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MICROCLONAL PROPAGATION OF LIRIODENDRON TULIPIFERA IN VITRO

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Abstract:

In vitro reproduction, a new direction in the field of biotechnology, is a way to reproduce a large number of plants in a relatively short time and without seasonal restrictions. Especially, woody plants can be propagated in vitro mainly by means of apical meristems or leaf primordia obtained from apical buds. Currently, this method is widely used for commercial purposes. This is due to the high variability and acclimatization of plants that reproduce slowly in natural conditions, including L. Tulipifera, for effective use in pharmaceutical, medical, industrial and agricultural fields, and the problem of rapid cultivation of its seedlings is currently being solved by modern microclonal in vitro propagation is the most effective method for mass production of this plant.

Keywords:

In vitro, Liriodendron tulipifera, meristem, explant, culture, photoperiodism, viroid, microcloning, regulator, ex vitro.

Introduction

Landscape plants are a group of cultivated and wild plants belonging to different botanical families used to satisfy people's aesthetic needs. Landscape plants are grown for the greening of cities and villages, recreation parks, social sphere objects, industrial buildings and residences, and urban planning. It is characterized by its beauty, the color of its leaves, flowers, fruits, and the strange shapes of its body[1].

Minutes of the 03-13-2 meeting of the Cabinet of Ministers of the Republic of Uzbekistan on May 12, 2011 "On measures to organize the cultivation of ornamental tulip tree seedlings in the conditions of the Republic of Uzbekistan" and on October 16, 2012 This research work serves to a certain extent to ensure the implementation of Decree No. 683 "On increasing the planting of tulip trees and Crimean pine seedlings" [2]. Taking into account the fact that a lot of attention is paid to the planting of tulips in the landscaping and improvement facilities of our republic, propagation in the in vitro laboratory is considered to be of practical importance.

Our research was conducted in order to study the effectiveness of in vitro propagation of genetically identical seedlings of L. Tulipifera in two different Mc Cown Woody plant and DKW (Driver& Kuniyuki) media. The laboratory experiments were conducted in the I.CH.AT building of the USAMV University of Cluj-Napoca, Romania, Faculty of Horticulture, and in the in vitro laboratories "Bogbon" belonging to "Sag Agro" LLC, Samarkand region of the Republic of Uzbekistan. In order to effectively use the useful aspects of the tulip tree in pharmaceutical, medicine, industry and agriculture, solving the problem of rapid cultivation of its seedlings by modern in vitro microclonal propagation has several positive properties.

-obtaining genetically identical plants;
-breeding of plants that are difficult to reproduce by traditional methods;
- production of a large number of seedlings in a short period of time;
-reduction of required areas for growing seedlings;
-continuous work throughout the year; [3]

Plant propagation in vitro has several advantages over conventional propagation. Including resistance to adverse conditions in plants, high productivity and quality, reproduction of valuable genotypes, recovery of plants from viruses and viroids, and breeding of plants that are difficult to reproduce from seeds in the traditional way.

2. MATERIALS AND METHODS.

The tulip tree (Liriodendron tulipifera) is a plant belonging to the Magnolia family and is one of the largest native trees of the eastern United States. The height is 20-58 m, the diameter of the trunk is 3 m, and the average height can reach from 21 to 30 meters. L. tulipifera is a unique decorative and medicinal plant. Its trunk is widely used for valuable wood, furniture for interior decoration of houses, construction materials. Paint is obtained from the bark. In ancient times, its inner shell was used for medicinal purposes against worms, arthrosis, cough and cholera. [7]. L. Tulipifera is widely used in urban planning to transform city streets and industrial areas into a beautiful landscape.

Microcloning, sterilization, mathematical statistical analysis, photoperiodism, in vitro and ex vitro methods were used in our research [8].

Figure 1. Liriodendron tulipifera seeds.

