International Journal of Horticultural Science and Technology
Journal homepage: http://ijhst.ut.ac.ir

Evaluation of Sucrose Benefits to Tuberization in 'Sante' Potato Cultivar *in vitro*

Naser Askari[1]*

1 Department of Horticulture, Faculty of Agriculture, University of Jiroft, Jiroft, Iran

ARTICLE INFO

Article history:

Received: 11 November 2023,
Received in revised form: 1 December 2023,
Accepted: 1 December 2023

Article type:

Research paper

Keywords:

Biomass allocation,
Explant growth,
In vitro tuberization,
Microtuber yield,
Shoot growth

ABSTRACT

Sucrose has a significant role in promoting microtuberization in potato. It acts as a crucial modifier, influencing the growth and development of microtuber in potato. Understanding the role of sucrose in microtuberization is vital for improving potato cultivation practices and enhancing crop productivity. In this experiment, different concentrations of sucrose (3, 6, 9, 12, and 15%) were investigated to determine their effects on the microtuberization of a potato cultivar 'Sante'. The findings revealed that high concentrations of sucrose (12 and 15%) inhibited microtuberization percentage, microtuber count, and shoot growth. Tuberization percentage reached its maximum value (100%) when exposed to a sucrose concentration of 9%. The microtuber count was 2 per explant and 4.6 per vessel. However, using a 12% sucrose concentration resulted in the highest microtuber diameter (5 mm), microtuber fresh weight (120 mg) and dry weight (26 mg), and microtuber yield (FW: 752 mg; DW: 170 mg). The highest explant fresh weight (44 mg) and dry weight (9.5 mg) occurred in response to 15% sucrose concentration. Biomass allocation was influenced by sucrose concentration, with higher concentrations leading to a greater biomass allocation to the microtuber and explant, rather than the shoot. Accordingly, it can be concluded that a sucrose concentration of 12% was an optimal treatment for 'Sante' potato microtuber production.

Introduction

Potatoes (*Solanum tuberosum* L.) are the third most crucial food crop, following rice and wheat in global human consumption. Over a billion individuals around the globe include potatoes in their regular dietary intake (Stark et al., 2020). The cultivation of potatoes takes place on a noteworthy scale in 130 countries, having amounted to a substantial gross production value of 63.6 billion US dollars in 2016. Moreover, world potato production reached 368 million tons in 2018 (Dolničar, 2021). Potatoes possess exceptional sustainability due to their capability to yield a larger quantity of sustenance more quickly, occupying a smaller area of land and necessitating a diminished amount of water than

*Corresponding author's email: na.askari@yahoo.com

other significant food crops (Devaux et al., 2014). Potatoes serve as a fundamental source of nourishment for numerous individuals across the globe, rendering them rich in vital nutrients (Zaheer and Akhtar, 2016; Beals, 2019). They are an exceptional reservoir of vitamins B1 and B6 and a commendable source of potassium, copper, vitamin C, manganese, phosphorus, niacin, dietary fiber, and pantothenic acid. Potatoes exhibit low sodium content (Górska-Warsewicz et al., 2021). Potatoes have been the focus of cultivation for numerous millennia, and an excess of 10,000 potato variations have been cultivated globally up to the present.

Notwithstanding this extensive array of variations, there remains a necessity for novel varieties, and the classical breeding of fresh potato varieties has experienced minimal alteration over decades and primarily varies in terms of technologies employed (Bradshaw, 2022). The technique of potato microtuberization is significant in its application to generate disease-free potato seed storage, transplant in vivo plantlets, preserve essential cultivars, and facilitate the exchange of healthy germplasm (Yagiz et al., 2020). Microtubers can remain in storage for approximately one year and be directly transported to the market without requiring fresh media transport or acclimatization (Aksoy et al., 2021; Nhut et al., 2022). *In vitro* microtuber production is an efficient approach for handling and storage compared to those acquired from sprouts (Mohamed and Girgis, 2023). Environmental factors, nitrogen supply, plant growth regulators (PGRs), genotypes, growth nutrients, photoperiods, temperature, source of explant, potato cultivar, and sucrose concentration all contribute to the *in vitro* tuberization of potatoes (García-García et al., 2019). Sucrose, an indispensable carbohydrate for plant development and maturity, plays a pivotal role in various vital functions as a primary energy source, a transporter of carbon, and a molecule involved in signaling and regulation (Chen et al., 2020). The significance of sucrose synthase in sugar metabolism is particularly notable in tissues that primarily function as sinks, and it exerts a profound influence on plant growth and development (Stein and Granot, 2019).

