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The modification of murashige and skoog media for efficient cultivation of gizella-5 and vsl-2 rootstocks in vitro

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Abstract. This paper reports the effect of modified nutrient media on biotechnologically growing rootstock plants (Gizella – 5 and VSL – 2) at the stages of introduction and grafting. Murashige and Skoog medium was modified by adding 25 mg/l Chelate-Fe and 500 mg/l of $\text{Ca}(\text{NO}_3)_3$ to the basic nutrient at the stage of introduction of the plant. At the grafting stage 0.1 mg of 6-Benzylaminopurine (6-BAP) was added to Murashige and Skoog, where the effect of regeneration was 2 times higher than in experimental control. The results obtained prove that modified Murashige and Skoog medium is to be the best nutrient media for root-plant introduction and regeneration processes in Vitro followed by Chelate-Fe, $\text{Ca}(\text{NO}_3)_3$ and 6-BAP respectively.

1. Introduction

The intensive technology of producing fruit crops at the present time is impossible without successfully cultivating a planting material, the production of which is based on biotechnological (virus – free) features. The allocation of agricultural gardening to a virus-free basis is dictated by the rules for international trade and the exchange only of virus-free planting material for cultivated plants according to particular certificates. Consequently, the development of biotechnology definite that non-virus planting material will ensure a significant increase in its quality, which leads the increase an economical profitability and intensity of the industry. In the conditions of present time gardening, microclonal reproduction of fruit crop roots plays a special role and frequently modern stocks propagate exclusively invitro. An example of this is the clone stocks of VSL-2 and Gisela-5.

“It is known fact that during microclonal propagation, propagated plants exhibit a type-specific response to cultivation conditions, especially to the mineral composition of the nutrient medium and the complex of vitamins and phytohormones that make it up” [8]. Therefore, optimisation of the process of microclonal propagation of the rootstocks of Gisela-5 and VSL-2 are relevant.

Based on literature data [11], [9], it can be concluded that the mineral composition of artificial nutrient media has not been relatively determined. The most widely used medium is based on mineral salts from Murashige and Skoog (1962), enriched with vitamins, amino acids, phytohormones and sugars. The most widely used nutrient medium is based on mineral salts from Murashige and Skoog, enriched with vitamins, amino acids, phytohormones and sugars. Often, researchers modify the composition of the medium Murashige and Skoog. Reducing the concentration of mineral salts by two



times shows affectivity by introducing into the crop of apple rootstock M 7 [6], and during the reproduction of *Valeriana officinalis* in vitro [7]. T. Cheng used the same nutrient medium for propagation of apple and pear rootstocks [4].

Scientists differ on the optimal ratio of the ammonium and nitrate forms of nitrogen in the nutrient medium. According to I. N. Pronina reducing the concentration of ammonium nitrogen by 2 times at the stage of rhizogenesis of apple rootstocks 54-118 and 3-17-38 accelerates the process of root formation by 2 weeks' time [13]. An ultimate fact was also established that a decrease in the concentration of the mineral composition of the Murashige and Skoog medium by 2–4 times at the stage of rhizogenesis improves a root development. Moreover, N.V. Solovykh and co-worker S.A. Muratova using an environment with a 2-fold increase in the concentration of NO_3 at the stage of proliferation of raspberries, blackberries and raspberry-blackberry hybrids have received an increased number of developed adventive shoots [16].

Therefore, researchers have not come to a common opinion about the optimal composition of the nutrient medium. In the same time, the study for the optimal mineral structure (salts, vitamins and etc.) of the nutrient medium for exact genotypes of propagated plants still needs to be founded.

2. Materials and methods

The study was carried out in the laboratory of biotechnology Scientific Production Firm (SPF) LLC "Sady Chechni". Objects of the study were clonal rootstocks of VSL - 2 and Gizella - 5 stone fruit crops (rootstocks).

3. Preparation of Murashige and Skoog nutrient medium

The basic Murashige and Skoog nutrient media was obtained by following the methodical recommendations from E.N. Dzhigadlo [5], U.G. Popov [12] and V.N. Podorozhniy, N.N. Kovalenko [18]. The Murashige and Skoog was modified by adding 25 mg/l Chelate-Fe and 500 mg/l of $\text{Ca}(\text{NO}_3)_3$ to the basic solution, where the obtained product was used as a nutrient media for the research. On the modified nutrient media, a clonal rootstock of Gizella-5 and VSL-2 stone fruit crops were inducted (planted) and this step was called a (plant introduction) stage.

At the second stage "plant grafting" the basic nutrient Murashige and Skoog prepared by the same way as at the previous stage and was modified by adding 6-Benzylaminopurine (6BAP), where the grown plant from "plant introduction" stage was grafted into a new modified Murashige and Skoog nutrient media.

