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IMPROVEMENT OF IN VITRO ROOTING OF MICROCUTTINGS SOME ROOTSTOCKS OF FRUIT CROPS

Sevil Suleimanova*

Research Institute of Fruit and Tea Growing of Ministry of Agriculture of Azerbaijan Republic, Guba, Azerbaijan

Abstract. The objects of the study were microcuttings of rootstocks of fruit crops Myrobalan 29C, MaxMa 14, Garnem 15, GF 677 and OHF 87, as well as MS and DKW nutrient media modified with various combinations and concentrations of auxins and cytokinins (IBA, NAA, BAP) in 5 variants. It was found that the optimal medium for rooting micro plants of MaxMa 14 rootstock was DKW medium with 1.0 mg/l IBA and 0.01 mg/l NAA (rooting - 82%, average number of roots - 4.2 pcs, average root length - 4.3 cm). Optimal rooting of micro plants of the rootstock Myrobalan 29C (rooting - 80.0%, average number of roots - 4.0 pcs, average length of roots - 3.5 cm), Garnem 15 (rooting - 88.0%, average number of roots - 3, 7 pcs, average root length - 2.9 cm), GF 677 (rooting - 80.0%, average number of roots - 4.7 pcs, average root length - 3.2 cm), and OHF 87 (rooting - 80, 0%, the average number of roots is 6.5 pcs, the average length of the roots is 2.9 cm) was noted on MS medium using IBA at a concentration of 1.0 mg/l and NAA at a concentration of 0.1 mg/l. In addition, there was no significant change in the efficiency of rootstock rooting of fruit crops under in vitro conditions, depending on the passage.

Keywords: *in vitro, rooting, rootstock, fruit crops, plant medium, auxin, cytokinin.*

Corresponding Author: *Sevil Suleimanova, Research Institute of fruit and tea growing of Ministry of Agriculture of Azerbaijan Republic, Zardabi settlement, Guba, Azerbaijan, Phone: +994552423178, e-mail: suleymanovas81@mail.ru*

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1. Introduction

Root development in plants in vitro culture is determined primarily by genotype (the ability to form roots in in vitro culture, as a rule, correlates with the rooting of cuttings by traditional methods). The composition of the nutrient medium has a great influence on rhizogenesis: mineral components at the stage of rooting and in the passages preceding rooting; source and concentration of carbohydrates; medium density; concentration and ratio of biologically active substances and the method of their influence on the explant (introduction into the composition of the medium, processing of micro cuttings, preliminary soaking of micro cuttings in BAS solutions); the degree of development of the micro cuttings and mechanical damage to the bases of the cuttings, and a number of other factors. This indicates the complexity of the process of rhizogenesis and the difficulty of managing it, however, as experimental data show, with such a large number of factors affecting the efficiency of rooting, there is a wide range of possibilities for optimizing rhizogenesis.

According to many researchers (Hepaksoy, 2017; Erfani *et al*., 2017; Fidanci *et al*., 2005; Kose & Canli, 2015; Muna, 1999; Papakosta, 2016; Rezaei & Hosseipour, 2015; Sadeghi, 2015; Shabani, 2015), the mineral composition of the nutrient medium according to the prescription Murashige-Skoog (Murashige & Skoog, 1962) is a more

suitable for the rhizogenesis of fruit plants. However, the high concentration of nitrogen in this nutrient medium can promote excessive growth of shoots and leaves, to the detriment of the root system. Therefore, most researchers in the process of rooting micro cuttings suggest using a medium with half the content of macronutrients. Another nuance is the correct selection of growth hormones and their concentration.

Thus, according to Erfani et al. (2017), the best medium for rooting micro cuttings of the Garnem rootstock is ½MS medium with the addition of 2 mg/l IBA. In another research paper (Aghaye & Yadollahi, 2012) with rootstock GF 677, the best rooting rate was obtained at 1/2MS supplemented with 3.0 mg/l IBA.

Vaez-Livari and Salehi-Soghadi, (2005) consider that the best hormone for root formation in GF rootstock is indole-3-acetic acid (IAA) at a concentration of 0.5 mg/l.

The in vitro rooting ability of pear rootstock OHF333 was studied using indole-3-butyric acid (IBA) (0; 0.5 and 1.0 mg/l), putrescine (160 mg/l) using 4 different rooting methods. The highest percentage of rooting (49.5%), number (4.09) and length of roots (2.26 cm) were obtained by cultivating micro cuttings on a medium containing IBA and putrescine for 35 days (Erturk, 2013).

