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OPTIMIZATION OF TRADITIONAL AND RESEARCH OF THE LATEST WAYS OF REPRODUCTION OF EUROPEAN FIR-TREE (PICEA ABIES (L.) KARST.) IN CONDITIONS OF *IN VIVO* AND *IN VITRO*

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Results of researches of optimization of traditional and microclonal reproduction of European fir-tree (Picea abies H. Karst.) are represented. Influence of nine preparations (on the basis of regulators of growth and development of plants) on germination of P. abies seeds is shown and their optimal concentrations are fixed. Substratums are defined which provide high indicators of germination energy, germination of seeds and growth of seedlings of P. abies. The biotechnology of microclonal reproduction of P. abies and their adaptations to conditions of in vivo is developed, which allows to receive large number of adapted regenerated plants in short time.

Picea abies H. Karst., growth regulators, seeds, germination, substratum, culture in vitro, nutrient medium, microclonal reproduction, adaptation of regenerated plants to conditions of in vivo

The main ecological role in gardening of the cities belongs to lignified plants which are characterized by high decorative effect within a year and resistance to technogenic conditions. In particular the fir-tree European (Picea abies H. Karst.) - the introduced species with high winter hardiness, are widely used in landscape construction, an orchard and for creation of artificial plantings in the conditions of the rights of the coastal forest-steppe, Polesye and the Ukrainian Carpathians. Breeds seed and vegetative are traditional ways of reprodaction. Among them the most widespread is seed as the available methods of vegetative reproduction are extremely

labor-consuming and ineffective and don't allow to receive necessary quantity of the taken roots shanks from selected trees [2, 7, 12]. Modern way of receiving landing material of wood plants is the method of culture of the isolated fabrics of plants in vitro which now is the main component of modern biotechnologies of microclonal propagation in forestry [8, 14, 15, 18]. However the available techniques of microclonal propagation of coniferous species of plants are limited only to separate biotechnological stages and don't allow to reproduce all process [5, 14, 18].

The purpose of researches is an optimization traditional ways and development new (technologies of microclonal propagation) ways of receiving plants *P. abies.*

Materials and technique of researches. For traditional reproduction used seeds of plants of P. abies which were collected from trees by age of 40 years in December, 2008. In GP "Hmelnitsky LG" of Vinnytsia OULOH. We applied (2011) solutions of nine preparations to increase of its similarity (the Humate + 7 (60-65 of % of humates, 0,4 % of Fe, 0,2 % of Cu, 0,2 % of Zn, 0,17 % of Mn, 0,018 % of Mo, 0,02 % with, 0,2 % in, 1,5 % of N (in the form of complex connections with humic acids); Zircon (mix hydroxycinnamic acid); heteroauxin (indole acetic acid 920 g · kg-1); Epin(contains epibrassinolide);

Ale (contains archdeacon acid); Amber acid (ethane-1,2- dicarboxylic acids); Kornevin (contains 3- indole butiric acid); Kornevit (N, P, K, Mg, Zn, Cu, gumat, amber acid); Reak (45 g · K2O l-1,45 g · l-1 of P2O5, 14 g · l-1 of Fe, 6,5 g · l-1 of Zn, 4,5 g · l-1 of Cu, 2,2 g · l-1 In, 4,8 g · l-1 of Mn, 0,08 g · Mo's l-1, 0,03 g · l-1 CO), which are the most widespread in specialized points of sales in the Ukrainian market.

In 2013 we were put a series of researches on definition of an optimum substratum for the purpose of providing high rates in similarity of seeds of P. abies and further growth of their ladder (seedlings). For experiments we used the seeds collected in 2011 from trees of P. abies V of a class of age.

Plant material was grown up in five substrate which are recommended by producers for cultivation of coniferous plants (selected for the most similar contents micro and macrocells and mechanical structure, but different indicators of acidity

(pH): option 1 – 4,0–4,5; 2 – 5,0–5,5; 3 – 6,0–6,5; 4 – 6,0–6,5 and option 5 – 6,5–7,0 (data are specified by producers). As control we used the soil which we took from under bed curtains of fir-tree planting. Viability of seeds was defined in accordance with GOST 13056.7-93, similarity - in accordance with GOST 13056.6-97 [9, 10]. Acidity of substrate was determined by agrochemical methods on the basis of a salt extract [6]. Height of seedlings was measured for the 30th days.