Sucrose is a crucial component in the process of microtuberization in potato plants. It serves as a source of energy and stimulates the growth and development of cells to ultimately form tubers (Rafique et al., 2014; Uchendu et al., 2016). The sucrose concentration in nutrient media can affect various factors, such as the percentage, timing, and weight of microtuberization (Khuri and Moorby, 1995). The 'Sante' potato variety, having originated in the Netherlands, is highly versatile and widely favored for its ability to cater to multiple applications, ranging from industrial processing to domestic culinary practices (Yagiz et al., 2020). Microtuberization, as a process, is highly dependent on genotype (Askari et al., 2023). However, the impact of sucrose concentration on the microtuberization of the 'Sante' cultivar has not received extensive attention. Currently, there is limited data available regarding the influence of sucrose on the micropropagation of this highly regarded variety. Therefore, the current research aimed to evaluate the effects of different concentrations of sucrose (3, 6, 9, 12, and 15%) on the microtuberization process of the 'Sante' potato cultivar.

Materials and Methods

Plant materials and culture conditions

A high-yielding potato cultivar (Sante®) was selected to conduct this experiment. A single nodal segment (1 cm) was excised from plants grown in a greenhouse and used as an explant. Decontamination procedures were carried out to ensure the explants were disinfected. This involved treating the explants with 1% sodium hypochlorite and a few drops of Tween® 20, followed by triple rinsing with sterile distilled water (Askari and De Klerk, 2020; Askari and Visser, 2022). The explants were then cultured in a medium containing 30 g L^{-1} sucrose and 7 g L^{-1} agar, and maintained at 25±2 ºC for two weeks under a 16-h light cycle. The newly regenerated shoots were sub-cultured every two weeks to ensure an adequate number of explants for the experiments. Nodal explants were cultured on MS medium containing varying concentrations of sucrose (3, 6, 9, 12, and 15%), with 7 g L^{-1} agar and 2 mg L^{-1} BA. The explants were maintained on the culture medium for three months and were incubated under the same conditions of the shoot multiplication stage.

Tuberization traits

Three months from the beginning of the experiment, an evaluation was conducted on explant-related variables, shoots, and tuberization. Measurable parameters were the fresh weight (FW) and dry weight (DW) of the explant and shoot, as well as the fresh weight (FW), dry weight (DW), and diameter of the microtuber. The fresh weight of the explants, shoots, and microtubers could be determined using an electronic balance (± 0.01 g; MXX-412; Denver Instruments, Bohemia, NY, USA). To obtain the dry weight, the shoots and roots were

subjected to a drying process in a ventilated oven. Initially, they were dried at 105 °C for 3 h, followed by further drying at 80 °C for over 72 h until no further weight reduction occurred. We measured the microtuber diameter with a digital caliper (Mitutoyo). Tuberization percentages were measurable under *in vitro* conditions through the following equation:

$$\text{Tuberization percentage (\%)} = \left(\frac{\text{number of explants with tuber}}{\text{Total number of explants}}\right) \times 100$$

Microtuber yield (Chen et al., 2018) in each container was calculated using the following formula:

$$\text{Microtuber yield (per vessel)} = \text{number of microtuber} \times \text{microtuber fresh (or dry) weight}$$

Tuberization timing was defined as the number of days required from incubation to microtuber emergence. Biomass allocation was calculated as the fresh and dry weight of the explants, shoots, and microtubers.

Statistical analysis

The current study operated through a completely randomized design (CRD), which involved various concentrations of sucrose (3, 6, 9, 12, and 15%). The experimental design had three replicates, with fifteen explants per replication. Data analysis included the ANOVA technique and comparison of mean values by Duncan's multiple range test ($P \leq 0.01$). Statistical analyses ran in SAS software (Version 9.0). All graphs appeared from calculations in Microsoft Excel.

