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Use of preparation Lizoformin 3000 for obtaining aseptic cultures of honeysuckle in conditions in vitro

Zapolskyi Ya.[1], Medvedeva T.[2], Natalchuk T.[3], Bublyk M.[4]

Institute of gardening of NAAS, Sadova Str., 23, Kyiv, 03027, Ukraine; e-mail: [1]ya.zapolskyi91@gmail.com, [2]medvedevatv@ukr.net, [3]tania87@meta.ua, [4]mbublyk@ukr.net

The purpose. To investigate influence of preparation Lizoformin 3000 against pathogenic microflora at obtaining aseptic cultures of honeysuckle (Lonicera edulis Turcz) and on the further regeneration of plants depending on method of sterilization and state of initial plants. **Methods.** Laboratory, mathematical, calculation-comparative. **Results.** Influence of sterilizing substances on obtaining aseptic cultures of honeysuckle in conditions in vitro is studied. Experiment were spent on grades: Alisia, Spokusa, Chaika, Nimfa, Dochka Veletnia, Karina. As sterilizing agents they used Lizoformin 3000 at different time of sterilization and solution of mercury bichloride as the control. Stages of sterilization on the control included the following components: 1) treatment of explants in solution of sodium hipochlorite — 20 min with subsequent washing in water; 2) sterilization with spirit (C_2H_5OH) — 20 sec with washing in water; 3) sterilization in solution of mercury bichloride ($HgCl_2$) — 2 min with 3-times washing in sterile distilled water. In the variant with studying preparation Lizoformin 3000 instead of solution of mercury bichloride they used the studied preparation at expositions of 5, 7 and 10 min. **Conclusions.** For the maximal obtaining of sterile and viable explants the sterilizing agent and its toxicity has great value. Preparation Lizoformin 3000 at corresponding concentration and duration of sterilization should be recommended for obtaining aseptic cultures of honeysuckle. On the background of application of that preparation with exposition of 5 min they allocated by efficiency of regeneration 3 groups of varieties of honeysuckle: with high reclaiming ability (94 – 96%) — Alisia, Karina and Spokusa; with average reclaiming ability (86 – 87%) — Chaika and Dochka Veletnia, and with low reclaiming ability (80%) — Nimfa. Preparation Lizoformin 3000 in concentration of 3% and with duration of exposition of 5 min provides optimum efficiency of sterilization and regeneration of explants of honeysuckles and does not reduce their factors of duplication. Preparation Lizoformin 3000 at corresponding concentration and duration of sterilization should be recommended for obtaining aseptic cultures of varieties of honeysuckle.

Key words: *honeysuckle, sterilization, Lizo-formin, explant, in vitro, introduction in culture, proliferation.*

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The *in vitro* culture (plant microclonal propagation) is a promise method which makes it possible to obtain high quality planting stock of the fruit and small fruit crops in great quantities during short terms on not large areas and irrespective of the weather conditions. The technology for the microclonal propagation of some crop includes four main stages – introduction of the initial form into the sterile culture, propagation properly, rooting of the propagated microshoots and their adaptation to the growing in the ground.

The introduction into culture is one the major stages which brings about great costs and losses. In order to carry out this stage most successfully it is necessary to choose the phase of the plant active physiological development and corresponding sterilizing agents. When choosing them it should proceed from its toxicity and influence on the further development of the plant on the whole. At present mercuric preparations remain most effective but toxicity depresses the further microplants development [1]. For instance, when using 0.10% $HgCl_2$ solution for obtain the aseptic culture of the honeysuckle cvsChyelyabinka and Duet the regeneration from the primary explants was 65.9% and 64.9% respectively [2]. The increase of the concentration to 0.15% provided the sterile explants yield at a level of 95%. 67.5% them later on renerated [3]. When utilizing 0.2% of $HgSO_4$ the proliferation of 54.43% of explants was observed, 4.57% of them did not develop at all [4]. Lizoformin3000 was applied

successfully for the sterilization of the explants of 20 cultivars, 14 species and 13 genii of the ornamental and fruit and small fruit crops which belong to 3 families [5]. Other sterilizing agents (calcium hypochlorite, sodium hypochlorite, hydrogen peroxide) did not ensure the sufficient sterile explants yield, therefore the search of effective and less toxical sterilizing agents for obtaining the aseptic culture is an actual problem.

