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THE EFFECT OF THE MINERAL CONTENT OF CULTURE MEDIUM AND THE TYPE OF AUXIN ON *IN VITRO* ROOTING OF MICROPROPAGATED PEAR PLANTS

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Introduction

In the last years the areas planted with pear trees in Bulgaria have been significantly reducing and they were decreased to the minimum. The reasons were the damages caused by Fire Blight disease (*Erwinia amylovora*) and the inefficient control of the attacks of pear psylla (*Psylla pyri*), as well as the difficult production of grafted planting material. The latter requires grafting on interstocks due to the later incompatibility between some major pear cultivars and the quince rootstock, which makes the production cycle 3-year long and the trees obtained become more expensive. An alternative for avoiding those disadvantages is offered by the *in vitro* method. It provides the impetus for accelerated production of huge amounts of top quality, authentic, virus-free certified planting material.

Micropropagation of the pear crop, including planting material for industrial scale production, was an object of study in a number of investigations [2-4]. The rooting stage of the micropropagated plants required a change in the content of the mineral elements in the nutrient substrate and the inclusion of growth regulators of the group of the auxins. The results in that aspect varied significantly depending on the propagated cultivars and rootstocks. Successful rooting of the cultivars Kaiser, Max Red Bartlett and Williams was achieved when the nutrient medium was supplemented with 1 mg/l IBA [8]. During micropropagation of the frost-resistant pear cultivar Gola, Dwivedi and Bist [5] established very good rooting when the microplants were grown in ½ MS nutrient medium Murasige and Scoog [9] with 1,0–2,0 mg/l of IBA. In that relation, when propagating three pear rootstocks of OH series, Bahri-Sahloul et al. [1] found out that the best rhizogenesis was achieved when adding IBA at a concentration of 10 µM. However, in the studies of Yeo and Red [11], over 80 % of rooting was achieved in the rootstock OH x F230, irrespective of whether IBA or NAA was used, while in OPR 260 the highest percentage of rooting (42,9%) was obtained when using 10 µM NAA. Nadosy [10], established the best rooting of OHF rootstocks, BA-29 quince rootstock, pear seedlings and pear cultivars Clapp's Favourite and Bartlett in the presence of higher concentrations of IBA. In our experiments with 5 *in vitro* propagated pear cultivars [7], the most significant effect on root formation was established in the presence of 2,5-3,0 mg/l IAA added to MS nutrient medium with ¼ strength macrosalts.

A typical characteristic in the process of *in vitro* rooting of the pear crop is the callus formation at the shoot base of the microplants, which is an undesirable factor [3, 6]. Due to that it is necessary to find out the proper auxin, which induces high rhizogenesis and does not provoke callus formation.

The major aim of the study was to establish the effect of the mineral composition of the nutrient substrate, the type of the participating auxin and its concentration on *in vitro* rooting of micropropagated pear plants, avoiding the induction of callus structures at the shoot base.

Objects and methods of investigation

The study was carried out in the Production Laboratory for *in vitro* propagation at the Fruit Growing Institute – Plovdiv with micropropagated plants of the pear cultivar Packham's Triumph. The investigations were directed to two aspects: the effect of the mineral composition in the nutrient medium and the type of the auxin used. In relation to the first aspect two nutrient media were tested – MS modification with a decreased content of ammonium nitrate and added calcium nitrate (medium A) and MS basal medium (medium B). Both media contained a quarter-strength macroelements, MS microelements, Thiamin hydrochloride 0,4 mg/l of the vitamins and FeNaEDTA 36,7 mg/l. IBA and IAA auxins were studied for induction of rhisogenesis in three increasing concentrations – 0,5 mg/l, 1,0 mg/l and 1,5 mg/l, grouped in 12 variants of nutrient media (Table).

The following indices were reported: percentage of rooting, mean number of roots formed; mean length of the rootlets (mm), vital status of the rooted microplants.

The plants were grown in a chamber at a temperature of 24°C, light intensity 3000 Lux provided by white light luminescent lamps and a photoperiod of 16/8 h, day/night.

Mathematical data processing was done by the ANOVA method.

Table

Experimental design

Mineral composition	IBA			IAA		
	mg/l					
	0,5	1	1,5	0,5	1	1,5
Medium A	V1	V2	V3	V7	V8	V9
Medium B	V4	V5	V6	V10	V11	V12

Results and discussion

Referring to the influence of the **mineral elements** in the nutrient medium, a better effect on root formation was observed when growing the microplants on the basal MS medium (medium B) regardless of the type of the auxin added (Fig. 1). The increase of **IBA** in the MS medium with modified mineral elements (medium A) led to an increase of the percentage of rooting from 50% at 0,5 mg/l to 70% at 1,5 mg/l but at the same time callus tissue was formed at the stem base, which is an undesired consequence. The participation of the same auxin in the medium with mineral substances MS (medium B) resulted in better rooting – 95% – 100% (Fig. 1 a) but it induced stronger callus formation.