Results

Various sucrose concentrations affecting microtuberization

The findings in Figure 1A demonstrated the notable influence of sucrose concentrations on microtuberization percentage. The most pronounced microtuberization occurred in response to a sucrose concentration of 6% and 9%, resulting in microtuberization rates of 94% and 100%, respectively. A further increase in sucrose concentrations led to a decrease in microtuberization, with the lowest microtuberization rate being 15% observed at a sucrose concentration of 15%. Microtuberization occurred faster with an increase in sucrose concentration. The 12 and 15% sucrose concentration caused microtuberization after 15 and 14 days of incubation, which were the earliest observed. The results indicated that higher sucrose concentrations elicited earlier microtuberization.

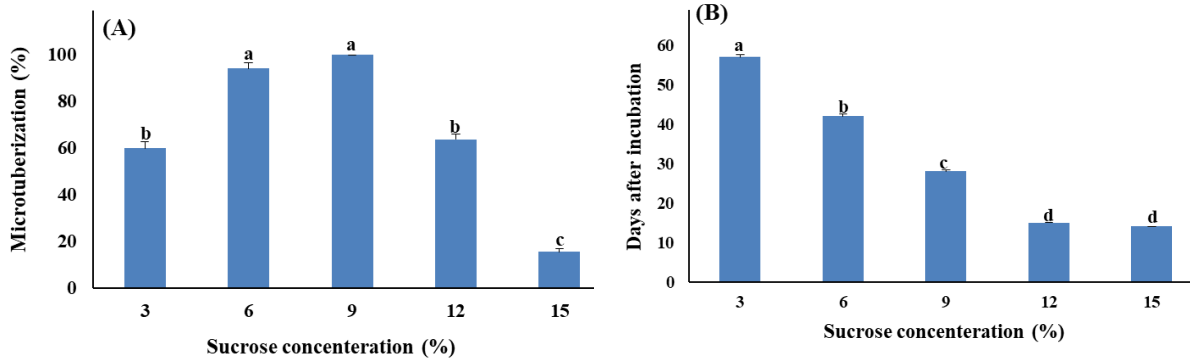

Fig. 1. Effects of different sucrose concentrations on potato microtuberization (%) *in vitro* (A) and microtuberization timing (B).

Various sucrose concentrations affecting microtuber quantity

Sucrose concentration significantly affected the *in vitro* production of microtubers (Figs. 2A and B). The highest number of microtubers, with an average of 2 microtubers per explant, occurred in response to a sucrose concentration of 9% (Fig. 2A). Conversely, the lowest number of microtubers, with only one microtuber per explant, occurred in response to a sucrose concentration of 15%. Furthermore, a similar trend appeared when considering the number of microtubers per tissue culture vessel. The 9% sucrose concentration resulted in the highest production of microtubers, with an average of 4.6 microtubers per vessel (Fig. 2B). On the other hand, the lowest number of microtubers per vessel, with an average of 1.6 microtubers,

occurred in response to a sucrose concentration of 15%. These findings highlighted the effects of sucrose concentration on microtuber quantity *in vitro*.

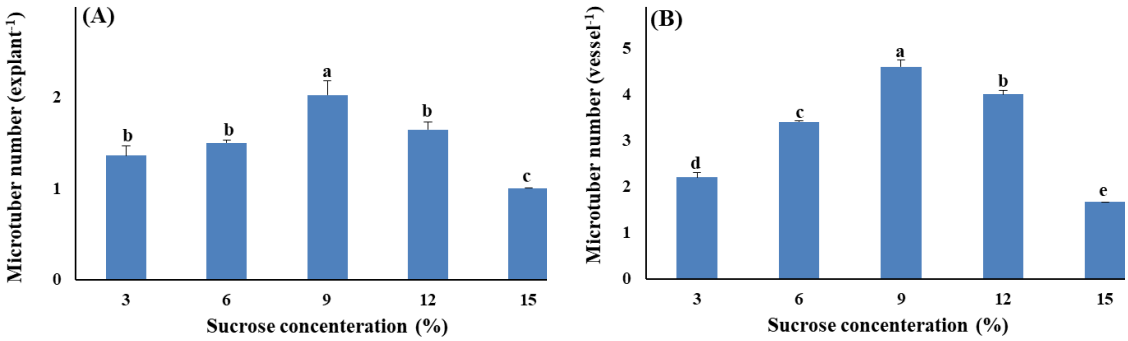

Fig. 2. Effects of different sucrose concentrations on the number of microtubers produced *in vitro*. Microtuber count per explant (A). Microtuber count per culture vessel (B).