The purpose was to explore the preparation Lizoformin 3000 influence on the pathogenic microflora when obtaining the studied crop aseptic culture and on the further plants regeneration depending on the sterilization method and initial plants state.

Materials and methods. The experiments were carried out in the Department of Virology, sanitation and Propagation of Fruit and Small Fruit Crops of the Institute of Horticulture of the National Academy of Agrarian Sciences of Ukraine in 2016-2017. For the sterilization the explants of the following *L. edulis*Turcz. Cultivars were used: Alicia, Spokusa, Chaika (Ukraine), Karina (Poland), Doch'Vyelikana, Nympha (Russia). The selection of hardwood shoots with dormant buds was conducted early in spring (February-March) [6,7,8]. Since for honeysuckle mixed buds are characteristic for the sterilization vegetive buds 5-10 mm were selected from each genotype after blossoming of the shoots germinated under the controlled conditions. The sterilization was carried out utilizing the sodium hypochlorite, spirit, mercuric bichloride solution (control) and the preparation Lizoformin 3000. The latest has bacterial (including, sporocidal), virocidal and fungicidal effect. The agent preserves its qualities even after freezing and further thawing and according to the toxicity parameters belongs to the third grade of the moderately dangerous substances.[9] The first sterilization variant included the following stages: 1) the explants treatment in the sodiumhypochlorite solution for 20 minutes with the subsequent washing in water; 2) sterilization with spirit (C_2H_5OH) for 20 seconds with washing in water; 3) sterilization in the mercuric bichloride solution ($HgCl_2$) for 2 min. with 3 fold washing with sterile distilled water. In the second variant the mercuric hipochloride solution was replaced with that of lizoformin and the sterilization was conducted for 5, 7 and 10 min. the explants were planted on the Murashige-Scoog medium.[10] which contained 0.5 mg/l 6-benzylaminopuryn (BAP).

The sterilization procedures efficiency was determined on the 21st day of the explants cultivation. During that time the aseptic explants began to develop and the infected ones were discarded.

Results. The researches showed that the sterilization efficiency was high (table 1). In the control variant and in those where Lizoformin 3000 was used with the 7 and 10 minutes exposition the efficiency was 100% but when utilizing the same preparation with the 5 minutes exposition (the cultivars Chaika, Doch'Vyelikana and Nympha) the full sterilization was not achieved although it exceeded 90%.

Table1. Sterilizing agents effect on the efficiency of the honeysuckle explants sterilization and regeneration, %

Cultivar	0,1% $HgCl_2$– (control)		Lizoformin 3000						For cultivars regeneration efficiency, LCD_{05} = 2,7
			Sterilization duration,min						
			5		7		10		
	Sterilization efficiency	**Regeneration efficiency**	Sterilization efficiency	**Regeneration efficiency**	Sterilization efficiency	**Regeneration efficiency**	Sterilization efficiency	**Regeneration efficiency**	
Karina	100	**69**	100	**95**	100	**97**	100	**95**	
Alicia	100	**67**	100	**96**	100	**95**	100	**92**	
Spokusa	100	**61**	100	**94**	100	**91**	100	**87**	
Chaika	100	**63**	95	**87**	100	**88**	100	**86**	
Doch'Vyelikana	100	**59**	90,8	**86**	100	**76**	100	**73**	
Nympha	100	**56**	93,6	**80**	100	**78**	100	**74**	
For regeneration efficiency (under the experiments), LCD_{05}= 2,1									

The vital explants amount varied considerably depending on cv and duration of the sterilization procedures. In the control treatment this indicator fluctuated from 56% to 69% and was determined by a pomological cultivar, where as when applying Lizoformin 3000 it was much higher. This comparison gives the reasons to conclude that the use of the mercuric bichloride solution as a sterilizing agent is unacceptable not only because of its toxicity but also due to its negative influence on the regeneration process.

