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THE INFLUENCE OF BIOMASS CONCENTRATION OF CYANOBACTERIA ON SHOOT MULTIPLICATION OF POTATO PLANT, DESIREE CULTIVAR, IN VITRO

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Abstract

This study was conducted to determine the optimum concentration of the biomass of the cyanobacteria (*Oscillatoria tenuis)* which supports the MS medium prepared for shoot multiplication and rooting of potato plant Desiree cultivar from the culture of sprout explants via in vitro. The results showed that all concentrations of the cyanobacteria biomass extract + MS medium led to a 100% response to shoot formation after 60 days of culture. The 60% of the cyanobacteria biomass extract to the MS medium led to recording the largest number of shoots, reaching 8.8 shoots per explant, The 20% and 40% cyanobacteria biomass concentrations + MS medium treatments were significantly superior in average shoot length and number of leaves compared to the other two treatments, which amounted to 10.02 cm and 5.1 leaves per shoot and 10.48 cm and 5.0 leaves per shoot, respectively. The control treatment and the 20% cyanobacteria biomass concentration + MS Medium treatment did not produce any stolons from the shoots after 60 days of culture. The 40% and 60% cyanobacteria biomass concentration + MS medium treatments had stolons growing from the shoot site in contact with the nutrient medium. The stolon tips began to swell until microtubers were formed 60 days after culturing. The 60% cyanobacteria biomass concentration + MS Medium treatment was significantly superior in the average number of stolons, the number of microtubers, and the microtuber weight, which amounted to 4.6 stolons per shoot, 4.6 stolons per shoot, and 0.32g, respectively. The shoots were cultured in all treatments responded to rooting 60 days after culturing. The treatment 60% cyanobacteria biomass concentration + MS medium recorded the highest response to rooting of the shoots, the average number of roots, and root length which reached 100%, 5.8 roots per shoot, and. 6.2 cm, respectively.

Keywords: *Biomass, microtuber, Oscillatoria tenuis, rooting, stolon, tissue culture.*

I. Introduction

The potato (*Solanum tuberosum* L.) plant belongs to the Solanaceae family. It is one of the most important vegetable crops that ranks fourth in the world in terms of economic importance (consumption and production) after wheat (*Triticum aestivum*), corn (*Zea mays*), and rice (*Oryza sativa*) (Chen et al., 2007). The original homeland of the potato plant is the Andes Mountains in Bolivia and Peru. There is a common belief that its original home is Chiloe Island, which is located on the southern coast of Chile. Potato cultivation spread in Arab countries in the late nineteenth century AD (Al-Khazali, 2006). Many people in the world depend on potatoes as a main food as it is a good energy source. Potatoes are a crop rich in carbohydrates and starch and contain many important vitamins, minerals, and proteins (Zamotaeva, 1997). It is the cheapest source of starch, as it is the main meal in many countries, especially European countries (Hassan, 2003). There are several methods for propagating potato plants, including seed propagation, and their use is limited to breeding and producing new cultivars (Mihaela et al., 2012). The other traditional method is vegetative propagation using tubers. Tubers are swell ground stems that grow below the soil from Stolons. One of the disadvantages of this method is the difficulty of storage and its need for large areas for production, in

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addition to its exposure to infection by viruses, fungi, and bacteria (Djurdjina et al., 1997). To overcome these weaknesses in these methods of sexual and asexual propagation was resorted to the technique of propagation by plant tissue culture in vitro. This method is followed in many countries of the world, including France, the Netherlands, India, and South Korea (Najjar, 1993). The success of plant tissue culture in vitro depends primarily on the components of the nutrient medium prepared for them and the source of the explants used in propagation (Ibrahim et al., 2013; Almusawy et al., 2015). Natural organic materials and extracts other than the components of the nutrient medium have been added in many studies to support the components of the nutrient medium and improve and stimulate the growth and development of plant tissues grown in vitro (Zaccro et al., 2006; Banerjee and Sharivastava, 2008). Many studies have found that adding extracts of some species of algae to nutrient media prepared for tissue propagation had a positive role in the multiplication and proliferation of horticultural plants. These species of algae produce bioactive substances such as auxin-like, cytokinin-like, gibberellin-like substances, and jasmonic acid, which stimulate plant cell division and differentiation. The investigators inferred that these algae can produce, release, and accumulate these substances from their cells. The investigators inferred that these algae can produce, release, and accumulate these substances from their cells (Gupta et al., 1973; Molnar and Ordog, 2005; Seema et al., 2011; Keerthiga et al., 2012). Shanab et al. (2003) through their in vitro culture of Nodal segment explants of the potato (Solanum tuberosum L.) plant, Sponta cultivar, showed that adding extracts of ten strains of cyanobacteria to the MS medium led to increased shoot multiplication. This study indicated the significant superiority of the MS medium treatment prepared with a concentration of 20% of Lyngbya valderianum strain extract compared to the other treatments in average shoot length (6.9 cm), average root length (4.0 cm), and number of leaves per shoot (4.0). Studies have shown that cyanobacteria extract contains auxin-like substances. This study was conducted to determine the ideal concentration of the biomass of the cyanobacteria *Oscillatoria tenuis*, which supports the MS medium prepared for shoot multiplication and rooting of potato plant Desiree cultivar from the culture of sprout explants via in vitro.