Various sucrose concentrations affecting fresh and dry microtuber weights

Experimental findings indicated that the fresh and dry weights of microtubers changed by varying concentrations of sucrose (Fig. 3A, B). The microtubers exhibited the highest fresh and dry weights when exposed to a sucrose concentration of 12%, resulting in 120 and 26 mg per microtuber, respectively. Conversely, the microtubers displayed the lowest fresh weight and dry weight when subjected to a sucrose concentration of 3%, yielding 37 and 7 mg per microtuber, respectively (Fig. 3A). The highest yield of fresh weight and dry weight per vessel was observed at a sucrose concentration of 12%, amounting to 752 mg and 169 mg, respectively. Conversely, the lowest fresh and dry weight per vessel was obtained at a sucrose concentration of 3%, resulting in 113 mg and 21 mg, respectively (Fig. 3B).

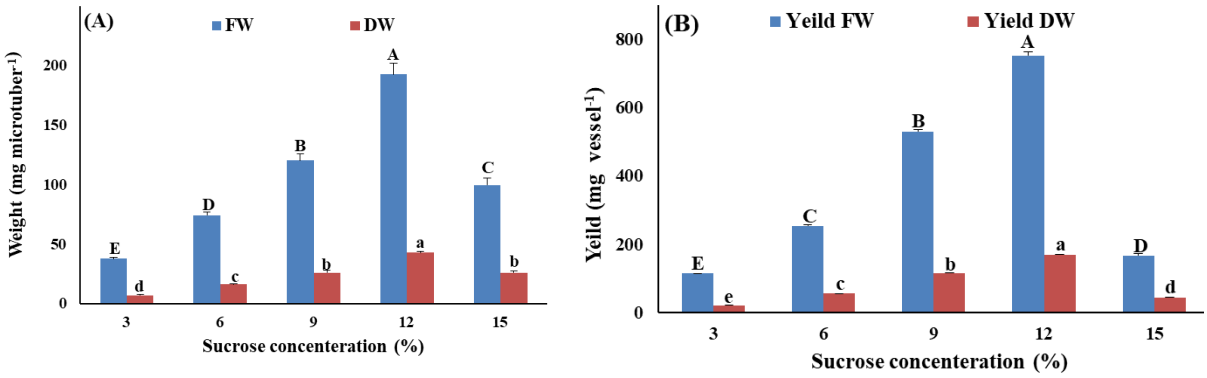

Fig. 3. Impact of different sucrose concentrations on the fresh and dry weights of microtubers (fresh weight and dry weight of microtubers per microtuber) (A) and yield (fresh weight and dry weight of microtubers per vessel) (B).

The effect of sucrose concentrations on microtuber diameter

According to Figure 4, the microtuber diameter was highest at a sucrose concentration of 12% and 15%, reaching a diameter of 5 mm. Conversely, the shortest microtuber diameter was observed at 3% and 6% sucrose concentration, reaching 1.7 mm and 1.4 mm diameters, respectively.

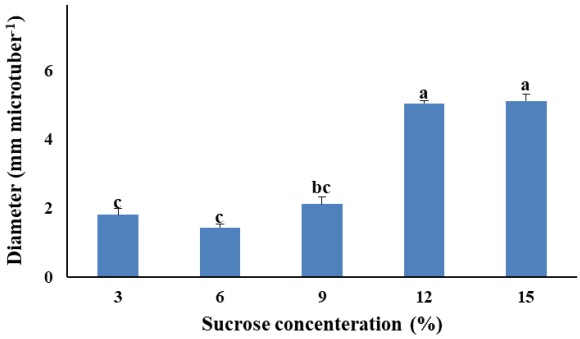

Fig. 4. Effects of sucrose concentrations on microtuber diameter *in vitro*.