As a biological material used for in vitro propagation of L. tulipifera (February and March is the period of active growth of buds), young shoots of Liriodendron tulipifera grown in the botanical garden of Babes Bolyai University Alexandru Borza Botanic Garden, Cluj-Napoca, Romania and apical meristem cells from side branch buds and leaf primordia from tip buds were used. The explants obtained were sterilized step by step to avoid contamination of the apical meristem cells of the separated apical and side branch buds.

2.1. Sterilization of the obtained biological materials.

The explants taken for the experiment were first thoroughly washed in running water and then rinsed with distilled water. The explants were sterilized by holding in 94% ethanol for 10 minutes, and the explants were sterilized by adding two drops of Tween to 200 ml of 6% chlorine solution in a laminar box for 20 minutes. Sterilization is reduced to half of the initial

time (10 minutes) for buds in the leafing stage and for apical buds in the period of full growth. [9].

Figure 2. Isolation and sterilization of explants.

The used instruments were sterilized in a drying oven (thermostat) at 120 0C for 2 hours. Explants were divided into sections in laminar boxes sterilized with bactericidal lamps. Before starting work, work areas, tables, binocular magnifiers and tripods with test tubes were wiped with alcohol.

Sterilization of artificial nutrient medium. The nutrient media prepared for planting L. tulipifera were saturated with steam under pressure. The mouth of the test tubes filled with nutrient media was closed with aluminum foil, wrapped in wrapping paper and kept in an autoclave at 120 oC at 1 atmosphere pressure for 20 minutes.

Before sterilizing the prepared nutrient medium in an autoclave, the pH of the medium should be equal to pH 5.8-5.9. But the pH level of the nutrient medium after autoclaving decreased to 0.4. Taking this into account, the pH level of the nutrient medium before sterilization was measured with a pH meter and raised to 6.2 with NaOH. After sterilization in the autoclave, the pH drops to 5.8-5.9, and this is considered the optimal pH medium. Planting of planting material in the nutrient medium is carried out in sterile conditions in laminar boxes. Each test tube or glass jar filled with nutrient medium is sterilized by heat before and after planting planting material in the oral laminar box, and then the lids are closed. The aim is to grow a pure plant from apical meristem cells obtained from sterilized, virus-free shoots.

Figure 3. Measurement of the pH level of nutrient media.

In order for the culture to grow, multiply, and take root, the room temperature of the culture should not exceed 21-240C, and the light intensity of the photoperiodism should be 16 hours. We need to provide room temperature and light intensity for each step to take place [10].

3.RESULTS

The main growth regulators FeNa-EDDHA, IBA, BAP, etc. Were used in the preparation of Mc Cown Woody plant nutrient medium, which was used to obtain microplants from explants isolated from Liriodendron tulipifera. Substances added to the feed medium and their concentration were taken in relation to the volume of 0.5 l. The content of nutrient medium for culturing apical meristem tissues isolated from L. Tulipifera was prepared based on STOC solution.

Table 1 Composition of Mc Cown Woody plant nutrient medium used to obtain microplants from explants isolated from Liriodendron tulipifera.

Basic tools	**Used growth regulators and their concentration in the nutrient medium**							
Mc Cown Woody plant, agar, sucrose, BAP, AiB	Agar	Sucrose	Mc Cown Woody plant	IBA	BAP	NAA	GA3	FeNa-EDDHA
	3,25 gr	15 gr	1,2313 gr	5 ml	10 ml	-	-	0,1 mg

The main growth regulators Mc Cown Woody plant, IBA, BAP, etc. were used in the preparation of Mc Cown Woody plant nutrient medium. The amount of additives and growth regulators contained in Mc Cown Woody plant nutrient medium was taken in relation to the volume of 0.5 l.

Mc Cown Woody plant 1.2313 g, IBA 5 ml, BAP 10 ml, agar 3.25 g, sucrose 15 g and FeNa-EDDHA 0.1 mg were used in the preparation of this nutrient medium. 15 explants from the apical meristem of Liriodendron tulipifera were planted in Mc Cown Woody plant nutrient medium.

Table 2 Composition of DKW (Driver & Kuniyuki) nutrient medium used to obtain microplants from explants isolated from Liriodendron tulipifera.

