IN VITRO PROPAGATION OF MULBERRY

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ABSTRACT

Propagation of mulberry seedlings suitable for the climatic conditions of the southern regions of our republic by in vitro method. *In vitro* breeding of mulberry cultivars and hybrids, environmental conditions for their explants, and in vitro breeding. *In vitro* propagation of explants of “Jarariq-6” varieties, determination of the composition of the necessary medium, sterilization, until the explants are propagated, rooting and from *in vitro* (*cultivation of sterile current material in an artificial environment*) to *in vivo* (*sterile current cultured material cultivation in natural conditions*) production of transfer methods. The practical importance of the development is based on the fact that the varieties and hybrids of the mulberry tree, which are fertile, productive and have high mobility, are rapidly multiplied by the *in vitro* method, and the strength of silk production is strengthened.

INTRODUCTION

Mulberry (*Morus* sp.), typical woody plant of the Mediterranean basin, belongs to the *Morus* genus and to the *Moraceae* family. It's cultivated for the tasteful fruits, for its potential pharmaceutical and cosmetic use and for its economic importance in silk industry for its foliage [1]. Mulberry is an invaluable tree of immense, which constitutes the chief food for the silkworm (*Bombyx mori* L). The improvement of productivity traits in mulberry plays a vital role in the progress of sericulture industry [2]. However, perennial nature of the plant coupled with prolonged juvenile period slows down the process of mulberry improvement [3].

Vegetative propagation of mulberry through grafting is not economically viable [4]. Mulberry tree improvement through conventional breeding is slow and also difficult due to its

heterozygous nature [5]. For targeted crop improvement through biotechnological approaches, attempts have been made to standardize *in vitro* regeneration protocols in different mulberry varieties [6]. Mulberry is a recalcitrant species in terms of tissue culture, and shoot regeneration is greatly dependent on the genotype, type of explant and combination of growth regulator used in the culture media. Using different explants such as stem, shoot tip and nodal segment, axillary bud, hypocotyl and cotyledon (Bhatnagar et al., 2001), leaf (Kapur et al., 2001; Vijaya Chitra and Padmaja, 2005).

In vitro regeneration has been attempted with various degrees of success. Since there are variations in regeneration among mulberry varieties. This study was designed to develop a protocol for rapid multiplication to produce micropropagated plants of mulberry (*Morus* sp.) cultivar. ”Jarariq-6” through in vitro culture using axillary buds’ explants which will be help to solve the poor rooting ability of stem cutting through conventional breeding.

MATERIALS AND METHODS

The steps of conducting experiments are as follows. Moderation of individual stages of the technology of microclonal reproduction of the above varieties of mulberry and development of effective regeneration methods from somatic tissues is carried out as follows.

Stage 1. The conditions for obtaining the initial sterile material for in vitro reproduction of mulberry and the moderate content of carbohydrates, macro and microelements that ensure the maximum level of regeneration during its micropropagation stage are determined.

Stage 2. Studying the laws of the moderate composition of growth regulators for micropropagation of mulberry trees in vitro and the moderate ratio of growth regulators for the induction of the process of root formation of mulberry varieties and hybrids, for a long-time in vitro storage options are determined.

Stage 3. During in vitro reproduction of mulberry trees, the processes of clarifying the specific characteristics of the morphogenesis process and optimizing the adaptation to in vitro and soil conditions are controlled.

Stage 4. Evaluation of the morphogenetic potential of the studied mulberry varieties and hybrids and the development of effective methods of adventitious organism induction. The growth patterns of the plant adapted to field conditions are studied.

Stage 5. Calculation of the economic efficiency of the microclan reproduction technology in obtaining healthy planting material and new developments in the laboratories of existing cell technologies in our republic are used. After sterilizing the mulberry varieties and hybrids selected for in vitro propagation, it is necessary to determine the optimal nutrient content for growing. During the research, MS (*Murasagi-Skug*), WPM (*Woody Plant Medium*) with different macro-micronutrient composition are used., DKW (*Driver and Kuniyuki*) nutrients are used.