Effects of sucrose concentration on potato shoot fresh and dry weights in vitro

The shoot fresh and dry weights decreased with an increase in sucrose concentration, as indicated by data in Figure 5. The highest fresh shoot weight occurred in response to 3% and 6% sucrose concentrations, reaching 371 and 61 mg, respectively. On the other hand, the lowest shoot fresh and dry weights occurred at a sucrose concentration of 15%.

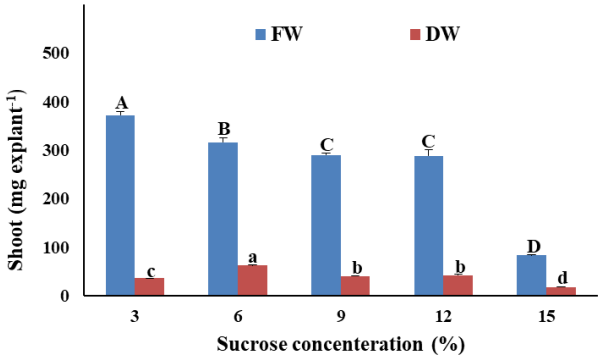

Fig. 5. Effects of different sucrose concentrations on the fresh and dry shoot weights of potato *in vitro*.

Effects of sucrose concentration on explant fresh and dry weight

Sucrose concentration affected explant growth (Fig. 6). The greatest fresh and dry explant weight (44 and 9.5 mg, respectively) were observed at 15% sucrose concentration. Conversely, the lowest fresh and dry explant weight (19.6 and 2.6 mg, respectively) occurred at 3% sucrose.

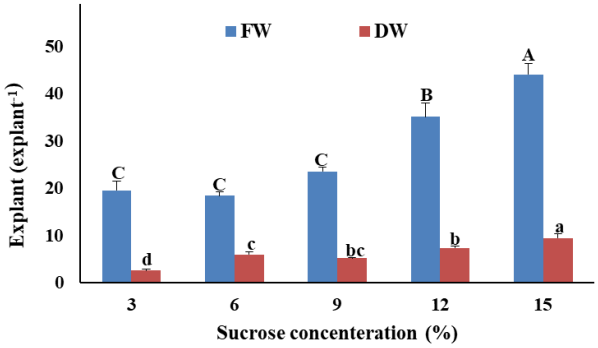

Fig. 6. Effects of different sucrose concentrations on the fresh and dry weights of single node potato explants.

Effects of sucrose concentration on biomass allocation

The sucrose concentration significantly affected biomass allocation in fresh and dry weights, as shown in Figures 7A and 7B. The microtuber, shoot, and explant all exhibited a similar trend in fresh and dry weights. As the sucrose concentration increased, the fresh and dry weights of microtubers and explants increased. Conversely, the fresh and dry shoot weight decreased in response to the increase in sucrose concentration.

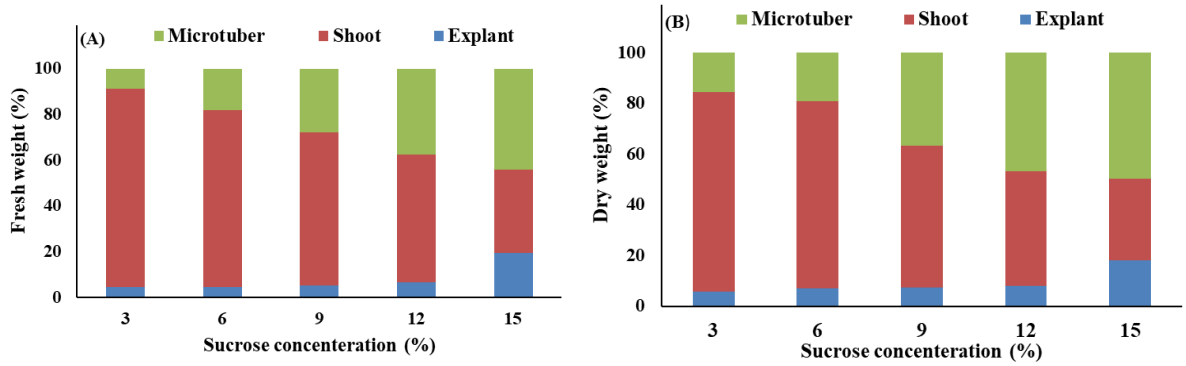

Fig. 7. Effects of different sucrose concentrations on biomass allocation in potato microtuberization *in vitro*.