For microclonal propagation of plants P. abies of part of tendril of the current gain 2-4 cm long were isolatde from thirty-year donor plants in June and July. Sterilization of plant material was carried out by 70 % ethanol (1 min.), 2,5 % of NaClO (10-20 min.), 1 % of AgNO3 (10-20 min.). Aseptic conditions created on the methods standard in biotechnology [1, 3]. Samples cultivated on basic hormone-free to a nutrient medium with Murasige and Skuga (MS) [17] and Makkouna-Lloyd (WPM) [16]. Regeneration abilities of microtendril investigated on MS with addition of regulators of growth by cytokinin (0,1 mg · l-1, 0,4 mg · l-1, 1,0 mg · l-1 of BAP, 2,0 mg · l-1 of a kinetin) and auxin (1,0 mg · l-1 of IVK and 0,1 mg · l-1 of NOC) action types. The indicator of acidity of the environment was brought to level 5,7-5,8. Plant material cultivated in the light room at a temperature of 25 ± 1 °C and lighting 2,0-3,0 with the 16-hour photoperiod and relative humidity of the air (GDP) of 70-75 %. Subcultivation of microtendril within the first two months was spent each 14-15 days, in future (depending on components of a nutrient medium) the cycle of cultivation made 30-90 days.

Plants - regenerants of P. abies were adapted for conditions of an open ground by a step way which included their endurance in the climatic camera on a substratum (4-5 days), and also cultivation in the conditions of the closed soil (28-30 days) and introduction to container culture (3-5 months). Plants landed to Phyto- capsula (volume of -200 cm3) in substrate: peat low-lying, sand river (1: 1); coconut substrate, perlite (1: 1); bark pine, charcoal, peat, sfagon moss (3: 2: 1: 1); cespitose soil. After adaptation and every 2-3 week a plant-regenerate fed with solution 1/2 macro - and MS microcells. As required plants were sprayed and watered. Containers with plant material maintained in controlled conditions of the adaptation room at a

temperature of 24 ± 2 °C and lighting 2,0-3,0 with the 16-hour photoperiod and GDP of 60-70 %.

Statistical processing of experimental data was carried out with use of a package of the analysis of MS Excel and by V. Schmidt's technique [13].

Results of researches.

Table 1 were made according to results of determination laboratory similarity of seeds a fir-tree European under the influence of preparations on the basis regulators of growth and development of plants.

1. Influence of preparations on the basis of regulators of growth and development of plants on laboratory similarity of seeds Picea abies

Option	Name of the product	Unit of measure	Concen-tration	Similarity, %	Option	Name of the product	Unit of measure	Concen-tration	Similarity, %
1	Humate +7	g/l	0,25	51,8±1,5	19	Ale	ml/l	1,5	48,1±1,7
2			0,5*	53,9±1,5	20			2,0	43,5±1,9
3			0,75	44,4±1,9	21	Amber acid	g/l	1,0	53,1±1,8
4			1,0	39,7±1,9	22			2,0*	57,8±1,2
5	Zircon	ml/l	0,15	25,8±2,7	23			3,0	57,1±1,2
6			0,3*	18,2±3,1	24			4,0	43,3±2,9
7			0,45	6,4±2,9	25	Kornevin	g/l	1,0	47,7±1,7
8			0,6	7,0±2,7	26			2,0*	52,8±1,6
9	Heteroauxin	g/l	0,5	51,5±2,4	27			3,0	58,1±1,0
10			0,1*	59,1±1,6	28			4,0	45,0±1,8
11			0,15	51,5±1,8	29	Kornevit	ml/l	3,5	50,0±3,9
12			0,2	42,6±3,1	30			7,0*	57,3±1,4
13	Epin	ml/l	0,15	57,5±1,7	31			10,5	50,0±4,0
14			0,3*	70,2±1,7	32			14,0	50,6±3,3
15			0,45	42,9±1,8	33	Reak	ml/l	1,25	50,5±2,1
16			0,6	31,4±2,7	34			2,5*	50,0±1,7
17	Ale	ml/l	0,5	50,5±3,4	35			3,75	50,0±2,5
18			1,0*	49,1±1,9	36			5,0	40,7±3,6
Control									53,7±1,2

* the concentration recommended by the producer.

