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Application of Disease-Free Tissue Culture For the Propagation of Sweet Potato (Ipomoea Batatas) Plants

Abstract

The aim of this study was to determine the most effective method for cultivating disease-free mother plants of sweet potato (*Ipomoea batatas* (L.) Lam.) through meristem culture. In this study, Murashige and Skoog (MS) medium (1962) was used as the basal medium. The medium was supplemented with different concentrations of BAP (1.0; 2.0; 3.5 mg/L) and 2,4-D (1.0; 2.0; 3.5 mg/L) to assess their effects on plant regeneration.The regeneration of apical meristems of sweet potato through tissue culture under *in vitro* conditions, followed by subculturing of shoots, enables the production of disease-free plants at a rapid rate. This is a crucial aspect of modern plant biotechnology. The genetic stability of the regenerated plants provides a significant advantage in agricultural production. The results indicated that the highest shoot formation (1.8 shoots per explant) was observed in the medium containing 3.5 mg/L BAP. The medium supplemented with 2.0 mg/L BAP showed superiority in terms of root number, leaf number (8.9), and node number (8.6). The regenerated shoots were transferred to subculturing media containing NAA (1-Naphthaleneacetic Acid). At this stage, the best results in terms of root number (2.5) and leaf number (4.5) were obtained in the medium containing 0.1 mg/L NAA.Despite the establishment of agricultural research infrastructure in Azerbaijan over the past decade, this study represents the first successful propagation of sweet potato using disease-free tissue culture methods in the country.

Keywords: *Sweet potato, in vitro, apical meristems, MS medium, subculturing, BAP*

Introduction

In this study, the in vitro development of sweet potato through meristem culture on nutrient media was investigated. Sweet potato (*Ipomoea batatas* (L.) Lam.) is a cultivated plant with diverse agricultural uses. Traditionally propagated through tuberous roots, sweet potato is susceptible to numerous diseases. One of the most effective approaches for obtaining disease-free mother plants is

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propagation through meristem culture.Sweet potato (*Ipomoea batatas*) is a species of the genus *Ipomoea*, belonging to the family Convolvulaceae in the order Solanales of the plant kingdom. It is rich in vitamins, minerals, and antioxidants (Wikipedia). Also known as sweet potato, it resembles the common potato and is widely cultivated in China, South America, Japan, India, New Zealand, Turkmenistan, and the North Caucasus region. Sweet potato is a heat-loving perennial plant. Its roots are elongated in shape, weighing between 0.3 and 1.5 kg. On average, it contains 72.5% water (range: 7–175%), 3.75% protein, 24% carbohydrates (of which 15–20% is starch and 1.2–2% is sugar), 0.8–1.5% minerals, and 1% cellulose. One hundred grams of sweet potato provides 466 kJ of energy. It contains 397 mg% potassium, 49 mg% phosphorus, 1 mg% iron, and the following vitamins (in mg%): vitamin C – 23, B1 – 0.10, B2 – 0.05, PP – 0.5, and 0.3 mg% carotene (Doroshenko, 2018).

Research

The flesh of sweet potato can be white, red, or pink. It is used in both sweet and savory dishes and is considered one of the beneficial plants for human health (Laveriano-Santos, Lopez-Yerena et al., 2022, p. 11).

Despite the establishment of agricultural research centers with appropriate infrastructure in Azerbaijan over the past decade, the first propagation of sweet potato via disease-free tissue culture methods in the field of biotechnology was carried out by our team. One of the key biotechnological approaches for producing disease-free plant material is propagation through tissue culture (Abbasi, Askari, Bhatti, Rabbani, 2001, p. 23). In addition to allowing for the mass production of plants in a short period, this method also enables the preservation of genetic material under controlled conditions with limited space and labor requirements (Rabbani, Islam, Lee, 2023, pp. 55-63). Producing healthy seedlings in large numbers remains a primary challenge in sweet potato cultivation. To address these challenges, it is essential to implement both traditional and biotechnological breeding programs (Thorpe, 1994, p. 36.). Tissue culture techniques allow the production of disease-free and genetically uniform plantlets (Sonia et al., 2022). Moreover, these techniques are not only used for increasing propagation rates but also for conserving and modifying existing genetic material (Villalba, A., & Martínez-Ispizua, 2024, p. 12).