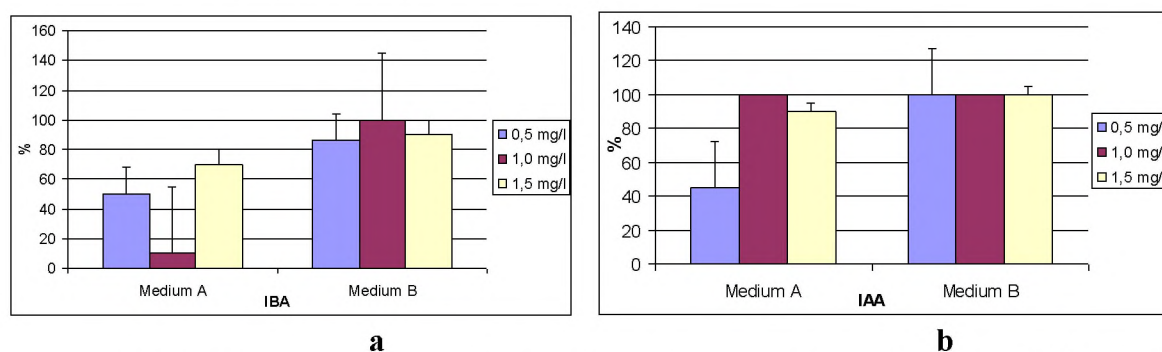


Fig. 1. Percentage of rooting of micropropagated plants, Packham's Triumph cultivar, grown in nutrient media with modified mineral content MS (medium A) and MS only (medium B) at increasing concentrations (0,5-1,5 mg/l) of IBA (a) and IAA (b). The bars show the standard error

Callus structure was not observed only when growing the microplants in the variants of nutrient media with **IAA**. Even more, regardless of the variation of mineral salts, the participation of IAA in both studied media contributed to achieving a very high percentage of rooting, which was within 90% – 100% (Fig. 1 b).

Observations on the **formation of primary roots** at the stem base of the microplants showed once again the better effect of the basal MS medium (Fig. 2). However, the effect of the separate auxins and their concentrations was not uniform. The increase of IBA to concentrations 0,5-1,5 mg/l in medium B caused an increased rootlet induction – 4,8-6,1. (Fig. 2-a). With the participation of IAA in the nutrient medium, the mean number of roots per plant in medium A was quite low – 2 to 3, while in medium B the biggest number of rootlets (6,3) was formed at 1,0 mg/l concentration of auxin (Fig. 2-b).

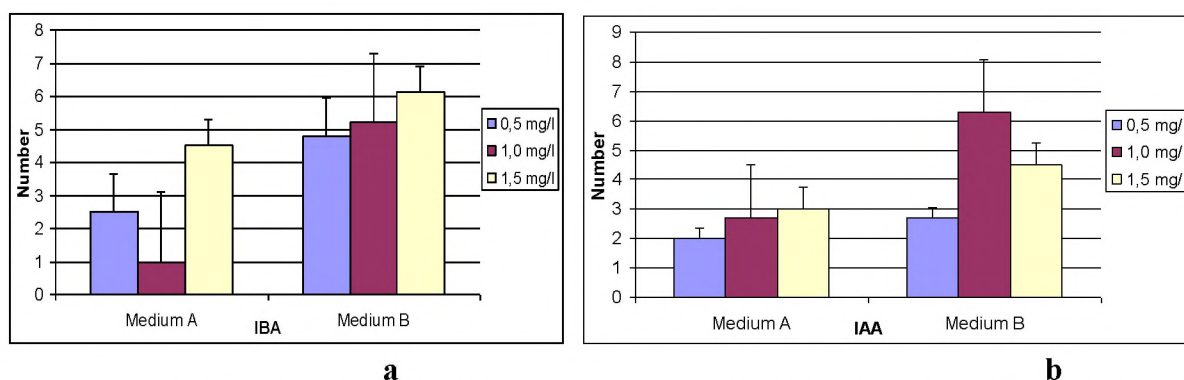


Fig. 2. Mean number of roots per plant, Packham's Triumph cultivar, grown on nutrient media with modified mineral content MS (medium A) and MS only (medium B) at increasing concentrations (0,5 - 1,5 mg/l) of IBA (a) and IAA (b). The bars show the standard error

Analogous results were obtained regarding the mean **length of the formed rootlets**. Again the better development of the roots in general was established when growing the microplants in MS medium without modification of the macroelements (Fig. 3 – medium B). The studied auxins also exerted different effects. When IBA was added to the nutrient medium, the biggest root length was established at the concentration of 0,5 mg/l – 20,8 mm. Increasing the auxin concentration to 1,5 mg/l led to shorter rootlets formed – 6,6 mm. The participation of IAA, regardless of the variation in concentration, induced the formation of longer rootlets – 23,7-25,0 mm.