With tab. 1 it is visible that the indicator of similarity three-year-old a seed on control makes 53,7 ± 1,2 %. High rates of similarity in comparison with control seeds killed, in preparations: Epin in concentration of 0,3 ml/l (70,2 ± 1,7 %), Kornevin - 3,0 ml/l (58,1 ± 1,0 %), heteroauxin- 0,1 g/l (59,1 ± 1,6 %), the Humate + 7 - 0,5 g/l (53,9 ± 1,5 %), Amber acid - 2,0 g/l (57,8 ± 1,2 %), Kornevit - 7,0 ml/l (57,3 ± 1,4 %). The smaller percent of similarity of seeds in comparison with control

was in the seeds killed in RRR solutions: Zircon, Ale and Reaky (in four various concentration) [11].

During research on selection of optimum acidity of a substratum we checked reliability of the indicators of acidity of substrata specified by producers with actual (option 1 -4,0; 2, 3 - 6,2; 4 - 6,0; 5 - 6,9; control - 6,2). We were established that in the majority of options the actual values don't go beyond specified on packing, only in option 2 acidity above the stated.

Table 2 were made according to the certain options of research to indicators of energy of germination, similarity of seeds and growth of seedlings.

2. Influence of acidity of a substrate on similarity of seeds and growth of seedlings a fir-tree European

Option of testing	pH-value	Energy of sprouts, %	Similarity, %	Mean height of seedlings after 30 days of growth, mm
1	4,0	41,0	56,7	21,9 ± 1,31
2	4,6	62,9	69,4	23,1 ± 1,21
3	6,2	66,2	75,7	24,2 ± 1,11
4	6,0	68,6	83,3	27,4 ± 1,90
5	6,9	52,4	64,3	22,1 ± 1,44
Control	6,2	81,0	83,3	26,7 ± 1,41

As a result of the conducted researches it is established that on control (soil from under bed curtains of fir-tree planting with pH-6,2) indicators of energy of germination (81,0 %) and similarity of seeds (83,3 %) are the highest in comparison with other options of experience. Rather higher percent of energy of germination of seeds recorded in options with use of substrata with acidity of pH-6,0 (68,6 %) and pH-6,2, (66,2 %); similarity indicators in these cases also high -83,3 % and 75,7 %. Very weak energy of germination of seeds and their viability were observed in strongly sour and neutral substrata of the first (41,0 %, 56,7 % are, less, than on control for 40,0 in compliance with 26,6 %) and the fifth (52,4 %, 64,3 %, are 28,6 controls less and in compliance with 19,0 %) options.

The best for growth of shoots of a fir-tree European were option 3 substrate (height of seedlings 24,2 ± 1,11 mm) - 4 (27,4 ± 1,90 mm) and control (26,7 ± 1,41

mm) with indicators of pH-6,0 and 6,2. The difference between heights of seedlings of these options and control is insignificant.

Negative influence on growth of seedlings was shown by a sour substratum (rn-4,0) of the 1st option, height of seedlings of this option made 21,9 ± 1,31 mm that is 17,9 % less concerning control.

Receiving the best indicators of similarity and growth of shoots on control with acidity of pH-6,2 in comparison with the 3rd option where acidity is similar, we consider it occurred at the expense of a mycorhiza which existence is usually characteristic for the flat ground of soils of fir-tree plantings.

So, as a result of the conducted researches it is picked up preparations on the basis of regulators of growth and development of plants, it is established their optimum concentration and the substrata accepted for cultivation P. abies which provide high rates of energy of germination of seeds, their similarity and growth of seedlings are defined.

We began microclonal propagation of plants of P. abies on sterilization of samples, for the reason that receiving aseptic viable plant material is problematic because of high contamination or allocation of secondary metabolites of an samples[1, 3, 5, 8, 16]. For this reason, were attracted a wide range of the sterilizing substances with a different exposition to achievement of an objective.

In table 3 were given received results according to options of sterilization samples of plants P. abies

3. Efficiency of sterilization samples of plants P. abies

Option	Sterilized Substance	Concentration, %	Exposition, min.	Input in culture *in vitro* samples, item.	Sterilization action, %
1	NaClO	2,5	10	30	41 ± 3
2	NaClO	2,5	20	30	23 ± 4
3	$AgNO_3$	1,0	10	30	37 ± 6
4	$AgNO_3$	1,0	20	30	57 ± 5

The highest percent (more than 90 %) of aseptic regeneration capable microtendril was observed at their endurance in 1 % - AgNO3 number within 10

min. with the subsequent transfer in 2,5 % - ny of NaClO of 15 min. (option 5, fig. 1, a).