MS, WPM, DKW media positions

№	Position 1		Position 2		Position 3	
	MS (*Murasage-Skug*)	concentra-tion, mg	DKW (*Driver va Kuniyuki*)	concentra-tion, mg	WPM (*Woody Plast Medium*)	concentra-tion, mg
1.	NH_4NO_3	1650,0	NH_4NO_3	1416,0	NH_4NO_3	46,6
2.	KNO_3	1900,0	KNO_3		KNO_3	
3.	K_2SO_4		K_2SO_4	1559,0	K_2SO_4	90,6
4.	$Ca(NO_3)_2 \times 2H_2O$		$Ca(NO_3)_2 \times 2H_2O$	1664,0	$Ca(NO_3)_2 \times 2H_2O$	471,36
5.	$CaCl_2 \times 2H_2O$	440,0	$CaCl_2 \times 2H_2O$	112,50	$CaCl_2 \times 2H_2O$	72,50
6.	$MgSO_4 \times 2H_2O$	370,0	$MgSO_4 \times 2H_2O$	361,19	$MgSO_4 \times 2H_2O$	100,54
7.	KH_2PO_4	170,0	KH_2PO_4	265,0	KH_2PO_4	170,0
8.	H_3BO_3	6,2	H_3BO_3	4.80	H_3BO_3	6,2
9.	$MnSO_4 \times 2H_2O$	27,7	$MnSO_4 \times 2H_2O$	33,80	$MnSO_4 \times 2H_2O$	22,30
10.	$CoCl_2 \times 6H_2O$	0,025	$CoCl_2 \times 6H_2O$		$CoCl_2 \times 6H_2O$	
11.	$CuSO_4 \times 5H_2O$	0,025	$CuSO_4 \times 5H_2O$	0,025	$CuSO_4 \times 5H_2O$	0,025
12.	$ZnSO_4 \times 7H_2O$	8,60	$ZnSO_4 \times 7H_2O$	17,0	$ZnSO_4 \times 7H_2O$	8,00
13.	$Na_2MoO_4 \times 2H_2O$	0,25	$Na_2MoO_4 \times 2H_2O$	0,39	$Na_2MoO_4 \times 2H_2O$	
14.	KJ	0,83	KJ		KJ	
15.	$FeSO_4 \times 7H_2O$	27,8	$FeSO_4 \times 7H_2O$		$FeSO_4 \times 7H_2O$	
16.	$Na_2EDTA2H_2O$	37,3	$Na_2EDTA2H_2O$		$Na_2EDTA2H_2O$	
17.	$Fe_2EDTA \times 2H_2O$		$Fe_2EDTA \times 2H_2O$	44,63	$Fe_2EDTA \times 2H_2O$	
18.	Innozitol	100,0	Innozitol	100,0	Innozitol	100,0
19.	Casein hydrolyzate	100,0	Casein hydrolyzate	100,0	Casein hydrolyzate	100,0
20.	Shugar	40,0	Shugar	20,0	Shugar	20,0
21.	Kinetin	5,4	Kinetin		Kinetin	
22.	Tiamin		Tiamin		Tiamin	

23.	Peredoxin		Peredoxin	0,50	Peredoxin	0,50
24.	Glitsin		Glitsin	1,00	Glitsin	1,00
25.	Fito agar	5,4-6,0	Fito agar	5,4-6,0	Fito agar	5,4-6,0
26.	BAP	0,50	BAP	0,50	BAP	0,50
27.	IMK	0,50	IMK	0,50	IMK	0,50

RESULTS AND DISCUSSIONS

Microclonal propagation of plants. Vegetative propagation of plants *in vitro* is called microclonal propagation. Seed plants reproduce bisexually; through seeds and vegetative. Both methods have advantages and disadvantages. Disadvantages of propagation by seeds: it takes a long time; the diversity of the genetics of the planting material. In vegetative reproduction, the genotype is preserved, it does not take a long time. However, most plants cannot be propagated vegetative.

The reason:

- most varieties (*for example, oak, birch, spruce, walnut trees, etc.*) do not reproduce effectively vegetative even during young shoots; they cannot be propagated vegetative;
- the complexity of obtaining standard ECU material; the complexity of the process of grafting older plants;
- it is difficult to obtain genetically homogeneous material throughout the year, and
- the technologies developed for this are also ineffective.

Plant cells and tissues are gray as a result of the achievements in the field of species, a new method of vegetative propagation of plants- microclonal propagation method was created. The essence of the method is based on the totipotency property of plants.

The method of microclonal reproduction of plants has a number of advantages over other traditional methods:

- it is possible to prepare genetically homogeneous equal material; due to the use of meristem tissue, the plant is free from viruses;
- high reproduction coefficient; reduction of selection time;
- "the plant goes to the reproductive development phase faster;

- it is possible to reproduce even plants that are difficult to reproduce by traditional methods;
- a large area of land is not required for planting material.

In the 50s of the XX century, George Morel for the first-time orchid was able to multiply his plant by microclonal method.

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