

# AGRICULTURAL SCIENCES

## EFFECT OF SUCCINIC ACID ON GISELA-6 PLANTS PRODUCED IN VITRO CONDITIONS

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### Abstract

This study investigates the effect of succinic acid as a biostimulant on the micropropagation of Gisela-6 (*Prunus cerasus* × *Prunus canescens*) cherry rootstock under in vitro conditions. In modern intensive horticulture, the production of high-quality, disease-resistant rootstocks is crucial, and micropropagation offers an efficient method for rapid, large-scale multiplication. The research compared a standard Murashige and Skoog (MS) nutrient medium against a modified MS medium where the cytokinin BAP was replaced with 0.5 ml of succinic acid. The experiment was conducted over 25 days, monitoring parameters such as propagation coefficient and shoot length. Results indicated that the nutrient medium supplemented with succinic acid significantly enhanced plant growth and reproductive capacity compared to the standard medium. Explants cultured on the succinic acid-modified medium exhibited increased shoot length (reaching 4-5.4 cm) and produced a higher number of quality microshoots (3-5 per explant). The findings suggest that succinic acid is a promising growth regulator for improving the efficiency and quality of in vitro propagation protocols for the Gisela-6 rootstock, potentially contributing to more sustainable and productive horticultural practices.

**Keywords:** Succinic acid, Gisela-6, in vitro, micropropagation, biostimulant, rootstock, Murashige and Skoog.

### Introduction

Drought, which is becoming increasingly important in the world due to climate change, has become a significant threat in the current era. The entire world is focused on eliminating global warming, oil dependence, and seeking bio-based solutions, which are the main causes of drought. Chemical synthesis of biostimulants applied during plant growth has a promising future in sustainable development and conservation of biodiversity in agriculture. According to the conducted research, succinic acid is used to stimulate the development of seed samples at the initial stage. Succinic acid shortens the period from sowing to germination and increases the rate of plant growth.

Succinic acid is considered a universal intermediate metabolite in the Krebs cycle. There are two main effects of succinic acid on plants during the growth period. The first is stimulation (increased productivity), the second is a protective effect on plants exposed to various stress factors. In addition, succinic acid supports the strengthening of leaf stems, improving the health of microshoots. It is used to treat problems encountered during microclonal propagation of plants in vitro, such as vitrification.

In modern horticulture, rootstocks and varieties used as rootstocks in fruit growing are of great

importance. Based on this, it is necessary to carry out the reproduction of virus-free and disease-resistant plants and to improve them using modern technologies. One of the most modern and promising ways of growing rootstock is its microclonal reproduction in vitro. Adaptation to soil and environmental conditions and durable resistance to biotic agents are among the main features considered in fruit production throughout the development period of new cherry rootstocks. The propagation of rootstocks and their varieties for cherry production using modern methods is a priority for agricultural producers all over the world.

In modern intensive horticulture, rootstocks are used for several different purposes:

ease of propagation and production of higher quality trees;

better adaptation to soil or field with various characteristics;

potential improvement in production through additional traits such as early flowering, high and quality yield, and greater or reduced root vigor, respectively.

Gisela-6 rootstock is becoming one of the main sweet cherry rootstocks in fruit growing today. In horticulture, it is difficult to ensure high quality production of Gisela-6 rootstock by using conventional methods

such as cutting and layering. Micropropagation is one of the most effective methods for accelerating the production of healthy, disease-free plants and obtaining large-scale production in a short period of time.

Gisela-6 (*P. cerasus* × *Prunus canescens*) which resistant poor-quality soil and low water supply compared to other cherry rootstocks. At the same time, when cherry varieties are grafted on Gisela-6 rootstock, they have superior of fruit and better colour, earlier harvesting, higher yield and 30% less height than other cherry varieties.

Microclonal propagation of cherry rootstocks in vitro with the participation of factors such as nutrient medium (macro and micronutrients, water, sugar, plant phytohormones, vitamins, plant agar, etc.), light, humidity, and temperature, is the most effective method.

Gisela-6 of cherry rootstock is taken into account promising for the improvement of contemporary intensive or super-intensive agriculture, because this rootstock has poor height quality.

Micropropagation of rootstock of stone fruits plays a vital role in wide-ranging production of stone fruits, in modern horticulture.

