CROP PRODUCTION

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RESULTS OF PRACTICAL APPLICATION OF COCONUT SUBSTRATE AT THE STAGE OF ADAPTATION OF MICROPLANTS

Research article

Abstract

The transfer of plants from in vitro to ex vitro conditions is the final and most stressful stage for microplants, which determines the success of the whole work. At this stage, up to 80% of the planting material often dies, since in vitro plants must adapt to non-sterile ex vitro conditions, which differ significantly from in vitro cultivation conditions (high humidity, carbon dioxide deficiency). The article presents the results of a study of the influence of coconut substrate on the morphological development of microplants of clonal rootstocks of fruit crops obtained by micropropagation. The objects of the study were clonal stocks of American and European selection used for laying intensive and super-intensive orchards – MaxMa14 (cherry), GF677 and Garnem15 (almond, peach, nectarine), Myrobalan 29C (plum, cherry-plum, apricot), OHF87 (pear, quince), Madrasa and Bayan Shirey (local Azerbaijani varieties of grapes). As a result of the study, it was found that, in general, the coconut substrate had a positive effect on the morphological development of regenerated plants. The substrate contributed to the intensification of the growth processes of the aerial and root parts of plants. Rootstock Garnem 15 showed the highest percentage of adapted plants (86,4%). Rootstock GF 677 was distinguished by a good stem formation of 3,8 cm and the longest roots of 3,54 cm. A largest number of formed roots was noted in MaxMa14 regenerated plants – 4,3 pcs.

Keywords: microplants, adaptation, ex vitro conditions, coco substrate.

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РЕЗУЛЬТАТЫ ПРАКТИЧЕСКОГО ПРИМЕНЕНИЯ СУБСТРАТА КОКОСА НА ЭТАПЕ АДАПТАЦИИ МИКРОРАСТЕНИЙ

Научная статья

Аннотация

Перенос растений из условий in vitro в ex vitro является заключительным и наиболее напряженным этапом для микрорастений, от которого зависит успех всей работы. На этом этапе часто погибает до 80% посадочного материала, поскольку растения in vitro должны адаптироваться к нестерильным условиям ex vitro, которые значительно отличаются от условий выращивания in vitro (высокая влажность, дефицит углекислого газа). В статье представлены результаты исследования влияния субстрата кокоса на морфологическое развитие микрорастений клоновых подвоев плодовых культур, полученных путем микроразмножения. Объектами исследования являлись клоновые подвои американской и европейской селекции, используемые для посадки интенсивных и сверхинтенсивных садов – MaxMa14 (вишня), GF677 и Garnem15 (миндаль, персик, нектарин), Myrobalan 29C (слива, алыча, абрикос), OHF87 (груша, айва), Madrasa и Bayan Shirey (местные азербайджанские сорта винограда). В результате исследования было установлено, что субстрат кокоса в целом оказал положительное воздействие на морфологическое развитие регенерированных растений. Субстрат способствовал интенсификации процессов роста воздушной и корневой части растений. Rootstock Garnem 15 показал самый высокий процент адаптированных растений (86,4%). Rootstock GF 677 отличился хорошим формированием стебля в 3,8 см и наиболее длинными корнями в 3,54 см. Наибольшее количество сформированных корней было отмечено у регенерированных растений MaxMa14 – 4,3 шт.

Ключевые слова: микрорастения, адаптация, условия ex vitro, субстрат кокоса.

1. Introduction

Roots formed in in vitro culture differ anatomically from ex vitro roots [2, P. 101–109]. They contain a low content of lignin, the surface of the roots and their apexes are densely covered with hairs. At this stage, the formation of numerous callus cells is likely. In the process of in vitro adaptation, the roots lose hairs and stop growing. Such roots grow rapidly and on ex vitro condition developed new roots that are able to absorb nutrients from the soil. New roots have a wide suction zone, a large number of hairs, and callus cells between the hairs are absent. However, these roots cannot provide the plant with all the necessary water, and therefore, increased humidity is created during the adaptation period [1, P. 133–129], [3, P. 1424–1427].

For leaves formed in culture in vitro, a weak development of the cuticle, epicuticular wax is characteristic. The conducting vessels of the xylem are poorly developed, and there are no transpiration flows that provide plants with water. The stomatal apparatus does not function under in vitro conditions. All this is a specific reaction of the plant organism to the conditions of excessive moisture in the cultural vessel [1, P. 133–139], [4, P. 409–412], [5, P. 480]. After transplanting into the soil, due to a decrease in atmospheric humidity, systems for maintaining turgor and the functioning of stomata are being established in the plant. Here, survival primarily depends on the ability of the plant to withstand low humidity. Since the leaf blades of test-tube plants lack an epicuticular wax layer, they are subject to very rapid dehydration when transplanted from in vitro to ex vitro conditions, which leads to the death of adaptable plants.