Hepaksoy, (2017) found that GF 677 rootstock reproduces better on MS medium supplemented with 2.0 mg/l BAP, 0.1 mg/l NAA and 0.1 mg/l GK_3. The best results at the stage of root formation were obtained with the use of MS medium with 1.0 and 1.5 mg/l of 1-naphthaleneacetic acid (NAA).

Fidanci et al. (2005) in their study on in vitro micropropagation of rootstocks for sweet cherry Gisela 5, MaxMa 14, micro shoots were rooted on the medium 1/2 MS + 1.0 mg/l IBA. As a result, after three weeks, 95-100% of fully formed micro plants were recorded.

6-benzyladenine (BA) stimulates increased callus development in Gizela5 micro cuttings (Aghaye, 2013) and Garnem 15 (Arab, 2014), hinders in vitro root formation in rootstocks Maxma 14 and F12-1 (Canli & Demir, 2014).

Thus, from the review of some literary sources, it was concluded that there is no uniform opinion of scientists about the technology of rooting micro plants and research on improving this technology still continues.

2. Material and methods

In order to evaluate the effectiveness of our study on the study of rhizogenesis of fruit rootstocks Myrobalan 29C, MaxMa 14, Garnem 15, GF 677 and OHF 87 under in vitro conditions, the following nutrient media and cultivation conditions were considered:

Variant I (control). Nutrient medium with macro- and microelements according to MS (Murashige and Skoog, 1962) with the addition of growth regulators - 0.1 mg/l BAP and 0.5 mg/l IBA, vitamins - 1 mg/ l B1 (thiamine), 0.5 mg/l B6 (pyridoxine), 0.5 mg/l PP (nicotinic acid), 2 mg/l glycine and 100 mg/l myo-inositol. Conditions for cultivation of micro plants in vitro: lighting 2500-3000 Lux, photoperiod 16/8 hours, temperature +22...+25°C.

Variant II. Nutrient medium with macro- and microelements according to MS (Murashige & Skoog, 1962) with the addition of growth regulators - 0.1 mg/l BAP and 0.5 mg/l IBA, vitamins - 1 mg/l B1 (thiamine), 0.5 mg/l B6 (pyridoxine), 0.5 mg/l PP (nicotinic acid), 2 mg/l glycine and 100 mg/l myo-inositol. Conditions for cultivation of micro plants in vitro: 10 days without illumination, 10 days with illumination of 2500-3000 Lux, photoperiod 16/8 hours, temperature +22...+25°C.

Variant III. Nutrient medium with macro- and microelements by MS (Murashige & Skoog, 1962) with the addition of growth regulators - 0.5 mg/l IBA and 0.1 mg/l NAA, vitamins - 1 mg/l B1 (thiamine), 0.5 mg/l B6 (pyridoxine), 0.5 mg/l PP (nicotinic acid), 2 mg/l glycine and 100 mg/l myo-inositol. Conditions for cultivation of micro plants in vitro: 10 days without illumination, 10 days with illumination of 2500-3000 Lux, photoperiod 16/8 hours, temperature +22...+25°C.

Variant IV. Nutrient medium with macro- and microelements by MS (Murashige & Skoog, 1962) with the addition of growth regulators - 1 mg/l IBA and 0.1 mg/l NAA, vitamins - 2 mg/l B1 (thiamine), 1.0 mg/l PP (nicotinic acid), 2 mg/l glycine and 100 mg/l myo-inositol. Conditions for cultivation of micro plants in vitro: 10 days without illumination, 10 days with illumination of 2500-3000 Lux, photoperiod 16/8 hours, temperature +22...+25°C.

Variant V. Nutrient medium with macro- and microelements according to DKW (Driver & Kuniyuki, 1984) with the addition of growth regulators 1 mg/l IBA and 0.01 mg/l NAA, vitamins - 2 mg/l B1 (thiamine), 1 mg/l PP (nicotinic acid), 2 mg/l glycine and 100 mg/l myo-inositol. Conditions for cultivation of plants in vitro: illumination 2500-3000 Lux, temperature +22...+24°C, photoperiod 16/8 hours.

In all nutrient media as the iron chelate was used FeEDDTA.

Rootstock micro cuttings were planted on modified MS (Murashige & Skoog, 1962) and DKW (Driver & Kuniyuki, 1984), taking into account 50 pieces for each variant (5 jars of 10 micro cuttings). The duration of the rooting process was 21-25 days. The effectiveness of the study was taken into account by the percentage of rooted microcuttings (%), the average number of roots formed (pcs) and their average length (cm) (Table 1).