Basic tools	**Used growth regulators and their concentration in the nutrient medium**								
DKW (Driver& Kuniyuki), glucose, BAP, AiB,nicotinic acid, glycine, pridoxina gidroxlorid, Mezo-inozitol, thiamine HCl	DKW (Driver& Kuniyuki)	Glucose	IBA	BAP	Nicotinic acid (B_3)	Thiamine HCl (B_1)	Glycine	Mezo-inozitol	Pridoxina gidroxlorid (B_6)
	2.73975 gr	15 gr	10 ml	20 ml	5 ml	10 ml	10 ml	50 mg	2,5 ml

DKW (Driver& Kuniyuki) nutrient medium consists of DKW (Driver& Kuniyuki), glucose, BAP, AiB, nicotinic acid, glycine, pyridoxine hydrochloride, Meso-inositol, thiamine HCl. The amount of additives and growth regulators contained in the DKW nutrient medium was taken in relation to the volume of 0,5 l.

Figure 4. Mc Cown Woody plant and L. Tulipifera explants planted on DKW medium.

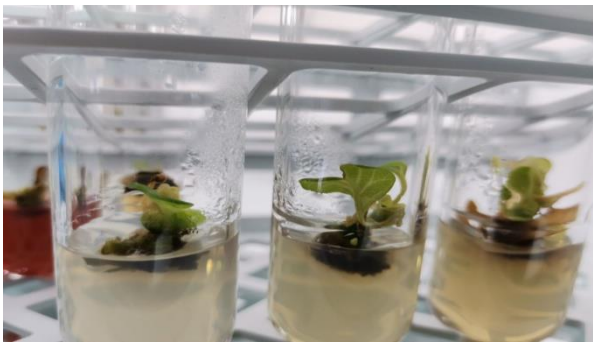
15 explants from the apical meristem of Liriodendron tulipifera were planted in DKW (Driver& Kuniyuki) medium.

For the experiment, a total of 30 explants, 15 for each medium, were prepared from the apical meristem of Liriodendron tulipifera. Out of 15 explants planted in Mc Cown Woody plant nutrient medium, the number of shoots and leaves was 10. The number of infected explants is 5. Explants in 10 test tubes produced leaves and achieved high results. These observations were made for 3 months.

Table 3 The process of leaf and bud formation in Liriodendron tulipifera explants.

Nutrient medium	**Total number of explants**	**Number of explants that produced buds and leaves**	**The number of explants that produced buds and leaves is in %**	**Number of infected explants**	**Number of affected explants in %**
Mc Cown Woody plant	15	10	66,6%	5	33,3%
DKW (Driver& Kuniyuki) ozuqa muhiti	15	8	53,3%	7	46,6%
Jami:	30	18	60%	12	40%

Out of 15 explants planted in DKW (Driver & Kuniyuki) nutrient medium, the number of shoots and leaves was 8. The number of infected explants is 7. The incidence of infection was higher than in Mc Cown Woody plant medium, but leaf formation and number were higher in DKW (Driver& Kuniyuki) medium.



DKW (Driver& Kuniyuki)

MC Cown Woody plant

Out of 30 explants of L. Tulipifera used in this experiment, 18 of them produced leaves in 60%, and a high index was reached. The number of infected was 12 40%.

4. DISCUSSION.

We used Mc Cown Woody plant and DKW (Driver & Kuniyuki) nutrient media in our experiments on microclonal reproduction of L. Tulipifera until the process of leaf and bud formation. In microclan propagation of L. tulipifera, leaf formation was slower in explants planted on Mc Cown Woody plant medium. In DKW (Driver & Kuniyuki) nutrient medium, there was a lot of infection damage, but the process of leaf formation was faster. DKW (Driver & Kuniyuki) nutrient medium was found to be optimal for obtaining L. Tulipifera microflora. The results obtained from this experiment clearly showed that L. Tulipifera propagation in vitro is an effective and alternative way to obtain seedlings in large numbers and adapted to climatic conditions.

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