Under test tube conditions, photosynthetic activity is limited, nutrition is photomixotrophic in nature (due to sucrose in the nutrient medium). In the process of adaptation to ex vitro conditions, changes occur in the photosynthetic activity of the leaf, in particular, in the content of chlorophylls, an increase in the content of which in the leaves of microplants improves their survival during adaptation [6, P. 629–631].

In connection with the morphological and physiological characteristics of microplants after in vitro culture, for successful adaptation in non-sterile conditions, a number of additional measures are recommended to improve the process of plant adaptation and increase their survival rate in non-sterile conditions [2, P. 101–109], [3, P. 1424–1427], [5, P. 480], [7, P. 569–575]: when preparing plants for transfer to the soil to increases the illumination to 10,000 lx; in the last stages before planting, to place the plants on a medium without growth regulators for a period of 3 weeks; to create conditions of high humidity during adaptation, with subsequent opening of the lid over the adaptable regenerants; to open test tubes for several days, treat plants with fungicides (0,2% benlate solution), to create an artificial dormant period for plants for 40–60 days before planting in non-sterile conditions, to keeping them in a refrigerator at a temperature of +5...+ 6ºC. After forced dormancy, plants transferred to the soil in culture chambers begin intensive growth, actively rebuilding the transpiration system, becoming less dependent on pathogenic microflora.

There are different opinions about the influence of the degree of root development on adaptation. In some studies [5, P. 480], the survival rate increased when plants adapted, the root system of which consisted of 3–4 roots 4–7 cm long with lateral branches, other authors [10, P. 320–323] noted that the roots were was longer than 1–2 cm are damaged when transplanted into pots.

There is also no unequivocal answer to the question of the optimal period of ex vitro adaptation for rootstocks of stone fruit crops. Some researchers [6, P. 629–631] recommend that this work be carried out in the spring (at the end of February, and preferably in March–beginning of April). Others [4, P. 409–412] recommend the spring–summer period (March–June), when the adaptation time was reduced to 4–5 weeks (in the autumn–winter period, woody plants underwent adaptation for a longer period of 6–7 weeks).

Commonly used substrates for transferring plants to ex vitro conditions [12, P. 402–419], [13, P. 22–28], [14, P. 128–131], [15, P. 35–39] are sterile mixtures of bottom peat and river sand or perlite in a ratio of 3:1 or 1:1, as well as substrates such as peat substrate (a mixture of peat and perlite in a ratio of 5:1), agroperlite (a product of grinding and heat treatment of volcanic rock) and coconut substrate (dried coconut peel).

2. Materials and methods

The objects of the study were non-rooted microplants of clonal rootstocks of fruit crops of American and European selection (Myrobalan 29C, Maxma14, GF677, Garnem15) and local grape varieties (Madrasa and Bayan Shirey), obtained in vitro by introducing segments of non-lignified shoot segments of 1,5–2,0 cm with one apical or axillary bud, as well as coconut substrate. The main purpose of this study was to study the adaptation of non-rooted plants in vitro to ex vitro conditions on coco substrate.

In our studies, the adaptation of regenerated plants to ex vitro conditions took place in four stages:

- adaptation in laboratory conditions;
- adaptation in greenhouse conditions;
- adaptation under the shadow grid;
- adaptation in open ground conditions.

Primary adaptation of plants was carried out in a culture room with illumination of 2500–3000 lux, temperature +21...+25ºC and photoperiod of 16/8 hours. Microplants were planted in mini-greenhouses with an interval of at least 3 cm or in plastic cassettes with cells (Figure 1). Cocopit (coconut substrate) was used as a substrate in the process of primary adaptation.

The cassettes with plants were covered with a transparent plastic cover, creating conditions of high humidity until they started to grow. A week later, the lid was slightly opened, gradually lowering the humidity and accustoming the plants to the conditions of the culture room.

Assessing the impact of the coconut substrate on the development of plants, biometric measurements of the following morphological parameters of plants were carried out:

1) percentage adapted of regenerated plants (%);
2) length of stem (cm);

3) length of roots (cm), the number of roots (pcs).
The experiments were carried out in 3-fold repeat.