Discussion

The tuberization process is a complex physiological phenomenon influenced by various factors. These factors include environmental conditions, nitrogen availability, PGRs, genetic composition, nutrient availability, photoperiodic cycles, temperature, explant source, potato cultivar, and sucrose concentration (Ramawat and Merillon, 2013). Sucrose is an essential element in the process of microtuberization in potato (Asmono et al., 2018). It is a significant source of energy that impacts the development of microtubers significantly (Wazir et al., 2015). The capacity of sucrose to induce potato microtubers is well-established, although its influence on microtuberization is contingent upon its concentration. While sucrose promotes microtuberization to a certain degree, excessive concentrations can impede microtuber formation (Ali et al., 2018). The ideal sucrose concentration for microtuberization varies depending on the specific potato cultivar and the growth regulators utilized (Sadek Hossain et al., 2017). For example, MS medium with twice the usual sucrose level (60 g) and without growth regulators reportedly enhanced tuber formation (Ali et al., 2018).

The results of the current study indicate that the microtuberization percentage of single-node potato explants improved by a moderate sucrose concentration. However, both low and high sucrose concentrations resulted in a significant decrease in microtuberization percentage (Fig. 1A). Conversely, a higher sucrose concentration promoted the initiation of microtubers on nodal explants. Notably, the microtubers appeared on the nodal explants approximately 40 days earlier when exposed to sucrose concentrations of 12% and 15% compared to 3% and 6% sucrose concentrations (Fig. 1B). The results of the current study are in line with other research which show that increasing in sucrose concentration from 1% to 8% can increase the percentage and earliness of microtuberization (Al-Hussaini et al., 2015). The number of microtubers produced *in vitro* via nodal explant increased by moderate sucrose concentrations (9%) in single nodal explant and culture vessels. The lower and higher concentrations of sucrose responded negatively to produce microtubers *in vitro* (Fig. 2 A, B). Ali et al. (2018) documented that sucrose functions vitally in microtuber development. The augmentation of sucrose levels within the medium fosters the generation of microtubers to a certain degree, while elevated concentrations (100 g) exhibited an adverse impact. The fresh and dry weight of microtubers increased with an increase in sucrose concentrations up to 12%. However, a sucrose concentration of 15% decreased the fresh and dry weight of microtubers, inhibiting their growth (Fig. 3A). This trend was also observed in fresh and dry yields (Fig. 3B). A study conducted by other researchers demonstrated that a higher concentration of sucrose (10%) hindered the process of tuberization (Sadek Hossain et al., 2017).

Furthermore, an investigation into the impact of varying sucrose concentrations on the fresh and dry weight of microtubers revealed that an elevated concentration of sucrose (8%)

decreased fresh and dry microtuber weights (Ebadi and Iranbakhsh, 2011). It seems sucrose improves microtuberization to a certain extent, but at very high concentrations, it reduces microtuber formation (Ali et al., 2018). The process of microtuberization depends on genotype, so the suppressive impact of sucrose on microtuberization varies depending on sucrose concentrations (Mulugeta Diro, 2013). The size of microtubers produced under *in vitro* conditions relies on their diameter. Our research revealed that an increased sucrose concentration results in larger microtubers, with the highest diameter (5 mm) observed when sucrose concentrations of 12% and 15% were used (Fig. 4). The positive impact of higher sucrose concentration on microtuber size has been well-documented in previous studies (Wazir et al., 2015). The current study showed that shoot growth declined by an increase in sucrose concentration (Fig. 5). The lowest shoot fresh and dry weights occurred at 15% sucrose concentration and the highest at 3% sucrose concentration. An elevated level of sucrose concentration reportedly impeded shoot growth within the context of potato tissue culture (Hou et al., 2022). The conducted experiments have demonstrated that a heightened sucrose concentration can stimulate tuber formation while concurrently inhibiting shoot growth (Asmono et al., 2018).