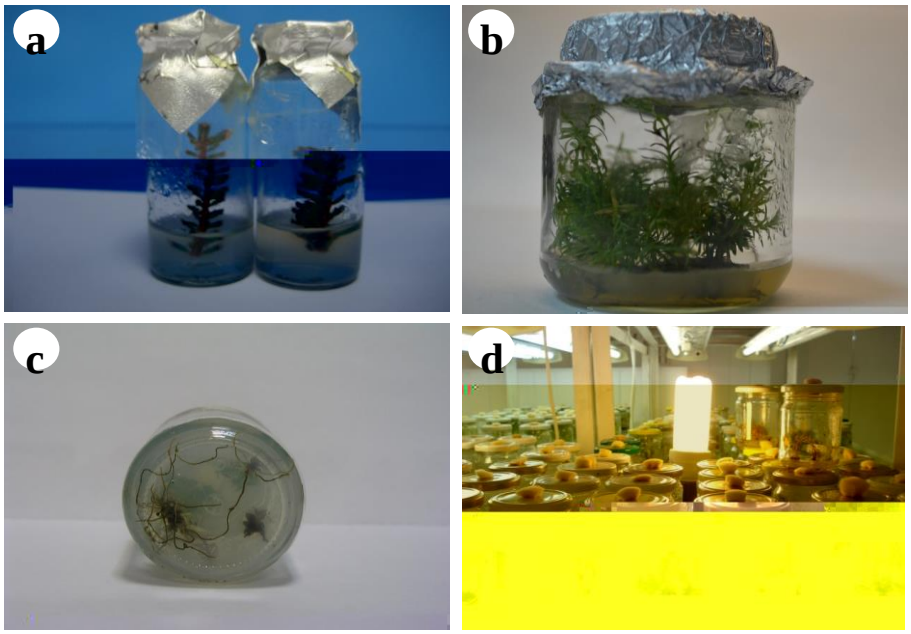

Fig. 1. Sequence of mikroklonalny reproduction of plants-regenerantov P. abies:

a − aseptic viable sample on a hormone-free nutrient medium of MS; b − plants-regenerantion 1/2 MS; c − root system of plants on MS with half concentration of macrosalts, an inozitola and glucose, with addition of 1,0 mg · l^{-1} of IVK and 0,1 mg BAP l^{-1}; d − large quantities multiplied by a graftage of stem culture and a direct morphogenetic of a plant in vitro.

It should be noted that for sterilization of samples of plants P. abies it is inexpedient to use 1, 2 and 3 options as in these procedures number of aseptic viable microescapes was insignificant (less than 50 %). Very low interest of efficiency of sterilization (23 %) fixed for use 2,5 % - NaClO foot a current 20 min. (option 2). At application of 1 % − AgNO3 foot throughout 20 min. (option 5) efficiency of sterilization of eksplantat made more than 50 % (see tab. 3)

It is known that management of processes of differentiation and a morphogenesis in culture of the isolated fabrics and bodies of plants in vitro happens by entering into a nutrient medium of exogenous regulators of growth - auxins, cytokinins and gibberellins [1, 3, 5]. Results of their influence on regeneration abilities plants microtendril of P. abies in vitro it is reflected in tab. 4.

4. Morphometric indicators of plants-regenerants of P. abies on nutrient mediums of various structure

Option	Structure of growing medium	Cycle duration of cultivation, days	Length of microtendril, cm	The number of rootage microtendril, %	:Rate of reproduction	Type of microclonal propagation
K[1]	MC hormone-free	30	2,5−3,0	90−100	1:2−1:4	a. g. m. e.[2]
1	MC with part concentration of solation, inositol and glucose 1,0 mg/l IOA, 0,1 mg/l BAP	90	1,2−1,9	90−100	1:5−1:10	a. g. m. e.
2	1/2 MC hormone-free	90	2,5−4,0	90−100	1:9−1:23	
3	MC 0,4 mg/l BAP 0,1 mg/l HOA, 20 mg/l of adenine	60 90	1,2−2,0 2,9−4,0	0 0	1:5−1:14 1:20−1:30	d. m.[3]
4	WPM 1,0 mg/l BAP, 2,0 mg/l of kinetin	60	0,5−1,0	0	1:8−1:10	d. m.