In the variant where Lizoformin 3000 was utilized with the 5 minutes exposition three groups of cvs were singled out concerning the regeneration capability (94-96%) – Alicia, Karina and Spokusa; average (86-87%) – Chaika and Doch'Vyelikana and low (80%) – Nympha.

When applying Lizoformin 3000 with the 7 and 10 min. expositions the cultivars peculiarities of the preserved on the whole – the cultivars Alicia and Karina distinguished themselves with the high regeneration capability while Doch'Vyelikana and Nympha with the low one.

The analysis of the sterilization duration with Lizofomin 3000 showed that its increase from five to seven minutes did not cause the substantial plants regeneration capability rise (cvs Alicia, Karina, Chaika, Nympha) or even decreased much this index (Spokusa, Doch'Vyelikana,). The further reduction of the regeneration capability of most of the cultivars was observed in the treatment where the sterilization duration with Lizoformin 3000 was 10 minutes.

So the variant with the least Lizoformin 3000 sterilization duration (5 minutes) should be recognized as the best one. However, sterilizing agent and their usage duration influenced not only the efficiency of the honeysuckle explants sterilization and regeneration but also the propagation coefficients of the latest ones (table 2). The investigations showed that the Lizoformin 3000 utilization for the sterilization increased considerably the propagation coefficient of the explants of all studied cvs in comparison with 0.1% of $HgCl_2$. For example, in the treatment with the Lizoformin 3000 application where the exposition longed 5 minutes on the average among the cultivars this index rose more than by 40%.

It should be noted that the explants propagation coefficients depended on a pomological cultivars: Karina had this indicator as the highest one (3.45), Doch'Vyelikana as the lowest (2.04). Other cvs had it as inter mediate, the cultivars of this group not having essential differences concerning that index.

Table 2. Coefficients of the honeysuckle cultivars explants propagation *in vitro*

Cultivar	0,1% of $HgCl_2$-(control)	Lizoformin 3000			under the cultivars LCD_{05} = 0.24
		Sterilization duration, minutes			
		5	7	10	
Karina	2,46	3,45	3,36	3,06	
Alicia	1,58	2,34	2,17	2,02	
Spokusa	1,92	2,58	2,21	2,1	
Chaika	1,66	2,47	2,02	1,99	
Doch'Vyelikana	1,5	2,04	1,93	1,87	
Nympha	1,66	2,38	1,99	1,89	
Under the experiment variants, LCD_{05}= 0.30					

The increase of the Lizoformin 3000 exposition also effected the explants propagation coefficient to a certain degree. For instance, in the variant with the 10 minutes exposition as compared to that where the exposition longed 5 minutes all cvs, except Doch'Vyelikana, had this indicator as much lower. When using the 5 minutes exposition the results did not appear so synonymous – the explants propagation coefficient of Spokusa, Chaika and Nympha decreased substantially, and the other cultivars had a tendency of this index decreasing.

Thus regarding the propagation coefficient it is Lizoformin 3000 utilizing with 5 minutes exposition should be recognized as the most acceptable treatment.

Conclusions

Our explorations have showed that the preparation Lizoformin 3000 in a concentration of 3% and with a exposition duration of 5 minutes provides the optimum efficiency of the honeysuckle explants sterilization and regeneration and does not reduce their propagation coefficients.

On the background of the Lizoformin 3000 application with the above mentioned exposition concentration concerning the regeneration efficiency studied honeysuckle cvs were divided into three groups: with the high regeneration capability (94-96%) – Alicia, Karina and Spokusa; with the average one (86-87%) – Chaika and Doch'Vyelikana; with low (80%) – Nympha.

Taking into consideration that the honeysuckle cultivars explants reaction to Lizoformin 3000 is different the researches should be continued on its new perspective cvs.

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