3. Results and Conclusion

Based on the results of the study presented in Table 1, the most suitable medium for root formation in MaxMa 14 rootstock was DKW medium with 1.0 mg/l IBA and 0.01 mg/l NAA (variant V). On this medium, the proportion of rooted regenerated plants of the rootstock was 82.0%, while on media I-IV, this figure varied from 32% to 64%. The maximum number of roots (4.2 pcs) of MaxMa14 rootstock regenerated plants was also obtained on the DKW medium: in the first variant, this figure was 2.02 pcs, in the second variant - 3.8 pcs, in the third variant - 3.0 pcs, and in the fourth variant - 3.8 pcs. The average length of the roots of microplants varied depending on the composition of the nutrient medium from 0.95 cm (variant I) to 4.3 cm (variant V).

The optimal rooting of micro plants of the rootstock Myrobalan 29C (80.0%) was noted on MS medium using IBA at a concentration of 1.0 mg/l and NAA at a concentration of 0.1 mg/l. The minimum percentage of rooting was recorded on the MS medium with 0.1 mg/l BAP and 0.5 mg/l IBA when cultivated under conditions of a photoperiod of 16/8 hours, while under conditions of 10 day etiolation and 10 day illumination, this figure was 43.3 %. A good percentage of rooting was also observed on the DKW medium - 70%. On MS medium supplemented with 0.5 mg/l IBA and 0.1 mg/l NAA, the proportion of rooted regenerated plants was only 50%.

The largest number of roots was noted on the DKW medium with rhizogenesis inducers 1.0 mg/l IBA and 0.01 mg/l NAA (5.2 pcs), while in the variant showing the highest percentage of rooting (variant IV), the average number of roots was 4.0 pcs. The maximum root length was recorded on MS medium (variant IV) supplemented with 1.0

mg/l IBA and 0.1 mg/l NAA. The minimum indicators of root formation of micro plants of the rootstock Myrobalan 29C were noted in the control variant MS (variant I) with the addition of 0.1 mg/l BAP and 0.5 mg/l IBA.

In rootstock Garnem 15, the best rooting was observed in variant IV (88.0%), which exceeds the rooting rate in the control variant (40.0%) by 48.0%. Also, between these study variants, there was a significant difference in the average number of roots and their length. So, on medium IV, the average number of newly formed roots per micro plant was 3.7 pcs, and their average length was 2.9 cm, while in the control variant, 1.3 pcs of roots were recorded, the average length of which was 0.8 cm. Nutrient medium DKW completely inhibited the process of rhizogenesis of micro cuttings of Garnem 15 rootstock.

For rootstocks GF 677 and OHF 87, the optimal composition of the nutrient medium, as well as for the rootstock Garnem 15, includes the mineral composition according to MS prescription, supplemented with auxins for root formation 1.0 mg/l IBA + 0.1 mg/l NAA (variant IV).

The proportion of rooted regenerated plants of the clonal rootstock GF 677 was 80.0% (on day 25 after planting on a nutrient medium with root formation regulators), the average number of roots formed for each micro plant was 4.7 pcs, their average length was 3.2 cm. The proportion of rooted regenerated plants rootstock OHF 87 was 80.0%, the average number of roots formed is 6.5 pcs, their average length is 2.9 cm. Such development of the root system is maximum for the studied clonal rootstocks and should ensure a high level of adaptation regenerants under non-sterile conditions, on substrates that differ sharply in physical and chemical parameters from nutrient media. It should also be noted that media I, II and V completely inhibited root formation in micro cuttings of these rootstocks.

In addition to all of the above, in the course of the study it was found (comparing the results of the 1st and 2nd variants) that the root formation of micro cuttings of rootstocks of fruit crops Myrobalan 29C, MaxMa 14, Garnem 15, GF 677 and OHF 87 occurs better under conditions 10 day etiolation followed by cultivation under normal illumination of 2000-3000 Lux and a photoperiod of 16/8 hours. So, already on the 7-10th day after planting on the medium for rooting and cultivation under etiolation conditions, 98% of the micro cuttings of the OHF 87 rootstock showed swelling of the bases, and on the 12-14th day, roots appeared (Figure 1).

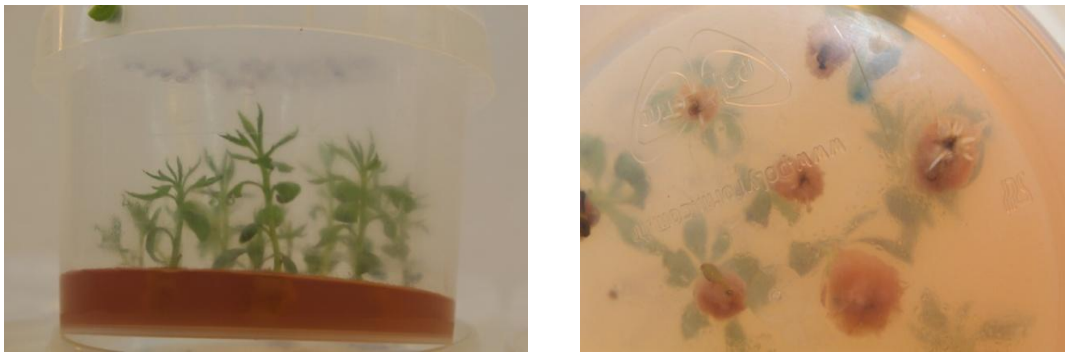

Figure 1. Microcuttings rootstock OHF 87 on the medium for rooting on the 14th day after planting

