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Microclonal Propagation of Apple Roots Suitable to the Soil and Climate Conditions of Samarkand Region

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Abstract Buds of MM.106-semi-small and MM.111-strong growing grafts suitable for the soil-climatic conditions of Samarkand region were grown in the selected nutrient medium in vitro. To do this, the shoots separated from the body of the samples were first washed in a soapy solution and running water for 10-15 minutes, then washed for 5 minutes in 1:1 chlorine water (chlorine bleach "Belizna") and sterilized with MS containing mineral salts. 1 mg/l gibberellic acid, pH 5.6 medium was placed in the container according to the established procedure. In the process of microclonal reproduction of the obtained aseptic samples, pH medium with MS containing 30 g/l sucrose, 0.5 mg/l BAP, 0.01 mg/l IMK, and 4 g/l agar was transferred to 5.7 feed.

Keywords Graft, Apple, Bud, BAP, IMA, Gibberellin and ascorbic acid, If

1. Introduction

Biotechnological methods are widely used to obtain pathogen-free plant seedlings resistant to external factors. Tissues or cells of plant organs are used in this [1-4]. The use of biotechnological in vitro methods in the preservation and reproduction of varieties and forms of plants with promising and valuable biological/economic characteristics, the possibility of reproduction throughout the year regardless of the season, short-term selection processes has advantages with increasing. The use of aseptic healthy plant samples obtained as in vitro products in the international exchange of germplasm facilitates the quarantine control procedure [2,4,5-8].

Micropropagation of plants in vitro includes several stages. The first step is to select and sterilize the primary explant. Then, it is important to choose the optimal nutrient environment for the growth and development of explants [1,5-6]. In vitro cultivation of woody plants, including apple trees, is difficult and requires considerable attention. This is due to the high percentage of infection in the body tissues and cells of the apple tree under natural conditions, the amount of phenolic compounds in the tissues, which leads to necrosis in isolation conditions. Infection of plant material is associated with high contamination of its bacterial, mycoplasmal and viral infections.

Several different viral diseases have been identified in the apple tree. The most harmful of them are chlorotic spots on the leaves of the tree, which causes decay of the wooden part of the tree [6,9,10]. Common fungal diseases of apple trees include scab, rust, canker, root canker caused by bacteria, black spot, and bacterial burn. To get rid of these infectious diseases, trees are cut down and burned [11-13].

Accordingly, it is important in terms of economic and environmental sustainability to establish nurseries and orchards with pathogen-free variety samples, which are propagated by microclonal propagation in vitro, in contrast to vegetatively propagated plantations in tree seedling institutions [14].

In our previous works, the results of the establishment of pathogen-free beds and plantations of some medicinally important [15], tree and shrub [16] plants obtained in vitro under biotechnological methods were described. In this work, we aimed to obtain pathogen-free seedlings based on in vitro microclonal reproduction of apple grafts resistant to external stress factors, introduced in order to breed promising apple varieties.

2. Research Objects and Methods

As an object for the research conducted in this work, samples of wild apple MM.106-semi-small and MM.111-strong growth, propagated in natural conditions, were taken to prepare grafts for promising apple varieties for the soil and climate conditions of Samarkand region.

MM.106-semi-small and MM.111-strong rootstocks belong to the group of rootstocks for grafting apple trees, and they were propagated at the East Malling Research Station, Southeastern Agricultural College, Kent, England. MM.106-half pea is very well propagated by Parkhish method. The main bush gives an average number of

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well-rooted branches. In semi-intensive gardens, it is used as a semi-small graft. Roots are relatively cold-resistant (-12℃). It does not grow from its roots. Trees come into harvest early. It is not very picky about the fertility of the soil, but it is demanding on the water permeability of the soil. Productivity is very high. MM.111-strong growing weld tag. The growth rate is slightly stronger than MM.106, Intensive apple. It reproduces very well by cuttings. The main bush produces many well-rooted branches, from which many seedlings are formed. Somewhat resistant to cold and drought. Trees come into harvest early. It is also used for cultivation in lands with low soil fertility.

Cuttings 20-30 cm long are cut from annual shoots in February-March (Fig. 1), washed in a soap solution and running water, then treated with a diluted bleach solution (1:1) for 5 minutes and washed in running water. The samples were a solution of Murashige and Skoog mineral salts (MS) [17] at pH 5.6 at ½ concentration with 1 mg/L gibberellic acid (GA) and 1 mg added to promote the development of stem-forming shoots. l is placed in containers and 1 mg/l ascorbic acid (AC) is added. After 3-4 weeks, the grown shoots 1-2 cm long are cut, treated in a laminar box in a 0.1% sulema (HgCl2) solution for 5 minutes, and washed in sterilized distilled water. Or sterilized in a 0.1% HgCl2 solution for 3 minutes, treated with "Belizna" bleach solution (1:1) for 2 minutes and washed in sterile distilled water.

Figure 1. Preparation of the tips of the meristematic tissues of the buds of apple grafts

Aseptic bud tip tissue wrapped in a sterilized filter with a pH of 5.7 and containing 30 g/l sucrose, 0.5 mg/l 6-benzylaminopurine (BAP), 0.01 mg/l indolyl butyric acid (IMK) , MS with 1 mg/l GC and 1 mg/l AC is placed in liquid nutrient medium. Buds are transferred to a new environment every day. After 3-4 weeks, the microshoots that have maintained their biological viability are transplanted into a solid MS nutrient medium supplemented with 0.5 mg/l BAP and 0.01 mg/l IMK. Experiments were conducted in 3 replicates, and 20 explants were used in each replicate. The statistical analysis of the experimental results was carried out based on the generally accepted method presented in the manual prepared by G.F. Lakin [18].

