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The Use of Different Hormone Concentrations During the Propagation of Tea Plant by Tissue Culture

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Abstract

Propagation of tea plants in vitro provides a fast technology for producing a large number of genetically superior and pathogen-free plant materials in a limited time and space. However, in comparison with ex vitro propagation methods, propagtion of plants in vitro reduces the acclimatization rate. The low survival rate of micropropagated plantlets after transfer to natural ex vitro condition has made the use of these propagation techniques economically unviable for many species (Nguyen et al., 1999).

Tea is one of the plants that is very difficult to reproduce in tissue culture. For this reason, the fact that the plant faces some difficulties in plant sterilization at the time of propagation by tissue culture causes this plant to be placed in the order of plants that are difficult to propagate for tissue culture.

The tea plant is a very important plant variety for our country, and traditional varieties are faced with the fear of extinction, making it necessary to try different conditions to increase this plant variety. One of these ways required the propagation of our local variety, which grows wild and civilized in nature in the province of Lankaran, by tissue culture. Although sterilization of the tea plant in tissue culture is a bit difficult, we made the plant materials sterile by using liquid soap, alcohol and mercury chloride in the laboratory environment and planted them in the nutrient medium in a laminar cabinet in the Modified MS environment. Different concentrations of BAP, IBA, IAA, NAA, GA3 hormones were tested in the nutrient medium during the growing period of the plant.
In the rooting part, different concentrations of IBA, IAA, NAA hormones were tried and the most suitable and the most appropriate dose was determined.

Keywords: Medium, tea, hormon, IBA, GA3, BAP, İAA, NAA

Abbreviations

BAP- Benzilamunapurine
MS-Murashige and Skoog
IBA-Indole 3 butyric acid
GA3-Gibberelic acid
İAA- Indole Acetic acid
NAA- Naphthalene Acetic Acid

Introduction

Tea is among the most consumed beverages in the world, valued not only for its flavor but also for its cultural and economic importance. In Azerbaijan, tea represents a core part of daily life and a key agricultural product. This article explores the characteristics of the tea plant (Camellia sinensis), the development of tea cultivation in Azerbaijan, and the nation's vibrant tea culture. The tea plant (Camellia sinensis) is an evergreen shrub from the Theaceae family, cultivated for its tender leaves and buds which are processed into tea. In natural conditions, the plant can reach several meters in height; however, in agricultural settings it is pruned to 1–1.5 meters to facilitate hand harvesting [1].

There are two major cultivated varieties:
• Camellia sinensis var. sinensis – characterized by small leaves and adaptability to cooler climates.

• Camellia sinensis var. assamica – larger leaves and preference for warm, humid conditions.
The ideal environment for tea includes high humidity, well-distributed rainfall, mildly acidic soils, and moderate temperatures (FAO, 2023).

Tea cultivation in Azerbaijan is primarily centered in the southern regions such as Lerik, Lankaran, Astara, and Masalli, along the Caspian Sea. These regions offer a humid subtropical climate, ideal for growing tea [2].

Commercial tea farming began in the early 20th century and expanded rapidly during the Soviet era. By the 1970s, Azerbaijan had become a major tea-producing region within the USSR. The industry declined after independence due to economic disruptions, but recent years have seen government-led revitalization programs focusing on tea sector development [3, 4].

The tea plant plays a vital role in both the agricultural and cultural fabric of Azerbaijan. With favorable climatic conditions in the southern coastal zones and a population that deeply values tea, the country has great potential for strengthening its tea industry. Strategic investments, modernization of agricultural practices, and state support are key to ensuring sustainable growth in the sector [4].

Materials and Methods

Many experiments have been conducted on the sterilization time of the tea plant in textural terms. The sterilization protocol was chosen according to the best result by creating a difference in the time of these experiences, the features used and the timing.

Experiments were carried out by adding all the materials used to 1 lt of distilled water, respectively.
30 materials were taken into 1 lt of distilled water to which 10 ml of liquid soap was added and kept in the mixer for 30 minutes. Then, 10 ml of Previkure was added to 1 lt of distilled water to these 30 materials and kept in the mixer for 15 minutes. Then, 0.5 g of Mercury II Clorine was added to 1 lt of distilled water and mixed for 15 minutes.

After repeating these experiences a few times, the desired number of materials was obtained with the material amounts and timing in Table -6.

Table 1

Material used	Quantity	Time
Liquid soap	10 ml	30 minute
Previkure	10 ml	15 minute
Mercury II chlorine	0.5 g	15minute

As a result of the sterilization used in Table 1, it was revealed that there was extra fungus in the plants within the first 3 days, and another method was used for this.