4. Results and Discussion

To compare the effectiveness of the nutrient medium, a series of experiments using a modified and initial Murashige and Skoog media were used. The graphical illustration was plotted where the explant development of Gisella – 5 and VSL -2 on basic and modified Murashige and Skoog were shown by grades from 0 (very bad) to 5 (excellent).

Note: the final score is the sum of both a developmental score (from 1 to 3) and a score for color intensity (from 1 to 2).

This research showed that, the growth and development of explants (VSL – 2 and Gizella – 5) of stone seed cultures in initial based Murashige and Skoog were considerably worse than in the modified nutrient medium. Consequently, this statement was expressed in the deficiency of green pigment in microshoots on initial-based Murashige and Skoog medium in the conglomerate and the growth of callus tissue, from which during following observation the development of single fasciated shoots, which were promoted. This phenomenon was categorized as a phenotypic in the cultivated plant.

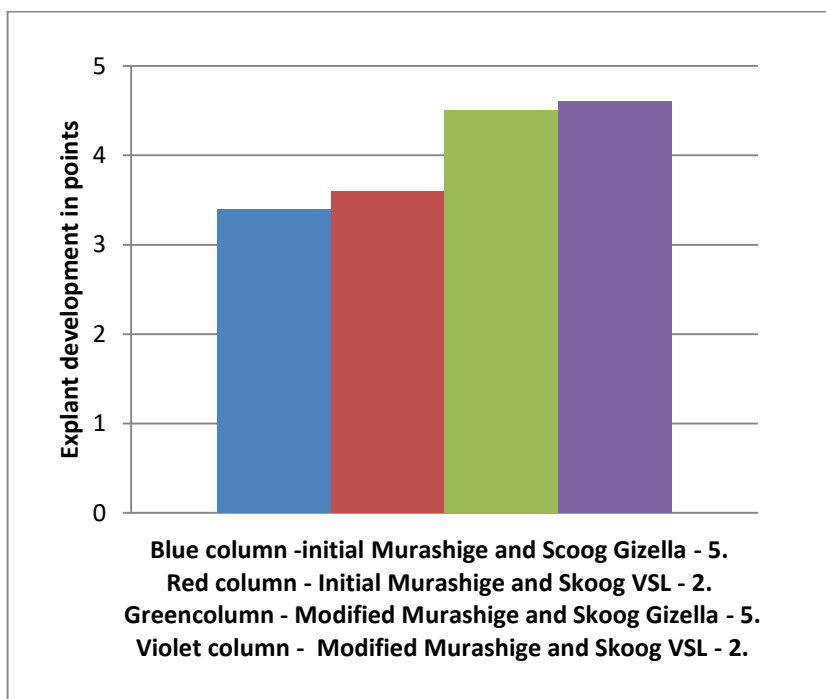


Figure 1. The degree of development of initial explants (Gizella – 5 and VSL – 2) on various nutrient media (modified vs control).

Modified Murashige and Skoog is much better than after cultivation on initial basic medium. The use of modified Murashige and Skoog nutrient medium with breeding in vitro stock of Gizella -5 also shows excellent results. Micro-shoot measurements at the proliferation stage, show an increase in shoot growth by 0.4-0.5 cm per passage (figure 2 and figure 3).



Figure 2. Growth of Gisela shoots on basic Murashige and Skoog medium (left) and modified Murashige and Skoog medium (left).



Figure 3. Growth of VSL-2 shoots on basic Murashige and Skoog medium (left) and modified Murashige and Skoog medium (right).

From the figures above figure 2 and figure 3 the influence of Chelate-Fe, $\text{Ca}(\text{NO}_3)_3$ and 6-BAP can be noticed, as in both cases the growth of plant shoots are significantly notable. However, the study of use modified Murashige and Skoog nutrient medium in the process of microclonal propagation of the clone stock of Gizella-5 and VSL – 2 is further requires more thorough analysis of its influence on the growth and development of micro-shoots at the proliferation stage. However, received data shows that

a modified nutrient medium needs to be used for wider in the process of studying and optimizing the technology for growing virus – free planting material of the basic category.

5. Conclusion

To sum up, a significant plant growth can be noticed on Gizella – 5 and VSL -2 fruit crops, where the results were experimentally obtained by applying a modified Murashige and Skoog with 25 mg/l Chelate-Fe and 500 mg/l of $\text{Ca}(\text{NO}_3)_2$ at the stage of “plant induction” and 0.1 mg of 6-Benzylaminopurine at the “grafting stage”. All the results show that a modified Murashige and Skoog nutrient medium suits promisingly well for steps “plant introduction” and “grafting stage”, consequently, the fact must be clearly stated that, an experimental work must be carried out for further detailed demonstration, for example, at the final step “rooting stage” of Gizella – 5 and VSL -2 fruit crops.

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