II. Materials and Methods

The research was conducted in the Plant Tissue Culture Laboratory, Fadak Plant and Animal Production Company, Basrah, Iraq. The potato plant, Desiree cultivar, was micropropagated in vitro using sprouts of potato tubers. Sprouts with a length of 2 cm were excised from the tubers using a sterile scalpel. These excised explants were washed with running tap water and liquid soap. Then, the process of surface sterilization of the explants was carried out by using the fungicide Caravan G, from the American company SYNGENTA, at a concentration of 10% for 10 minutes by immersing the explants with continuous shaking. These explants were then placed in an antibiotic solution consisting of 50 mg each of Tetracycline and Rifampicin dissolved in 500 ml of sterile distilled water for 10 minutes. Then it was immersed in a solution of 1.05% sodium hypochlorite (NaOCl) and 5 drops of Tween20 for 15 minutes with continuous stirring. Then the sprout explants were washed with sterile distilled water three times. They were then kept in a sterile glass container until cultured in vitro.

Biomass of *Oscilatoria tenius* grown in BG11 medium was obtained (Mackinney, 1942). 5 g of algae biomass was taken after collecting it by filtering it on Whatmann N0.1 type, filter papers. Then the biomass was re-suspended in 100 ml of phosphate buffer solution (7), under sterile conditions. Biomass was extracted from algae cells by breaking down the cells using the processes of freezing and thawing until the solution changed, and these processes were repeated several times. After that, the solution was centrifuged at 3500rm for 45 minutes, then the clear solution was taken, and the deposit was left behind (Medina-Jaritz et al., 2011). The filtrate was sterilized by filtering, and it was added to the MS media at different concentrations (0, 20, 40, and 80 ml L^{-1}) for shoot regeneration, tuberization, and rooting stages.

The nutrient medium for shoot regeneration was prepared, consisting of ready-formula MS salts (Murashige and Skoog, 1962) supplemented with vitamins with a concentration of 4.43g L^{-1}, produced by the American Caisson Company. Organic compounds consisting of 40g L^{-1} of sucrose, 100mg L^{-1} of

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activated charcoal, 50mg L^{-1} of adenine sulfate, 1.5mg L^{-1} of kinetin (Kn), 1.5mg L^{-1} of naphthalene acetic acid (NAA), and 0.2mg L^{-1} gibberellic acid (GA_3) were added to it. Then the biomass of cyanobacteria algae was added at different concentrations to the nutrient media prepared for the cultures (0, 20, 40, and 60 ml L^{-1}). The pH of the nutrient medium was adjusted to 5.7, and then phyto-agar was added at a concentration of 6 g L^{-1}. After that, the MS medium was sterilized using an autoclave device at a temperature of 121°C and under a pressure of 1.05 kg cm^2 for 20 minutes.

Indicators

Number of shoots (shoots per explant)

Shoot length (cm)

Number of leaves (leaves per shoot)

Number of stolons (stolons per shoot)

Number of microtubers (microtubers per shoot)

Microtuber weight (g)

Shoot response to rooting (%)

Number of roots (roots per shoot)

Root length (cm)

Statistical analysis

Simple experiments were designed using a completely randomized design. The data were analyzed statistically by analysis of variance. Each experimental treatment was repeated ten replicates. The treatment means were compared using a least significant difference test at the 1% probability level, based on Al-Rawi and Khalafallah (2000).