The number of materials obtained at the end of this method was as follows.

Table 2

First amount of plants	The number of plants remaining as a result
30	11

Since the number of materials obtained was low, another method was used.

This time, in the method used, the doses of ingredients were increased and a new experience was obtained.

Table 3

Material used	Quantity	Time
Liquid soap	10 ml	40 minute
Previkure	10 ml	25 minute
Mercury II chlorine	1 g	25 minute

As a result, burning occurred in the materials because the dosages were excessive.

Table 4

First amount of plants	The number of plants remaining as a result
30	10

As a result of the method used, most of the materials were burned because the timing was too high when Mercury II Chlorine was used.

As a result of additional experience, the appropriate number of materials for the desired number was obtained as a result of the dosages and timing in Table-5.

Table 5

Material used	Quantity	Time
Liquid soap	10 ml	40 minute
Previkure	10 ml	25 minute
Mercury II chlorine	1 g	25 minute

The number of materials obtained at the end of this method was as follows.

Table 6

First amount of plants	The number of plants remaining as a result
30	23

For the propagation of the tea plant by tissue culture, plant materials from the local variety were collected and cleaned from the leaves in the laboratory and kept under tap water for one hour. Afterwards, it was first sterilized by mixing with a shaker for 30 minutes in a mixture of liquid soap and sterile distilled water. After completing this process, the materials that were kept in the previcure for 35 minutes were washed 3 times with sterile distilled water in a laminar cabinet and cleaned. Finally, sterilization was terminated by keeping it in a laminar cabinet in mercury II chloride for 15 minutes and the materials passed through sterile distilled water 3 times were planted in the nutrient medium.

During the propagation phase, BAP, IBA and GA3 were used in different doses and many experiments were carried out for the best results in accordance with the number of new plants obtained. All used hormones were added to 1 liter of Modified MS nutrient medium and tests were carried out.

Different concentrations of BAP hormone were tried to form number of new plants obtained in the plant during the propagation stage. IBA hormone was added to match the cytokine and auxin, causing callus formation in the plant. And different concentrations of GA3 hormone were tried for faster growth of top shoots. As a result, the number of new plants obtained and the height of the plant were obtained in the most appropriate nutrient medium by adding BAP, IBA, GA3 hormones in the Modified MS medium.

As a result, we came across the following result (mg/l Modified MS medium)

Table 7

Name of the hormone used	Quantity	Number of new additional plants
BAP	0.5 mg	0
İBA	0.5 mg	
GA3	0.1 mg	

With the amount of hormone used in Table 7 no new additional plants were obtained after 22 days in the plants planted in the new nutrient medium. The normally expected result was the emergence of new plants after 22 days. 10 plants were planted in the jar, and after 22 days the number remained at 10.

The new method was applied to obtain a higher number of new additional plants by increasing the amount of BAP and the result was as follows.

With the amount of hormone used in Table-8 20 plants were obtained after 22 days from 10 plants planted in the glass jar, meaning that each plant was able to produce 1 new additional plant appropriately.

Table 8

Name of the hormone used	Quantity	Number of new additional plants
BAP	0.8 mg	1
İBA	0.1 mg	
GA3	0.1 mg	

Finally, the cytokine and auxin balance was harmonized and good results were obtained.

The desired result was achieved with the hormone amounts used in Table-9. After 22 days, 30 new plants were obtained from 10 plants planted in the glass jar.

Table 9

Name of the hormone used	Quantity	Number of new additional plants
BAP	1 mg	3
İBA	0.35 mg	
GA3	0.2 mg	

Finally, the result obtained by adding the hormones in table-9 to the Modified MS medium used was accepted.

Although IBA, IAA and NAA hormones are used separately during the rooting stage, the best results were obtained in MS nutrient medium where all three hormones were used. Good results have not been achieved in the nutrient medium using only one hormone.

Of the 10 plants planted in Modified MS medium with the Auxin amounts used in Table-10 no rooting occurred after 22 days, on the contrary, burns appeared at the ends of the plants.
Then, only swollen calluses are formed after 22 days from 10 plants planted in Modified MS medium using the Auxin amounts specified in Table-11.

Finally, the amount of IBA hormone used was 1 mg/l and at the end of 22 days, roots formed in 8 of the 10 plants planted in the Modified MS nutrient medium in the glass jar, and the other 2 plants developed callus.