3. Obtained Results and Their Discussion

15 out of 20 shoots (75%) from MM.106 cuttings and 16 out of 20 shoots taken from MM.111 cuttings were taken from 20 of MM.106 - semi-small and MM.111 - strongly growing cuttings and sterilized samples involved in our research. the regeneration process was observed.

It is known that plants produce phenol and their compounds in order to protect themselves from the effects of external factors during the development stage, especially the newly forming shoots. Such a situation also occurs in the buds of an apple tree, which are introduced to in vitro conditions. The most important way to reduce the effect of toxic phenolic compounds released by micro-shoots on the environment during introduction into the in vitro environment is to keep them in a liquid nutrient medium for 3 or 4 weeks. In this case, the toxic phenols and compounds released from the explants are evenly distributed in the liquid medium, and due to the reduction of their toxicity, the healthy specimens adapt to the nutrient medium. During the research, it was noted that the correct selection of the liquid nutrient medium and its composition is more effective at the stage of introducing the apple tree in vitro.

As mentioned above, the composition of the nutrient medium is of great importance when introducing samples in vitro. In this case, the addition of GK to the nutrient medium of MS is important in the first stages of in vitro introduction of buds. Acceleration of the development of buds based on the addition of GC to the nutrient medium was also noted in the works of some researchers [19-21]. But at this point it should be noted that the addition of GK at later stages also causes the growth of vegetative organs.

The addition of ascorbic acid to the nutrient medium reduces the amount of phenolic compounds produced or released by apple explants. Taking this into account, we added ascorbic acid to the nutrient medium at the initial stage of in vitro introduction. In this case, the composition of the liquid nutrient medium during in vitro introduction was as follows: MS contained 30 g/l sucrose, 0.5 mg/l BAP, 0.01 mg/l IMK, 1 mg/l GC and 1 mg/l AK. The pH of the liquid nutrient medium was 5.6, in which good development of buds was observed.

We used a solid nutrient medium for subsequent microclonal propagation. In this case, we added 0.5 mg/l BAP, 0.01 mg/l IMK, and 4 g/l agar to MS solid nutrient medium containing 30 g/l sucrose. The pH of the nutrient medium was 5.7, and positive changes were observed in the development of buds (Fig. 2).

A,B-stem regeneration, C-root formation process in vitro, D,E-transfer of root-formed pathogen-free seedlings to soil conditions, F-pathogen-free seedlings adapted to soil conditions.

It is possible to use pathogen-free seedlings produced in vitro as grafts or to use them in microcloning to obtain grafts.

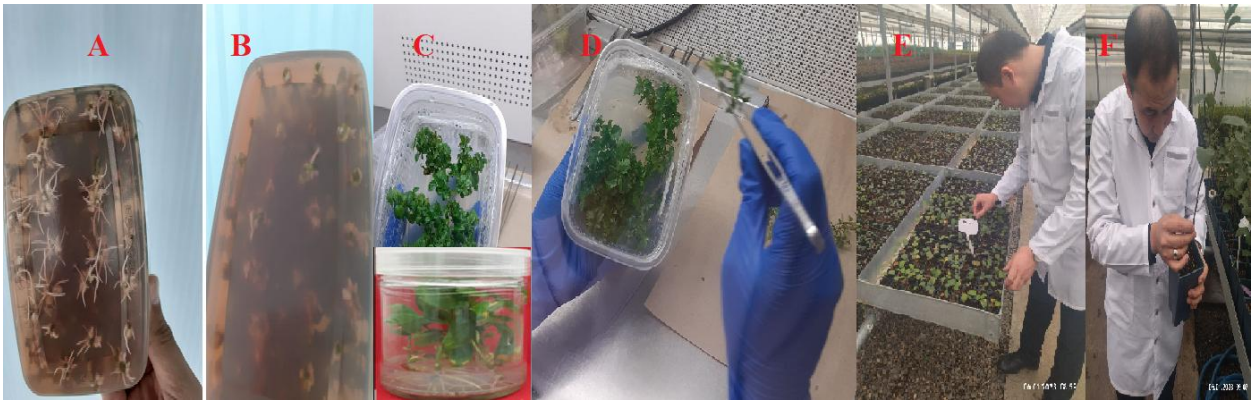

Figure 2. Obtaining pathogen-free healthy seedlings of apple grafts in vitro

4. Conclusions

As a result of studies on the introduction of apple tree grafts in vitro, it was shown that when explants were obtained by growth promotion, the highest percentage of healthy shoots and the lowest percentage of infection were in the first method of introduction.

It is important to keep the buds in a liquid nutrient environment for 3-4 weeks when introducing the apple tree grafts in vitro. In this case, toxic phenols and compounds released by the explants are evenly distributed in the liquid medium, and the poisoning of the buds is reduced.

The introduction of ascorbic acid into the nutrient medium reduces the amount of formation or release of phenolic compounds released by apple explants.

Samples were transferred from liquid nutrient medium to MS solid nutrient medium containing 30 g/l sucrose, adding 0.5 mg/l BAP, 0.01 mg/l IMK, 4 g/l agar, pH indicator of nutrient medium 5, When it reaches 7, positive changes are observed in the development of buds.

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