Notation. K[1] – Control; a. g. m. e.[2] − activation of growth meristems explants; d. m.[3] – direct morphogenesis

It is established that in control, and also in 1 and 2 options of nutrient mediums regeneration microtendril in vitro P. abies occurred by activation of growth of the available meristems of sample. Significant amount of plants-regenerants (coefficient of reproduction 1: 9-1: 23 at a cycle of cultivation of 90 days) it is received when using a hormone- free nutrient medium of 1/2 MS (fig. 1, b,c)

It should be noted that application of options 3 and 4 was caused intensive microtendril which occurred by a direct morphogenesis, 60 – 90-day cycle of cultivation. However, such options didn't stimulate regeneration of root system. It is shown that cultivation microtendril - within 90 days led P. abies in option 3 to significant increase in coefficient of reproduction microtendril (by 2,5 times) and their lengths (by 2,0 times) in comparison with 60-day endurance (distinctions are statistically significant on $\alpha = 0,05$).

So, for use of various types of microclonal propagation (activation of growth of meristems of sample, a direct morphogenesis) we received a significant amount of plants-regenerants of P. abies for short terms (fig. 1, d).

The final stage of microclonal propagation is adaptation of plants-regenerants to conditions of an open ground. In the course of adaptation of plants after culture of in vitro providing appropriate levels of food of plants is important: mineral, air, water and observance of gradual change of temperature and humidity the air of environment. Among them the substratum is important [1, 5]. Results of influence of structure of a substratum on efficiency of adaptation of plants-regenerants it is reflected in tab. 5.

5. Efficiency of adaptation of plants-regenerants of P. abies on substrate (duration of adaptation of 30 days)

Option	Structure of substrate	Efficiency of adaptation plants-regenerants , %
1	turfs, river sand (1:1)	50−60
2	river sand	20
3	coconut substrate, perlite (1:1)	70−80
4	bark pine, charcoal , turfs, sfagon moss (3:2:1:1)	90−100
5	cespitose soil	10

The analysis of experimental data testifies that use for adaptation of plants-regenerants P. abies of a unicomponent substrate (options 2 and 5) is inexpedient as received extremely small efficiency (doesn't exceed 20 %). A significant amount of the adapted plants-regenerantov (more than 90 %) received 4 substrate in option (fig. 2).

Fig. 2 Adapted for conditions of the closed soil P. abies plant-regenerants

To sum up, as a result of the conducted researches we were developed biotechnology of microclonal it is multiplied plants of P. abies and their adaptation to conditions in vivo which allowed to receive in a short time a significant amount of plants-regenerants for various target use.

Conclusions

1. It is optimized traditional and developed new (technology of microclonal propagation) ways of receiving landing material of a fir-tree European (Picea abies H Karst.).

2. It is established that it is expedient to carry out traditional cultivation of the landing material P. abies on subacidic substrate (pH-6,0 or pH-6,2). Seeds before crops needs to be soaked in solution of one of the following preparations: in 0,3 mg · l-1 Epin, 3,0 mg · l-1 Kornevin , 0,5 g · l-1 the Humate + 7, 0,1 g · l of-1 heteroauxin, 2,0 g · l-1 of Amber acid, 7,0 mg · l-1 to Kornevit an extent of 18 hours.

3. Conditions of sterilization of sample P. abies (serial excerpts in 1 % - AgNO3 number within 10 min. with the subsequent transfer in 2,5 % - ny of NaClO of 15 min.) from 90 % are defined - ache efficiency of receiving viable microtendril.

4. A significant amount of plants-regenerants of P. abies is received on a nutrient medium of 1/2 MS of a 90-day cycle of cultivation. An intensive direct morphogenesis in microtendril P. abies it is recorded on MS with addition 0,4 mg · in l-1 of BAP of 0,1 mg · in l-1 of NOC and 20 mg · in adenine l-1.

5. Optimum conditions of adaptation of plants-regenerants of P. abies are established to in vivo conditions (excerpts of regenerant an extent of 4-5 days at the increased humidity on a substrate which contains pine bark, charcoal, peat and a sfagon moss (3: 2: 1: 1), cultivation in the conditions of the closed soil within 28-30 days and introduction to container culture for 3-5 months)

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