Table 10

Name of the hormone used	Quantity	Rooting result
İBA	0.1 mg	0
NAA	0.1 mg	
İAA	0 mg	

Table 11

Name of the hormone used	Quantity	Rooting result
İBA	1 mg	There was only callus formation
NAA	0.5 mg	
İAA	0.1 mg	

Table 12

Name of the hormone used	Quantity	Rooting result
İBA	5 mg	Rooting has occurred
NAA	0.5 mg	
İAA	0.5 mg	

Results

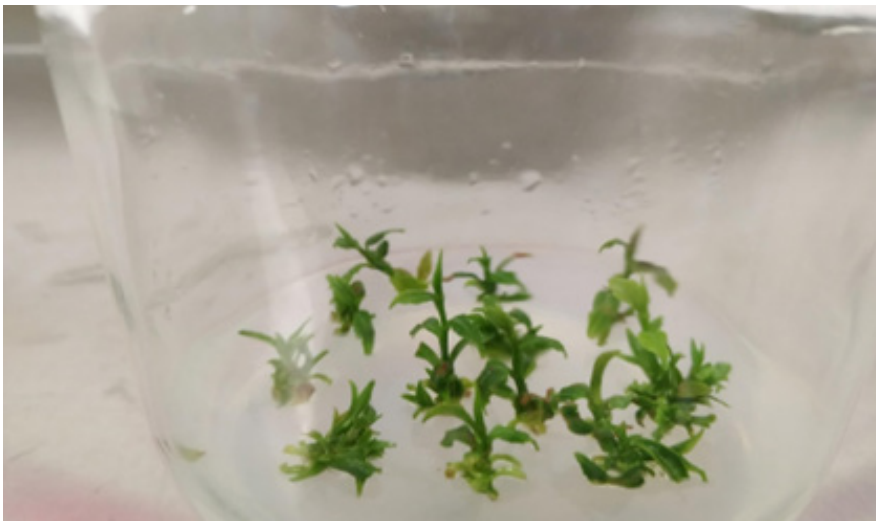

Figure 1: First green appearance 14 days after meristem removal
0.5 mg of BAP, 0.02 mg of IBA, and 0.1 mg of GA3 in MS Modified.

Plant materials taken from the meristem were planted by adding 0.5 mg BAP, 0.02 mg IBA, 0.1 mg GA3 hormones to the MS Modified nutrient medium, and after 14 days, the first green tissues started to form(ımage-1)

Figure 2: 0.8 mg BAP, 0.1 mg IBA, 0.1 mg GA3, MS Modified
Plant materials in the propagation phase were added to the MS modified nutrient medium after 14 days by adding 0.8 mg BAP, 0.1 mg IBA, 0.1 mg GA3 hormone (ımage-2).

Figure 3: 1 mg BAP, 0.1 mg IBA, 0.35 mg GA3, 0.2 mg iron chelate in MS Modified

Plant materials at the propagation stage were placed in MS Modified nutrient medium after 22 days with the addtion 1 mg BAP, 0.1 mg IBA, 0.35 mg GA3 hormone

The medium used as nutrient medium was prepared by adding 0.5 mg BAP, 0.02 mg IBA and 0.1 mg GA3 to the MS Modified medium and the meristems were planted.

Finally, the shoots were transferred to the newly prepared nutrient medium by adding 1 mg BAP, 0.1 mg IBA, 0.35 mg GA3, 0.2 mg to the MS Modified medium in laminar cabinets [5].

Figure 4: mg of IBA, 0.5 mg of IAA, 0.5 mg of NAA in MS Modified Medium.

During the rooting stage, the nutrient medium was prepared by adding 5 mg IBA, 0.5 mg IAA, 0.5 mg NAA hormones to the MS Modified nutrient medium, and rooting occurred after 40 days(ımage-4).

After the shoots had a certain lenght for all 40 days, they were planted in the nutrient medium prepared by adding 1 mg of IBA, 0.5 mg of IAA, 0.5 mg of NAA to the MS Modified medium, and the roots were split in the shoots for all 40 days.

Discussion

Tea as one of the important plants, is considered resistant to in vitro culture where genetic determinism complicates the different stages of micropropagation, along with other minerals such as media maintenance. Three different ML approaches were applied, along with PSO optimization measures, to assemble these clusters, identify and predict the management of genotype and environment setting throughout replication. Tea micropropagation can be improved by including different points in measurement and treatment. Tea micropropagation in consistently narrowed pores has not been extensively investigated. Different study on tea tissue culture has concluded that some chemicals such as phloroglucinol and FeEDDHA diffuse into MS Modified or (MS) basal medium with different PGRs in various tasks of the media. removal of agar , aeration and aeration of sucrose ,but some of the studies that led to the medium, including the interaction of mineral nutrients, vitamins and PGRs on proliferation quality and quantity[6].

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