities in somatic embryogenesis caused by 2,4-D: An overview. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *137*. https://doi.org/10.1007/s11240-019-01569-8

Ghomari, S., Lotmani, M., Labdi, M., Haddad, M., & Khaled, M. B. (2013). Effect of 2.4-D Phytohormone on Neoformation of Somaclonal Variants in *Solanum tuberosum* L. *Advances in Environmental Biology*, *7*(14), 4697–4702.

JayaSree, T., Umate, P., Ramesh, M., Rao, A., Reddy, K., & Abbagani, S. (2001). 34. T. JayaSree, U. Pavan, M. Ramesh, A.V. Rao, K. Jagan Mohan Reddy and A. Sadanandam (2001). Somatic embryogenesis from leaf cultures of potato (Solanum tuberosum L.) Plant Cell Tissue & Organ Culture, 64(1): 13-17. *Plant Cell Tissue and Organ Culture*.

Kaur, A., Reddy, M. S., & Kumar, A. (2018). Direct somatic embryogenesis of potato [*Solanum tuberosum* (L.)] cultivar 'Kufri Chipsona 2'. *Plant Cell, Tissue and Organ Culture (PCTOC)*, *134*(3), 457–466. https://doi.org/10.1007/s11240-018-1435-4

Khalafalla, M., Elaleem, K., & Modawi, R. (2010). Callus formation and organogenesis of potato (*Solanum tuberosum* L.) cultivar Almera. *Journal of Phytology*, *2*, 40–46.

Khatun, N., Bari, M.A., Islam, R., Huda, S., N.A.Siddique, Rahman, M.H., & Mollah, M.U. (2003). Callus Induction and Regeneration from Nodal Segment of Potato Cultivar Diamant. *Journal of Biological Sciences, 3*, 1101-1106.

Kumlay, A. M., & Ercisli, S. (2015). Callus induction, shoot proliferation and root regeneration of potato (*Solanum tuberosum* L.) stem node and leaf explants under long-day conditions. *Biotechnology & Biotechnological Equipment*, *29*(6), 1075–1084. https://doi.org/10.1080/13102818.2015.1077685

Leljak-Levanić, D., Bauer, N., Mihaljević, S., & Jelaska, S. (2004). Changes in DNA methylation during somatic embryogenesis in *Cucurbita pepo* L. *Plant Cell Reports*, *23*(3), 120–127. https://doi.org/10.1007/s00299-004-0819-6

Loschiavo, F., Pitto, L., Giuliano, G., Torti, G., Nuti-Ronchi, V., Marazziti, D., Vergara, R., Orselli, S., & Terzi, M. (1989). DNA methylation of embryogenic carrot cell cultures and its variations as caused by mutation, differentiation, hormones and hypomethylating drugs. *TAG. Theoretical and Applied Genetics. Theoretische Und Angewandte Genetik*, *77*(3), 325–331. https://doi.org/10.1007/BF00305823

Manisha, D., & Nailwal, T. (2015). High efficiency macropropagation of potato (*Solanum tuberosum* L.) cv. Kufri Jyoti in Kumaun Hills. *Journal of Plant Breeding and Crop Science*, *7*, 203–210. https://doi.org/10.5897/JPBCS2015.0507

Murashige, T., & Skoog, F. (1962). A Revised Medium for Rapid Growth and Bio Assays with Tobacco Tissue Cultures. *Physiologia Plantarum*, *15*(3), 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Raghavan, V. (2004). Role of 2,4-dichlorophenoxyacetic acid (2,4-D) in somatic embryogenesis on cultured zygotic embryos of Arabidopsis: Cell expansion, cell cycling, and morphogenesis during continuous exposure of embryos to 2,4-D. *American Journal of Botany*, *91*(11), 1743–1756. https://doi.org/10.3732/ajb.91.11.1743

Sharma, S. K., & Millam, S. (2004). Somatic embryogenesis in *Solanum tuberosum* L.: A histological examination of key developmental stages. *Plant Cell Reports*, *23*(3), 115–119. https://doi.org/10.1007/s00299-004-0814-y

Sharma, S. K., Millam, S., Hedley, P. E., McNicol, J., & Bryan, G. J. (2008). Molecular regulation of somatic embryogenesis in potato: An auxin led perspective. *Plant Molecular Biology*, *68*(1–2), 185–201. https://doi.org/10.1007/s11103-008-9360-2

Shiwani, K., Kumar, R., Rana, A., Kumar, D., Sharma, N., & Singh, N. (2021). *Recent advances in potato propagation* (pp. 255–262).

Singh, A., Sharma, M., & Sengar, R. (2017). *High frequency in vitro callus induction in potato (Solanum tuberosum L.) Genotypes*.

Spooner, D. M., McLean, K., Ramsay, G., Waugh, R., & Bryan, G. J. (2005). A single domestication for potato based on multilocus amplified fragment length polymorphism genotyping. *Proceedings of the National Academy of Sciences of the United States of America*, *102*(41), 14694. https://doi.org/10.1073/pnas.0507400102

Tokuji, Y., & Masuda, H. (1996). Duration of Treatment of Carrot Hypocotyl Explants with 2,4-Dichlorophenoxyacetic Acid for Direct Somatic Embryogenesis. *Bioscience, Biotechnology, and Biochemistry*, *60*(5), 891–892. https://doi.org/10.1271/bbb.60.891

Vondráková, Z., Eliášová, K., Fischerová, L., & Vágner, M. (2011). The role of auxins in somatic embryogenesis of Abies alba. *Open Life Sciences*, *6*(4), 587–596. https://doi.org/10.2478/s11535-011-0035-7

Westra, A., Nolte, P., Whitworth, J. L., & Durrin, J. (2020). Seed Potato Production and Certification. In J. C. Stark, M. Thornton, & P. Nolte (Eds.), *Potato Production Systems* (pp. 65–86). Springer International Publishing. https://doi.org/10.1007/978-3-030-39157-7_4