III. Results and Discussion

The research results indicated that all concentrations of the cyanobacteria biomass extract added to the MS medium led to a 100% response to shoot formation of the potato plant Desiree cultivar in the shoot multiplication stage after 60 days of in vitro culture. Data from Table 1 show that adding 60% of the cyanobacteria biomass extract to the MS medium led to recording the largest number of multiplying shoots, reaching 8.8 shoots per explant, which was significantly higher than the other treatments. The two treatments, 20% and 40% cyanobacteria biomass concentration + MS medium, also differed significantly from the control treatment (MS medium without the addition of cyanobacteria biomass extract). These two treatments did not differ significantly from each other. The MS Medium treatment without the addition of biomass extract recorded the lowest average number of shoots, reaching 2.2 shoots per explant.

It is clear from the data in Table 1 that the 20% and 40% cyanobacteria biomass concentration + MS medium treatments were significantly superior in average shoot length and number of leaves per shoot compared to the other two treatments, which amounted to 10.02 cm and 5.1 leaves per shoot and 10.48 cm and 5.0 leaves per shoot, respectively. The control treatment recorded the lowest average shoot length and number of leaves per shoot, which amounted to 6.24 cm and 2.0 leaves per shoot. The 60% of cyanobacteria biomass extract + MS medium treatment differed significantly from the control treatment in average shoot length and number of leaves per shoot (8.36 cm and 3.2 leaves per shoot).

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Table 1: Effect of cyanobacteria biomass concentration (%) on shoot multiplication stage of potato plant Desiree cultivar after 60 days from culturing

Treatment	Number of shoots per explant	Shoot length (cm)	Number of leaves per shoot
MS medium	2.20	6.24	2.0
20% CBME*+MS medium	6.00	10.02	5.1
40% CBME+MS medium	6.40	10.48	5.0
60% CBME+MS medium	8.80	8.36	3.2
LSD (p≥0.01)	1.44	2.10	0.62

CBME: Cyanobacteria biomass extract.

Data from Table 2 indicate that the control treatment and the 20% cyanobacteria biomass concentration + MS Medium treatment did not produce any stolons from the shoots of the potato cultivar Desiree after 60 days of in vitro culture. The 40% and 60% cyanobacteria biomass concentration + MS medium treatments had lateral shoots growing from the shoot site in contact with the nutrient medium. They extended horizontally and formed what are called stolons. After the growth of the stolons was completed, their tips began to swell until microtubers were formed 60 days after culturing. The 60% cyanobacteria biomass concentration + MS Medium treatment was significantly superior in the average number of stolons per shoot, the number of microtubers per shoot, and the weight of the microtuber, which amounted to 4.6 stolons per shoot, 4.6 stolons per shoot, and 0.32g, respectively. The treatment 40% cyanobacteria biomass concentration + MS Medium recorded the lowest average number of stolons per shoot, number of microtubers per shoot, and tuber weight (3.4 stolons per shoot, 3.4 stolons per shoot, and 0.28g, respectively).

Table 2: Effect of cyanobacteria biomass concentration (%) on tuberization stage of potato plant Desiree cultivar after 60 days from culturing

Treatment	Number of stolons per shoot	Number of microtuber per shoot	Microtuber weight (g)
MS medium	-	-	-
20% CBME*+MS medium	-	-	-
40% CBME+MS medium	3.4	3.4	0.26
60% CBME+MS medium	4.6	4.6	0.32
LSD (p≥0.01)	Significance	Significance	Significance

CBME: Cyanobacteria biomass extract.

It is clear from the data in Table 3 that the shoots of the potato cultivar Desiree that were cultured in all treatments responded to rooting 60 days after culturing. The treatments 40% and 60% cyanobacteria biomass concentration + MS medium recorded the highest response to rooting of the shoots, which reached 100% in each of them. The control treatment led to the lowest percentage of rooting of the shoots, reaching 60%. The 60% cyanobacteria biomass concentration + MS medium treatment recorded a significant increase in the average number of roots compared to the other treatments, which amounted to 5.8 roots per shoot. The two treatments, control and 20% cyanobacteria biomass concentration + MS medium recorded the lowest average number of roots formed, reaching 2.2 roots per shoot in each of them. The treatments 40% and 60% cyanobacteria biomass concentration + MS Medium recorded a significant increase in average root length compared to the other two treatments,

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which reached 6.0 and 6.2 cm, respectively. The control treatment recorded the lowest average root length of 2.2 cm.

