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CYDONIA OBLONGA PLANT-REGENERANTS ACCLIMATIZATION UNDER CONDITIONS *EX VITRO,* THEIR ADAPTATION IN THE HOTHOUSE INSULATOR

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The optimal acclimatization conditions of Cydonia oblonga plant-regenerants that cause increase in graft level from 82,5 % (IC 2-10, IC 4-12 and Sido) to 95,0 % (quince Anzherska) were defined. The incentive effect of boric acid and potassium permanganate on Cydonia oblonga plant-regenerants acclimatization in the hothouse insulator was revealed.

Key words: *Cydonia oblonga, clonal micro-reproduction process, adaptation, acclimatization to conditions ex vitro*

Reproduced plants adaptation to unsterile conditions is the final stage of clonal micro-reproduction. The possibility to enroot fruit plants under conditions *ex vitro* evokes significant interest in the branch of agricultural biotechnology. It is connected with clonal micro-reproduction process acceleration and reduction of expenses, providing the sanitized planting material is received. The absence of reliable adaptation technology can bring all the effort, spent on reproduction in culture *in vitro,* to nothing. Dying of plants after transplanting can be connected with mild transpiration control by microplants themselves and organotrophic way of nutrition [8, 9, 10].

Plant adaptation to unsterile conditions depends on culture, growth regulators, the time plants are put into conditions *in vivo,* way plants enroot, environment composition, way of leaves and roots adaptation [1].

During conditions change from *in vitro* to *in vivo* the plants are susceptible to stress, result of the entire succession of microclonal plants anatomico-physiological particularities: neuter wax cuticle of leaves, passive stomatal mechanism, low photosynthetic activity, vitrifaction and imperfect vascular link between the root and the shoot, imperfect or absent root hair that complicates water absorption and nutrition elements transportation [3, 4]. Such peculiarities of microclone composition and cultivation conditions *in vitro* stipulate high transpiration level of leaves and as a result their dehydration and fading [4, 6].

The strict control of air, temperature, water and nourishing regimen is needed during acclimatization of plants cultivated under conditions *in vitro.* Mineral nutrition is the most important element, which comes to fulfillment of plastic, electrochemical, osmotic and catalytic functions. For professional gardening it is important to support the necessary pH level and optimal amount of nutriments in substrata on turf base [7].

There are considerable amount of schemes on microplants transfer into unsterile conditions. For efficient plant adaptation special equipment which maintains the set humidity and temperature, aeration degree of soil substratum is used in modern laboratories.

Research materials and methods. The research was conducted during 2013-2014 years in the laboratory of biotechnology and reproduction of fruit cultures in the hothouse complex of the Ukrainian scientific-research station of plant quarantine IPP NAAS.

The research objects were the *Cydonia oblonga* vegetative wildings, Anzherska in particular, MC, BA 29, Sido and IC 4-6, IC 4-12, IC 2-10.

The following schemes were used for *Cydonia oblonga* microclonal plants acclimatization to conditions *ex vitro* [5]:

1. *Cydonia oblonga* plant-regenerants transplanting into unsterile substratum with mix of turf, sand, soil and perlite in proportion of 1:1:1:1, moistened by mineral salts solution of Murashige-Skoog nourishing environment without vitamins, growth regulators and sucrose – control.

2. *Cydonia oblonga* plant-regenerants transplanting into sterile substratum with mix of turf, sand, soil and perlite in proportion of 1:1:1:1, moistened by mineral salts solution of Murashige-Skoog (MS) nourishing environment without vitamins, growth regulators and sucrose.

3. *Cydonia oblonga* plant-regenerants transplanting into double-layer substratum with filling the 2/3 of hole capacity by unsterile substratum with mix of turf, sand and soil in proportion of 1:1:1. A third left was filled with sterile perlite and moistened by mineral salts solution of MS nourishing environment without vitamins, growth regulators and sucrose. Plant roots were thoroughly cleaned from remains of nourishing environment in potassium permanganate solution. Plants were transplanted into substratum so the roots did not touch the soil substratum.

Transplanted *Cydonia oblonga* plants were covered by glass caps. Plant acclimatization to conditions *ex vitro* went on for two months. The first month we maintained the temperature of 23±2 °C, relative humidity (RH) – 90 %, illumination 2,0 klx and photoperiod 16 hours. Second month – RH 50%, temperature, illumination and photoperiod the same as during the first.

Adaptation of mericlonal plants to cultivation conditions *in vivo* was conducted in the hothouse insulators. Acclimatized plant-regenerants were transplanted onto perlite which once in seven days was moistened by mineral salts solution of MS nourishing environment without vitamins, growth regulators and sucrose, previously dissolved in 10 times. Before transplanting the plants were treated by 0,01% solutions of boric acid, potassium permanganate and Peroxide M agro. In order to prevent dying of plants due to high transpiration level they were sprinkled with water every day.

Mathematical planning and statistic processing of received experimental data was conducted using methods of Dospehov, 1985 [2].