#### Materials and methods

First of all, 50 ml of distilled water was added to a 250 ml laboratory beaker, maintaining an equivalent weight ratio. Then, it was mixed by heating at 70-80 °C temperature. Then, the weight ratio of 0.12 g (0.01 mol) of succinic acid was weighed on an analytical balance and added to boiling water. In order to replace hydrogens (H) with sodium in the prepared solution, 0.84 g of NaHCO<sub>3</sub> was weighed and added to the solution. The solution was heated and mixed on a magnetic stirrer at 70-80 °C for 2 minutes. It is prepared to be added to the nutrient medium after cooling to 20 °C at room temperature.

**Table 1**

**Composition of the standard nutrient medium**

|                                 |         |
|---------------------------------|---------|
| NH <sub>4</sub> NO <sub>3</sub> | 10 ml   |
| KNO <sub>3</sub>                | 10 ml   |
| KH <sub>2</sub> PO <sub>4</sub> | 10 ml   |
| CaCl <sub>2</sub>               | 10 ml   |
| MgSO <sub>4</sub>               | 10 ml   |
| Microelement stock              | 1 ml    |
| Fe chelat stock                 | 5 ml    |
| B <sub>1</sub>                  | 0.6 ml  |
| B <sub>6</sub>                  | 0.6 ml  |
| PP                              | 0.6 ml  |
| C                               | 1.3 ml  |
| BAP                             | 0.7 ml  |
| GA                              | 0.02 ml |
| Sugar                           | 30 gr   |
| Agar                            | 7 gr    |

**Table 2**

**Composition of the nutrient medium by applying succinic acid**

|                                 |         |
|---------------------------------|---------|
| NH <sub>4</sub> NO <sub>3</sub> | 10 ml   |
| KNO <sub>3</sub>                | 10 ml   |
| KH <sub>2</sub> PO <sub>4</sub> | 10 ml   |
| CaCl <sub>2</sub>               | 10 ml   |
| MgSO <sub>4</sub>               | 10 ml   |
| Microelement stock              | 1 ml    |
| Fe chelat stock                 | 5 ml    |
| B <sub>1</sub>                  | 0.6 ml  |
| B <sub>6</sub>                  | 0.6 ml  |
| PP                              | 0.6 ml  |
| C                               | 1.3 ml  |
| Succinic acid                   | 0.5 ml  |
| GA                              | 0.02 ml |
| Sugar                           | 30 gr   |
| Agar                            | 8 gr    |

According to Table 1, BAP (6-benzylaminopurine acid) hormone was substituted from the standard Murashige and Skoog (MS, 1962) plant nutrient medium applied to the Gisela-6 rootstocks. Instead, the effect was studied by applying succinic acid in the volume of 0.5ml (500 µl). Based on the standard protocol and also using succinic acid, nutrient media were prepared in a volume of 200 ml. Then, 20 explant samples were planted in each nutrient medium under a laminar box.

First, the apical shoots of the selected plant samples were chosen for introduction in vitro culture. The length of the explants introduced in vitro culture was 1-1.5 cm. When preparing the nutrient media, the amounts of chemicals based on specific protocols were used. The nutrient medium based on the Murashige and Skoog protocol was autoclaved in an autoclave at 121 °C and 105 kPa pressure for 20 minutes.

Sterilization and subculture of plant material was carried out under the laminar cabin. The transplanted micro-shoots were kept in climate rooms under standard photoperiod of 16/8 (day - 16 hours, night - 8 hours), 2500-3000 lux illumination, temperature of 21+/-3°C and humidity of 50-60%. New plants obtained every 21-22 days were recultured in other nutrient media.

The effect of the tested biological growth regulator on plants was studied both independently in hor-

mone-free medium and in combination with Gibberellic acid (GA<sub>3</sub>). To assess the effectiveness of the process during microclonal propagation, the propagation coefficient (the ratio of the total number of shoots formed to the number of initial explants cultured), the presence of stems, the intensity of leaf colour (chlorosis or healthy, intensive green colour of the leaves), the number of leaves in the explant, and the general condition of the roots were taken into account.

### Conclusion

In terms of the effect of succinic acid on the growth of Gisela 6 microshoots in vitro, the standard medium showed significantly superior results. Compared to the standard nutrient medium, the nutrient medium supplemented with succinic acid had a better effect on plant growth and reproductive capacity during microclonal propagation. Observations were made for 25 days. The length of the plants that successfully completed the initiation stage increased to 4-5.4 cm, and 3-5 microshoots were formed in each explant, showing a better quality result compared to the control. The development dynamics of subcultured microshoots in nutrient medium containing succinic acid gave better results compared to the development of plants grown in standard nutrient medium. (Figure 1, Figure 2)



**Figure 1.** Plants grown on standart nutrient medium



**Figure 2.** Plants grown on nutrient medium modified with succinic acid

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