Sweet potato has shown poor responsiveness to tissue culture and regeneration. Since it is primarily propagated vegetatively, this leads to the spread of virus-infected material, which in turn reduces yield and increases agro-technical costs, discouraging farmers from cultivating this crop (Smith, 2021, p. 4). Additionally, obtaining sufficient vegetative propagules becomes challenging, especially in large-scale farming.The regeneration rate and nature of sweet potato depend on several factors, including genotype and the type and concentration of different plant growth regulators. Thorpe (1994) emphasized the crucial role of plant growth regulators in callus formation, organogenesis, and somatic embryogenesis (Ivanov, 2021). Together with nitrogen sources, these regulators form the basis of the nutrient media, providing essential minerals required for plant growth and development. In this study, the in vitro development of sweet potato via meristem culture on nutrient media was examined. Sweet potato (*Ipomoea batatas* (L.) Lam.) is an agriculturally valuable plant with various uses. It is commonly propagated through tuberous roots, which makes it vul nerable to many diseases. Propagation via meristem culture is one of the most effective methods to obtain disease-free mother plants. Apical meristems of sweet potato were cultured on Murashige and Skoog (MS) medium supplemented with different concentrations of BAP (6-Benzylaminopurine) and 2,4-D (2,4-Dichlorophenoxyacetic acid). The results showed that the highest number of shoots (1.8 per explant) was obtained on medium containing 3.5 mg/l BAP (Klimov, 2019). Meanwhile, the medium with 2.0 mg/l BAP was superior in terms of root number, leaf number (8.9), and node number (8.6). The obtained shoots were subcultured on media containing NAA (1-Naphthaleneacetic acid). At this stage, the best results for root number (2.5) and leaf number (4.5) were obtained on medium with 0.1 mg/l NAA.Through the regeneration of apical meristems under in vitro conditions, subculturing of shoots was performed, leading to the rapid and disease-free multiplication of sweet potato plants. The genetic stability of the resulting plantlets is considered one of the key advantages in sweet potato production (Kiryushin, 2015).

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Materials and Methods

Preparation, Sterilization, and In Vitro Cultivation of Genetic Material

The study utilized the *Nancy Gold* yellow sweet potato genotype. The storage roots of this genotype were cultivated at the Plant Clinic of the Azerbaijan State Agricultural University, from which sprouts were obtained. These sprouts underwent sterilization procedures. Initially, sweet potato sprouts were washed with tap water, followed by surface sterilization. Subsequently, apical meristems were isolated under a binocular microscope with 10×40 magnification and transferred to a nutrient medium (Naidoo, Laurie, 2022).



Figure 1. Rooting of sweet potato plants



Figure 2. Obtaining sweet potato seedlings in invitro conditions

MS (Murashige and Skoog, 1962) medium was used as the basal medium in this study, prepared with different concentrations of BAP (1.0; 2.0; 3.5 mg/L) and 2,4-D (1.0; 2.0; 3.5 mg/L). The cultivation process was conducted at 26°C with a light intensity of 1500–2000 lux, following a 16-hour light and 8-hour dark photoperiod. The in vitro cultivation process was designed according to a Randomized Complete Block Design with two replications. The nutrient media were sterilized by autoclaving at 121°C and 1 atm pressure for 20 minutes.Measurements were conducted for regenerated plantlets, including shoot number, shoot length (cm), root number, root length (cm), leaf number, and internode number. The obtained in vitro plants were transferred to MS (control) and MS + NAA (0.1; 0.5 mg/L) media for microclonal propagation (Mahmood, Moore, Tommasi, Simone, Colman, Hay, Pizza, 1993).

Results and Recommendations

Statistical analysis of the *Nancy Gold* sweet potato genotype under control and nutrient media conditions revealed that shoot number, root number, and internode number were significant at $p \leq 0.01$.In terms of shoot number, the MS + 3.5 mg/L BAP medium exhibited the highest result, with 1.8 shoots per explant. The media containing 1.0 and 2.0 mg/L BAP, along with 1.0 mg/L 2,4-D, showed identical results with 1.0 shoot per explant (Pasha, Arshad, Ahmad, Raza, 2022).

Regarding root number, the MS + 1.0 mg/L BAP medium yielded the highest value, with 5.0 roots per explant. In terms of leaf number and internode number, the MS + 1.0 mg/L BAP medium also exhibited the highest values, with 9.5 and 5.5, respectively. The cultivation of *Ipomoea batatas (L.) Lam.* apical meristems in nutrient media containing different concentrations of BAP and 2,4-D demonstrated that the medium with 3.0 mg/L BAP was the most effective in terms of shoot number, while the medium with 1.0 mg/L BAP was superior in root number, leaf number, and internode number. Based on all evaluated parameters, it was determined that media containing low concentrations of 2,4-D promoted shoot formation, whereas higher concentrations inhibited shoot

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development and predominantly stimulated callus formation (Statista, 2024). These findings suggest that BAP-containing media promote shoot development, whereas 2,4-D-containing media enhance both shoot development and callus formation. The in vitro regenerated sweet potato plantlets were subcultured, and the medium containing 0.1 mg/L NAA demonstrated superior results in shoot height, root number, root length, and leaf number. For the *Nancy Gold* genotype, the application of cytokinins at low concentrations in in vitro conditions is recommended. Additionally, media containing low doses of auxins were found to be suitable for subculturing and significant for commercial production due to their cost-effectiveness (Zhou, et al., 2020).



Figure 3. Obtaining sweet potato seedlings under in vitro conditions

Conclusion

In addition, obtaining disease-free sweet potato seedlings through apical meristem culture can provide a significant advantage for commercial production. This approach allows for the establishment of healthy mother stocks and the enhancement of sweet potato production potential through in vitro methods. Consequently, this could promote the commercial cultivation of this relatively unknown crop in our country. Furthermore, an efficient and economically viable laboratory protocol for the in vitro regeneration of sweet potatoes can be developed.

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