Table 1. Influence of the composition of nutrient media and cultivation conditions on rooting rootstocks of fruit crops in vitro

Variants		Cultivation conditions	Rooting, %					Average number of roots, pcs					Average length of roots, cm				
			MaxMa14	Myrobalan 29C	Garnem 15	GF677	OHF87	MaxMa14	Myrobalan 29C	Garnem 15	GF677	OHF87	MaxMa14	Myrobalan 29C	Garnem 15	GF677	OHF87
I	MS + 0,1 mg/l BAP + 0,5 mg/l IBA + 1,0 mg/l B_1 + 0,5 mg/l B_6 + 0,5 mg/l PP + 2,0 mg/l glycine +100 mg/l myo-inositol (control)	Lighting	32,0	36,0	40,0	0	0	2,02	1,85	1,3	0	0	0,95	0,54	0,8	0	0
II	MS + 0,1 mg/l BAP + 0,5 mg/l IBA + 1,0 mg/l B_1 + 0,5 mg/l B_6 + 0,5 mg/l PP + 2,0 mg/l glycine +100 mg/l myo-inositol	10 days darkness / 10 days lighting	58,0	43,3	44,0	0	0	3,8	2,2	1,8	0	0	1,02	1,0	1,7	0	0
III	MS + 0,5 mg/l IBA + 0,1 mg/l NAA + 1,0 mg/l B_1 + 0,5 mg/l B_6 + 0,5 mg/l PP + 2,0 mg/l glycine + 100 mg/l myo-inositol	10 days darkness / 10 days lighting	56,0	50,0	14,0	14,0	27,0	3,0	2,1	1,5	2,8	4,7	1,7	1,03	1,8	1,3	1,5
IV	MS + 1,0 mg/l IBA + 0,1 mg/l NAA + 2,0 mg/l B_1 + 1,0 mg/l PP + 2,0 mg/l glycine + 100 mg/l myo-inositol	10 days darkness / 10 days lighting	64,0	**80,0**	**88,0**	**80,0**	**80,0**	3,8	4,0	**3,7**	**4,7**	**6,5**	3,8	**3,5**	**2,9**	**3,2**	**2,9**
V	DKW + 1,0 mg/l IBA + 0,01 mg/l NAA + 2,0 mg/l B_1 + 1,0 mg/l PP + 2,0 mg/l glycine + 100 mg/l myo-inositol	10 days darkness / 10 days lighting	**82,0**	70,0	0	0	0	**4,2**	**5,2**	0	0	0	**4,3**	3,0	0	0	0

Using rootstock OHF 87 as an example, we studied the rooting efficiency depending on the passage of in vitro cultivation on a modified MS medium supplemented with 1.0 mg/l IBA and 0.1 mg/l NAA.

Table 2. The effectiveness of rhizogenesis of the rootstock OHF 87 depending on the passage

Passage	Nutrient medium	Number of microshoots, pcs	Number of rooted microshoots,	
			pcs	%
2	MS + 1.0 mg/l IBA + 0.1 mg/l NAA	43	31	72
3		55	45	82
4		76	52	68
5		42	29	69
6		63	44	70
Sum/average		279	201	72,2

It was shown that when planting on a nutrient medium for rooting regenerative plants 2-5 cm in size, on average, 72.2% of regenerative plants rooted. There was no significant change in the rooting efficiency of the clonal rootstock for pear OHF 87 depending on the passage - from 68% at the fourth passage to 82% at the third passage (Table 2).

Thus, the developed hormonal composition of nutrient media made it possible to obtain 80-82% of rooted micro plants, which subsequently underwent successful adaptation to non-sterile environmental conditions.

It was also found that the rooting of Myrobalan 29C, MaxMa 14, Garnem 15, GF 677 and the OHF 87 is better under conditions of 10 daily etiolation with subsequent cultivation at normal lighting 2000-3000 Lux and photoperiod 16/8 hours.

In addition, there was no significant change in the effectiveness of rooting fruit crops in vitro, depending on the passage was not observed.

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