Research results. In order to receive planting material of good quality, suitable for transplanting into conditions of open soil, it is necessary to develop methods of regenerants acclimatization to soil conditions. The quince plants are better cultivated on hoed, aired and sufficiently moistened soils. That is why, while selecting substratum we took into account certain agro physical characteristics, namely porosity of composition and high hygroscopicity, which stipulates their influence on mechanical structure of soil. In such case the gradual nutrition reorganization of transplanted plant to the side of autotrophy took place, since environment, surrounding roots, has similar salt content, but does not have exogenous sugars. As long as perlite does not contain organic fertilizer that decays it makes roots lesion impossible and so the mushroom microflora development round roots before their transplanting into unsterile substratum did not take place. The roots developed thick and resilient, light brown with side offshoots. Besides that we observed growth enhancement of overground shoots and leaf surface enlargement in comparison with the control (fig.1.1).

The effectiveness of using one-layer substratum for *Cydonia oblonga* acclimatization varied from 35,0 % (quince Sido) to 62,0 % (quince BA 29). For quince BA 29 the graft level of plant-regenerants under these conditions was at the level 62,5 %, IC 4-12 – 57,5 %, IC 4-6 – 55,0 %, IC 2-10 – 52,5% and quince MC – 44,0 %.

Acclimatization process caused increase of the graft level of plant-regenerants from 82,5 % (IC 2-10, IC 4-12 and Sido) to 95,0 % (quince Anzherska), as long as we use double-layer substratum. For plant-regenerants of quince BA 29 this index was 92,5%, quince MC, IC 4-6 – 85,0 % (fig.1.2).

Fig. 1.1 *Cydonia oblonga* plant-regenerants acclimatization under conditions *ex vitro*

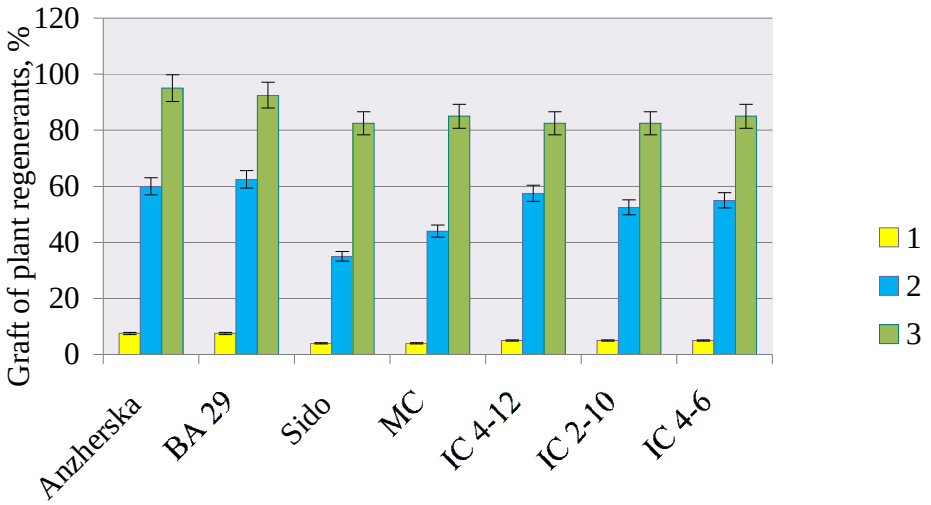

Fig.1.2 *Cydonia oblonga* plant-regenerants acclimatization effect under conditions *ex vitro*

Note: - unsterile substratum (control), - one-layer, - double-layer

High humidity and substratum dampness create perfect conditions for development of mushroom infection that causes dying of transplanted plants.

In order to prevent mushroom infections and to increase the graft level of plant-regenerants to natural cultivation conditions we treated *Cydonia oblonga* plant-regenerants with 0,01% antiseptic solutions (table 1).

The received results confirm the effectiveness of *Cydonia oblonga* plants treatment with the solutions of boric acid and potassium permanganate. The effectiveness of applying $KMnO_4$ as antiseptic was from 95 % (quince Anzherska) to 60 % (quince Sido).

The percentage of adapted plants in case of applying antiseptic HBO_3 varied from 90 % (quince Anzherska, BA 29) to 50 % (quince Sido).

Negative result was received by plant treatment with 0,01% Peroxide M agro solution. The percentage of adapted plants in given case was 15 % (quince Anzherska, BA 29) and 5 % for other *Cydonia oblonga* forms, besides quince Sido plant-regenerants for which this preparation turned out to be ineffective.