Nevertheless, it is necessary to note that the influence of sucrose on shoot growth can be significantly affected by various other factors, such as the presence of fluridone, which acts as an inhibitor of abscisic acid synthesis (Harvey et al., 1994). The *in vitro* experiment demonstrated a positive correlation between the sucrose concentration and the fresh and dry weights of the nodal explant (Fig. 6). Specifically, the highest fresh and dry weight of nodal explants occurred at 15% sucrose concentration. The distribution of biomass among different plant components, such as roots, stems, leaves, and tubers, is referred to as biomass partitioning. Our results indicated that (Fig. 7A, B) the nodal explant, shoot, and microtuber exhibited a similar biomass allocation pattern in fresh and dry weights. When sucrose concentrations increased, nodal explants and microtubers increased simultaneously with fresh and dry weight biomass allocation while inhibiting shoot growth. Gopal et al. (2004) found that 6-8% sucrose concentration promoted microtuber production and dry biomass allocation in the microtubers.

Conclusion

Our research findings demonstrated that an increased sucrose concentration significantly improved fresh and dry weights of *in vitro* potato microtubers. It is crucial to acknowledge that lower sucrose concentrations increased the number of microtubers and microtuberization percentage. Thus, the optimal treatment for microtuber production in the 'Sante' potato cultivar is a sucrose concentration of 12%.

Funding

This work was supported by University of Jiroft, Iran.

Author Contributions

NA directed the experiment, fulfilled the project, designed the experiment, analyzed the data, improved the manuscript, and drafted the manuscript.

Acknowledgements

The author acknowledges the University of Jiroft for supporting this research initiative and Shiva Yegane for her help during data collection in the laboratory.

Conflict of Interest

The author indicates no conflict of interest in this work.

References

Aksoy E, Demirel U, Bakhsh A, Zia MAB, Naeem M, Saeed F, Çalişkan S, Çalişkan ME. 2021. Recent advances in potato (*solanum tuberosum* L.) breeding. Advances in Plant Breeding Strategies: Vegetable Crops 8, Bulbs, Roots and Tubers, 409–487.

Al-Hussaini Z, Yousif S., Al-Ajeely S. 2015. The role of sucrose and light duration on *in vitro* tuberization for two cultivars of Potato *Solanum tuberosum* L . International Journal of Current Microbiology and Applied Sciences 4, 277–283.

Ali S, Khan N, Nouroz F, Erum S, Nasim W. 2018. Effects of sucrose and growth regulators on the microtuberization ofcip potato (*Solanum tuberosum*) germplasm. Pakistan Journal of Botany 50, 763–768.

Askari N, Ghahramani R, Reisi A, Sadat-Hosseini M, Parsa Motlagh B. 2023. The role of thermal stress on *in vitro* Potato microtuber induction. Journal of Vegetables Sciences 6, 73–84.

Askari N, De Klerk GJ. 2020. Elimination of epidermal wax from explants increases growth in tissue culture of lily. Scientia Horticulturae 274, 109637.

Askari N, Visser RGF. 2022. The role of scale explants in the growth of regenerating lily bulblets *in vitro*. Plant Cell, Tissue and Organ Culture 149, 589–598.

Asmono SL, Sari VK, Djenal. 2018. The effects of different concentration of sucrose and various auxin on *in vitro* shoot and microtuber formation of red potato

(*Solanum tuberosum* L. var Desiree). IOP Conference Series: Earth and Environmental Science.

Beals KA. 2019. Potatoes, Nutrition and Health. American Journal of Potato Research 96, 102–110.

Bradshaw JE. 2022. A brief history of the impact of potato genetics on the breeding of tetraploid potato cultivars for tuber propagation. Springer Science and Business Media B.V.

Chen L, Lu Y, Hu Y, Xue X. 2020. RNA-Seq reveals that sucrose-free medium improves the growth of potato (*Solanum tuberosum* L.) plantlets cultured *in vitro*. Plant Cell, Tissue and Organ Culture 140, 505–521.

Chen L, Xue X, Yang Y, Chen F, Zhao J, Wang X, Khan AT, Hu Y. 2018. Effects of red and blue LEDs on *in vitro* growth and microtuberization of potato single-node cuttings. Frontiers in Agricultural Science and Engineering 5, 197–205.

Devaux A, Kromann P, Ortiz O. 2014. Potatoes for sustainable global food security. Potato Research 57, 185–199.