Table 3: Effect of cyanobacteria biomass concentration (%) on rooting stage of potato plant Desiree cultivar after 60 days from culturing

Treatment	Response to rooting (%)	Number of roots per shoot	Root length (cm)
MS medium	60	2.2	2.2
20% CBME*+MS medium	90	2.2	4.1
40% CBME+MS medium	100	4.4	6.0
60% CBME+MS medium	100	5.8	6.2
LSD (p≥0.01)	2.40	0.43	0.41

CBME: Cyanobacteria biomass extract.

The inducement of the shoot and root regeneration characteristics of the Desiree potato cultivar in vitro is due to the optimum concentration of cyanobacterial biomass extract (60%) that was added to the nutrient medium prepared for shoot multiplication and rooting stages. This is due to the bioactive extracellular substances produced by cyanobacteria and the compounds that enter into their cellular structure, which are contained in the aqueous extract of biomass and which support the components of the nutrient medium. These substances are necessary for plant tissue growth and cell division in vitro culture. These result in stimulating and activating bioactivities such as cell division, differentiation, and organogenesis (Molnar and Ordog, 2005; Seema et al 2011; Keerthiga et al 2012; Mani et al., 2014; Shar et al., 2017).

The reason for the significant improvement in vegetative and root characteristics is due to supplementing the nutrient medium with the aqueous extract of the biomass of cyanobacteria, which enriches it with substances similar to plant hormones such as substances of auxin-like, cytokinins-like, gibberellin-like, and jasmonic acid produced by algae, that presence in the aqueous extract of the biomass contributed to stimulating and promoting their tissues for cell division, growth, and differentiation. The role of abscisic acid, which is one of the components of the aqueous extract of cyanobacterial biomass, also contributed to enhancing root growth (Molnar and Ordog, 2005; Seema et al., 2011; Keerthiga et al., 2012; Abul-Soad and Jatoi, 2014; Hegazy, 2014).

The growth of stolons and the formation of microtubers in the potato plant, Desiree cultivar, when the shoots are cultured on the MS medium supplied with two concentrations of 40% and 60% of Cyanobacterial biomass extract, after 60 days of culturing, is due to the role of hormones and bioactive substances produced by these algae, which interfered with the plant growth regulators that existing in the components of the nutrient medium, which together, in their ideal concentrations, contributed to the growth of the stolons, the swelling of their tips, and the formation of microtubers (Molnar and Ordog, 2005; Seema et al., 2011; Keerthiga et al., 2012). The reason for the lack of formation of stolons and microtubers in the control treatment and the 20% treatment of cyanobacteria biomass extract + MS may be because the concentrations of hormones and bioactive substances in those media were insufficient to induce and form stolons and microtubers (Kamarainen-Karppinen et al., 2010; Mani et al., 2014; Koleva Gudeva et al., 2012; Mamiya et al., 2020).

IV. Conclusion

Sprout explants of potato plant Desiree cultivar that were grown on MS medium supplemented with 60% cyanobacterial biomass extract led to a high response in the formation of shoots per explant, the number of shoots per explant, the number of stolons per shoot, and the number of microtubers per shoot, and a high response in the number and length of roots.

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V. REFERENCES

Abul-Soad AA, Jatoi MA. Factors affecting in vitro rooting of date palm (*Phoenix dactylifera* L.). Pak. J. Agri. Sci. 2014 Jun 1;51(2):467-74.

Al-Khazali, F. H. I. (2006). Producing seeds of the highest ranks of potatoes, Diamant, and Desiree cultivars, using different techniques. Doctoral Dissertation, University of Baghdad, Iraq.

Almusawi AH, Alshnawa AS, Alkhalifa AA, Almesfer RF. Induction of direct somatic embryogenesis in date palm (*Phoenix dactylifera* L.) cv. Hellawi. AAB Bioflux. 2015 Sep 1;7(2):80-9.

Al-Rawi KM., Khalafallah AM (2000). [Design and Analysis of Agricultural Experiments]. Dar Alkuteb for Press and Publishing, Mosul University, Iraq, 488 p. [In Arabic].

Banerjee M, Shrivastava S. An improved protocol for in vitro multiplication of *Bacopa monnieri* (L.). World Journal of Microbiology and Biotechnology. 2008 Aug;24:1355-9.

Chen Q, Su J, Nandy S, Kereliuk G. Screening potato genotypes for antioxidant capacity and total phenolics. InPlant Canada Congress 2007 (pp. 75-79).