Fig. 1 – Bayan Shirey grape variety at the stage of ex vitro adaptation:
A – immediately after planting, *B* – 2 weeks after planting

3. Results and discussion

As a result of the research, an assessment was made of the adaptation of micro plants of clonal rootstocks of fruit crops and grape varieties in ex vitro condition (Table 1).

Table 1 – Morphological indicators of rootstock development at the stage of adaptation

Variety	Index	
MaxMa14	Percentage adapted of the plants, %	85,0±0,21
	Average length of the stem, cm	3,5±0,39
	Average length of the root system, cm	2,54±0,05
	Average number of the roots, pcs	4,3±0,2
Myrobalan 29C	Percentage adapted of the plants, %	78,3±0,75
	Average length of the stem, cm	2,9±0,65
	Average length of the root system, cm	2,06±0,08
	Average number of the roots, pcs	3,5±0,2
GF 677	Percentage adapted of the plants, %	72,5±0,28
	Average length of the stem, cm	3,8±0,31
	Average length of the root system, cm	3,54±0,13
	Average number of the roots, pcs	3,8±0,1
Garnem 15	Percentage adapted of the plants, %	86,4±0,23
	Average length of the stem, cm	2,8±0,43
	Average length of the root system, cm	3,1±0,09
	Average number of the roots, pcs	4,0±0,12
OHF 87	Percentage adapted of the plants, %	75,0±0,26
	Average length of the stem, cm	2,6±0,31
	Average length of the root system, cm	2,68±0,11
	Average number of the roots, pcs	2,9±0,08
Madrasa	Percentage adapted of the plants, %	55,0±0,22
	Average length of the stem, cm	2,2±0,36
	Average length of the root system, cm	2,7±0,09
	Average number of the roots, pcs	3,7±0,05
Bayan Shirey	Percentage adapted of the plants, %	63,0±0,45
	Average length of the stem, cm	2,4±0,21
	Average length of the root system, cm	3,4±0,13
	Average number of the roots, pcs	3,94±0,12

In general, the coco substrate had a positive effect on the morphological development of all regenerated plants (Table 1). The substrate contributed to the intensification of the growth processes of the aerial and root parts of plants. A good activity of the root system on the substrate was noted: the formation of lateral and adventitious roots and the elongation of the root system, which in turn contributed to the good development of the aerial part of the plant (Figure 2). The share of adapted plants on coco substrate was 55,0–86,4%. The maximum indicator was noted in Garnem15 plants (86,4%), while the minimum indicator was noted in plants of the grape variety Madrasa (55,0%). The average stem length of regenerative plants on coco substrate was 2,2–3,8 cm. Rootstock GF677 plants were noted by their height of 3,8 cm, while Madrasa grape varieties plants had the lowest average stem length of 2,2 cm. The coco substrate also had a positive effect on the development of the root system of regenerated plants: the longest root system was in GF677 rootstock plants – 3,54 cm, and the highest average number of roots was in MaxMa14 plants – 4,3 pcs. Whereas, the minimum corresponding indexes were noted in Myrobolan 29C (2,06 cm) and OHF87 (2,9 pcs) plants.

The good development was also noted after 45 days of adaptation, when the rooted plants were transferred to the greenhouse (2nd stage of adaptation).

Fig. 2 – Regenerated plant (Garnem 15) after primary adaptation

Under greenhouse conditions, regenerated plants were transplanted into pots with a substrate prepared from soil, peat and perlite, in a ratio of 2:2:1 (Figure 3). The regenerated plants were kept at a temperature of +20...+25°C, periodically watered and fed with fertilizers. When the plants reached a height of 30–35 cm, good development and the beginning of lignification of the stems and leaf apparatus, they were taken out under the shade net.

Fig. 3 – The second stage of adaptation of rootstock regenerated plants in protected ground conditions (greenhouse)

The removal of regenerated plants from the greenhouse under the shade net (3rd stage of adaptation) was carried out not earlier than the end of April – beginning of May. Adaptation under the shade grid is an intermediate adaptation between adaptation in closed (greenhouse) and open ground (field) conditions. It is necessary in order to protect the plants from the direct rays of the sun for some time and adaptation to environmental conditions took place without loss. The process of adaptation of plants under the shade net takes at least 10 days.

Next, the plants were planted on the first field of the nursery, established according to the technology of two-line rootstock cultivation on a black impenetrable film.

Conflict of Interest **Конфликт интересов**

None declared. Не указан.

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