Dolničar P. 2021. Importance of potato as a crop and practical approaches to potato breeding. *Solanum tuberosum*: Methods and Protocols 3–20.

Ebadi M, Iranbakhsh A. 2011. The induction and growth of potato (*Solanum tuberosum*. L) microtubers ('Sante' cultivar) in response to the different concentrations of 6-benzylaminopurine and sucrose. African Journal of Biotechnology 10, 10626–10635.

García-García JA, Solano-Campos F, Azofeifa-Bolaños JB, Orozco-Rodríguez R. 2019. Effect of two cytokinins and a growth inhibitor on the *in vitro* tuberization of two genotypes of *Solanum tuberosum* L. CvS. Atlantic and Alpha. Uniciencia 33, 1–12.

Gopal J, Chamail A, Sarkar D. 2004. *In vitro* production of microtubers for conservation of potato germplasm: effect of genotype, abscisic acid, and sucrose. *In vitro* Cellular and Developmental Biology-Plant 40, 485–490.

Górska-Warsewicz H, Rejman K, Kaczorowska J, Laskowski W. 2021. Vegetables, potatoes and their products as sources of energy and nutrients to the average diet in Poland. International Journal of Environmental Research and Public Health 18, 1–23.

Harvey BMR, Bowden G, Reavey C, Selby C. 1994. Stimulation of *in vitro* root and shoot growth of potato by increasing sucrose concentration in the presence of fluridone, an inhibitor of abscisic acid synthesis. Plant Cell, Tissue and Organ Culture 37, 271–276.

Hou YX, Hu YS, Chen CM, Wu HC. 2022. Growth of potato plantlets in response to ventilation, sucrose, and paclobutrazol in modified temporary immersion vessels. HortScience 57, 1424–1429.

Khuri S, Moorby J. 1995. Investigations into the role of sucrose in potato cv. Estima microtuber production *in vitro*. Annals of Botany 75, 295–303.

Mohamed AE-S, Girgis ND. 2023. Factors affecting *in vitro* tuberization of potato. Bulletin of the National Research Centre 47.

Mulugeta Diro MF. 2013. The effects of sucrose on *in vitro* tuberization of potato cultivars. Advances in Crop Science and Technology 01, 1–3.

Nhut DT, Ngan HTM, Mai NTN, Tung HT. 2022. *In vitro* hydroponic culture system in plant micropropagation. Plant Tissue Culture: New Techniques and Application in Horticultural Species of Tropical Region. Springer Nature, 191–206.

Rafique T, Jaskani M, Raza H, Abbas M. 2014. *In vitro* studies on microtuber induction in potato *in vitro* studies on microtuber induction in potato. academia.edu 4–7.

Ramawat K, Merillon J. 2013. Bulbous Plants: Biotechnology. CRC Press.

Sadek Hossain M, Mofazzal Hossain M, Hossain T, Moynul Haque M, Zakaria M, Dulal Sarkar M. 2017. Varietal performance of potato on induction and development of microtuber in response to sucrose. Annals of Agricultural Sciences 62, 75–81.

Stark JC, Love SL, Knowles NR. 2020. Tuber Quality in Potato Production Systems. Springer International Publishing 479–497.

Stein O, Granot D. 2019. An overview of sucrose synthases in plants. Frontiers Media S.A.

Uchendu EE, Shukla M, Saxena PK, Keller JER. 2016. Cryopreservation of potato microtubers: the critical roles of sucrose and desiccation. Plant Cell, Tissue and Organ Culture 124, 649–656.

Wazir A, Gul Z, Hussain M, Khan ZU, Saleem M, Khurshid I. 2015. Effect of sucrose on inducing *in vitro* microtuberization in potato without using any growth hormone. International Journal of Agronomy and Agricultural Research 7, 118-124.

Yagiz AK, Yavuz C, Tarim C, Demirel U, Caliskan ME. 2020. Effects of growth regulators, media and explant types on microtuberization of potato. American Journal of Potato Research 97, 523–530.

Zaheer K, Akhtar MH. 2016. Potato production, usage, and nutrition—a review. Critical Reviews in Food Science and Nutrition 56, 711–721.