1. Antiseptics influence upon *Cydonia oblonga* regenerants adaptation process to conditions *in vivo*

Vegetative wilding of *Cydonia oblonga*	Plants adapted, %			
	Antiseptics solution , 0,01%			
	Control	KMn O_4	HBO_3	Peroxide M agro
1	2	3	4	5
Anzherska	20,0±0,6	95,0±2,8	90,0±2,7	15,0±0,6
BA 29	20,0±0,6	90,0±2,6	90,0±2,8	15,0±0,5
MC	10,0±0,4	65,0±2,0	60,0±1,9	5,0±0,3
Sido	5,0±0,2	60,0±0,5	50,0±1,6	0
Quince 4-6	15,0±0,5	85,0±2,7	80,0±2,5	5,0±0,2
Quince 2-10	15,0±0,6	80,0±2,5	80,0±2,6	5,0±0,3
Quince 4-12	15,0±0,5	90,0±2,7	85,0±2,7	5,0±0,2

Sprinkled with the solutions of boric acid and potassium permanganate in the process of adaptation the plant-regenerants were protected from mushroom infection and had positive impact on their growth.

Conclusion

Applying double-layer substratum on acclimatization stage *ex vitro* initiates graft process of plant-regenerants from 82,5 % (IC 2-10, IC 4-12 and Sido) to 95 % (quince Anzherska). Moreover, the incentive effect of boric acid and potassium permanganate on *Cydonia oblonga* plant-regenerants adaptation process in the hothouse insulator was detected.

LITERATURE

1. Деменко В. И. Адаптация растений, полученных *in vitro* к нестерильным условиям / В. И. Деменко, В. А. Лебедев // Известия ТСХА, 2011. – Вып. 1. – С. 60–69.

2. Доспехов Б. А. Методика полевого опыта / Б. А. Доспехов // Агропромиздат, 1985. – 351 с.

3. Калинин Ф. Л. Методы культуры тканей в биохимии и физиологии растений / Калинин Ф. Л., Сарнацкая В. В., Полищук В. Е. – К. : Наук. думка, 1980. – 488 с.

4. Медведєва Т. В. Проблеми акліматизації культивованих рослин / Т. В. Медведєва // Физиология и биохимия культурных растений. – 2008. – 40, № 4. – С. 299–300.

5. Патент 2366153 Российская Федерация, МПК7, A01G1/00, Способ доращивания *in vitro* растений ягодных и декоративных кустарников в нестерильных условиях / Аладина О. Н., Акимова С. В., Ковалева И. В.; заявитель и патентообладатель ФГОУ ВПО РГАУ – МСХА им. К. А. Тимирязева; заявл. 2004 ; опубл. 2006.

6. Сковородников Д. Н. Адаптация полученных *in vitro* растений малины к нестерильным условиям / Д. Н. Сковородников, И. А. Райков, Д. Н. Челяев // Вестник Орел ГАУ. – № 2. – 2012. – С. 70–72.

7. Ташматова Л. В. Укоренение и адаптация груши в условиях *in vitro* [Электронный ресурс] / Л. В. Ташматова // Современное садоводство. – 2013. – № 1. – Режим доступа : http://vniispk.ru/ news/zhurnal/article. php 1. – Название с экрана.

8. Orlikowska T. Propagation of quince *S 1 (Cydonia blonga* Mill.) *in vitro* / T. Orlikowska // Fruit Sc. Rep. Skierniewice. – 1988. – T. 15, №4. – P. 157–165.

9. Pierik R. *In vitro* culture of higher plants /R. Pierik. – 1987. – V. 5. – 344 p.

10. Pocock S. Procedures and problems associated with the transfer of tissue-cultured plants / S. Pocock // Intern. Plant Propagators Soc, – 1984. – 33. – P. 316–320.

АКЛІМАТИЗАЦІЯ РОСЛИН-РЕГЕНЕРАНТІВ АЙВИ ДОВГАСТОЇ В УМОВАХ *EX VITRO* ТА ЇХ ПОДАЛЬША АДАПТАЦІЯ В ТЕПЛИЧНИХ ІЗОЛЯТОРАХ

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Визначено оптимальні умови акліматизації рослин-регенерантів айви довгастої, що спричинюють підвищення рівня їх приживлюваності від 82,5 (ІС 2-10, ІС 4-12 та Sido) до 95,0 % (айва Анжерська). Виявлено стимулювальний ефект розчинів борної кислоти та перманганату калію на процес адаптації рослин-регенерантів айви довгастої в тепличних ізоляторах.

Ключові слова: *Cydonia oblonga, процес клонального мікророзмноження, адаптація, акліматизація в умовах ex vitro*

АККЛИМАТИЗАЦИИ РАСТЕНИЙ-РЕГЕНЕРАНТОВ АЙВЫ ПРОДОЛГОВАТЫЕ В УСЛОВИЯХ EX VITRO И ИХ ДАЛЬНЕЙШЕЙ АДАПТАЦИИ В ТЕПЛИЧНЫХ ИЗОЛЯТОРАХ

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Определены оптимальные условия акклиматизации растений-регенерантов айвы продолговатой, которые вызывают повышение уровня их приживаемости от 82,5 (ИС 2-10, ИС 4-12 и Sudo) до 95,0% (айва Анжерская). Выявлено стимулирующий эффект растворов борной кислоты и перманганата калия на процесс адаптации растений-регенерантов айвы продолговатой в тепличных изоляторах.

Ключевые слова: *Cydonia oblonga, процесс клонального микроразмножения, адаптация, акклиматизация в условиях ex vitro*