Djurdjina, R., Milinkovic, M. and Miloševic, D. (1997). In vitro propagation of potato (*Solanum tuberosum* L.). Acta Horticulturae, 462, 959-964. https://doi.org/10.17660/ActaHortic.1997.462.152

Gupta AB, Agarwal PR. Extraction, isolation, and bioassay of a gibberellin-like substance from *Phormidium foveolarum*. Annals of Botany. 1973 Sep 1;37(4):737-41.

Hassan, A. A. (2003). Potatoes. Arab House for Publishing and Distribution, Cairo, Egypt.

Hegazy, A.E. (2014). Promising protocol for in vitro direct organogenesis of date palm cv. Ekhlass. Fifth International Date Palm Conference , Abu Dhabi - UAE; 16 – 18 March 2014.pp. 207-218.

Ibrahim MA, Waheed AM, Al-Taha HA. Plantlet regeneration from root segments of date palm tree (*Phoenix dactylifera* L. cv. Barhee) producing by in vitro culture. AAB Bioflux. 2013 May 1;5(1):45-50.

Kamarainen-Karppinen T, Virtanen E, Rokka VM, Pirttila AM. Novel bioreactor technology for mass propagation of potato microtubers. Plant Cell, Tissue and Organ Culture (PCTOC). 2010 May;101:245-9. https://doi.org/10.1007/s11240-010-9679-7

Keerthiga M, Anand SP, Nandagopalan V, Doss A, Senthilkumar SR. In vitro propagation of *Wedelia trilobata* (L.) using *Phormidium subincrustatum* extracts: A novel approach. Asia Pac. J. Mol. Biol. Biotechnol. 2012;20(4):147-52.

Koleva Gudeva L, Mitrev S, Trajkova F, Ilievski M. Micropropagation of Potato *Solanum tuberosum* L. Electronic Journal of Biology. 2012 Aug 15;8(3):45-9.

Mackinney G. Absorption of light by chlorophyll solutions. Journal of biological chemistry. 1941 Aug 1;140(2):315-22.

Mamiya K, Tanabe K, Onishi N. Production of potato (*Solanum tuberosum*, L.) microtubers using plastic culture bags. Plant biotechnology. 2020 Jun 25;37(2):233-8. https://doi.org/10.5511/plantbiotechnology.20.0312a

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Mani F, Mhamdi M, Bettaieb T, Hannachi C. Shoot regeneration, micropropagation and microtuberization of potato (*Solanum tuberosum* L.) cultivars. Journal of new sciences. 2014 Jul 1.

Medina-Jaritz NB, Perez-Solis DR, Ruiloba de Leon SL, Olvera-Ramírez R. Antimicrobial activity of aqueous and methanolic extracts from Arthrospira maxima. Science against microbial pathogens: communicating current research and technological advances. A. Méndez-Vilas (Ed.). 2011:1267-71.

Mihaela C, Anca B, Andreea N, Monica P. Production of seedling tubers from true potato seed (TPS) in protected area. Journal of Horticulture, Forestry and Biotechnology. 2012;16(4):136-41.

Molnar Z, Ordog V. The effect of cyanobacterial compounds on the organogenesis of pea cultured in vitro. Acta Biologica Szegediensis. 2005 Jan 1;49(1-2):37-8.

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

Najjar KF. Commercialization of the patented microtuber multiplication system of potato. InThe second Arab conference on perspectives of modern biotechnology 1993 Apr (pp. 24-28).

Seema T, Arnold R, Tiwari A, Mishra RM, Chauhan UK. Cyanobacterial extract and MS media as a novel tool for in vitro regeneration of *Stevia rebaudiana* Bertoni. Journal of Algal Biomass Utilization. 2011;2(2):24-40.

Shar AH, Qambrani MA, Shar PA, Memon S, Shar AG, Liu J, Shen F, Kalhoro SA, Marri SN, Shahen M. Regeneration of Potato Plantlets Through Shoot Tip Culture Comparison Between GA3 and BAP. American Journal of Biology and Life Sciences. 2017;5(3):13-20.

Zaccaro MC, Kato A, Zulpa G, Storni MM, Steyerthal N, Lobasso K, Stella AM. Bioactivity of Scytonema hofmanni (Cyanobacteria) in *Lilium alexandrae* in vitro propagation. Electronic Journal of Biotechnology. 2006 Jun;9(3):0-.

Zamotaeva G., 1997 [Potato Production Guide]. Agropromizdat Publishing House, Moscow, 348 pp. [In Russian].