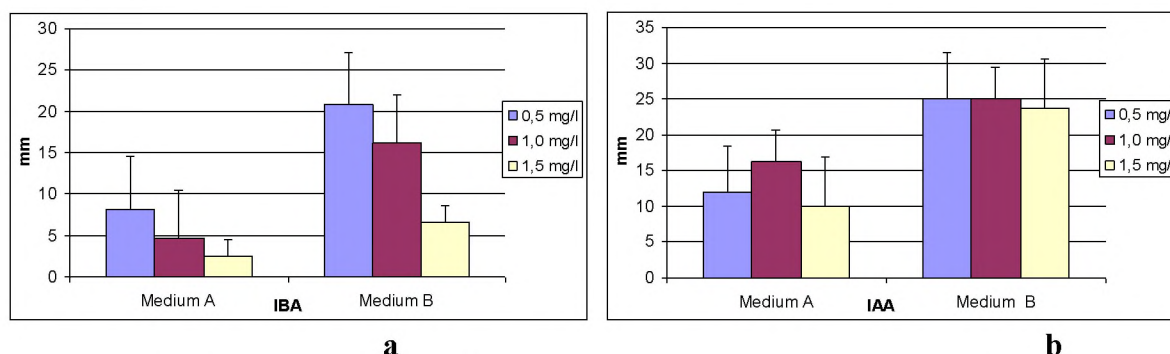


Fig. 3. Average length of the rootlets per plant, Packham's Triumph cultivar, grown on nutrient media with modified mineral content MS (medium A) and MS only (medium B) at increasing concentrations (0,5-1,5 mg/l) of IBA (a) and IAA (b). The bars show the standard error

The **vital status** of the microplants grown in the two nutrient media was good and it

showed an actively growing tip and fresh dark green leaves. The tendency towards a better development of the plants, expressed in a bigger stem height, was established in those grown with the participation of IAA.

The rooted plants in the tested media were successfully planted and adapted to non-sterile *ex vitro* conditions.

Conclusions

MS nutrient medium with ¼-strength macroelements without decreasing the content of the ammonium nitrate and additional inclusion of calcium nitrate, created better conditions for *in vitro* rooting of micropropagated plants of the pear cultivar Packham's Triumph.

The increase of IBA concentration in the nutrient medium led to a higher rooting percentage but at the same time it was accompanied by an increased induction of callus tissue at the stem base.

A high percentage of rooting without callus structure was established when growing the microplants in the variants with the presence of IAA (0,5-1,5 mg/l).

The mean number of rootlets formed per plant and their length, respectively, showed higher values in the MS nutrient medium with mineral elements without modifying them. In the presence of IBA in the medium, the biggest number of rootlets was established at a concentration of 1,5 mg/l (6,1), while in the presence of IAA that characteristic had the highest value at a concentration of 1,0 mg/l.

The participation of the separate auxins exerted an effect on the length of the rootlets formed. The increase of IBA caused the formation of shorter rootlets, while the increase of IAA did not have an effect on the root length and the rootlets induced were of optimal values – 23,7-25,00 mm.

The best development of the microplants concerning their vital status, stem height, number and length of the induced rootlets, can be obtained when growing them in MS medium containing ¼-strength macroelements with IAA.

The presented results gave the grounds to recommend the use of the basal MS nutrient medium at the stage of rooting pear cultivars – a stage of the *in vitro* propagation technological process.

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ХЕМОСЕЛЕКЦИЯ ВИНОГРАДА НА НАЛИЧИЕ АРОМАТА

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Введение

В ягодах винограда, а также в плодах других культур, имеется особая группа веществ, называемых эфирными маслами. Эти вещества обуславливают аромат и участвуют в образовании букета готовой продукции переработки, в частности, вина. В связи с этим представляют большую ценность для виноделия и могут являться критерием селекционного отбора.

В настоящее время из эфирных масел различных сортов винограда выделено около 1500 соединений, относящихся к спиртам, карбонильным соединениям, кислотам, ацеталам, сложным эфирам и углеводородам. Наиболее ценными ароматобразующими соединениями винограда, несомненно, являются терпеновые соединения. Они представлены углеводородами (мирцен и лимонен), спиртами (линалоол, α -терпинеол, цитронеллол, нерол и гераниол), а также ацетатами этих спиртов. Эти компоненты определяют у ягод винограда и в вине нежный цветочный аромат, которым отличаются мускатные сорта винограда. β -фенилэтиловый спирт, обладающий запахом розы и его ацетат также принимают участие в образовании аромата винограда [2].

По данным некоторых исследователей возможное содержание общих терпенов составляет 0,816-2,0 мг/дм³ в зависимости от сорта и зоны выращивания винограда [1]. Позднее С.И. Красохиной [4] были изучены некоторые сорта и гибридные сеянцы винограда межвидового происхождения в условиях Нижнего Придонья, и было установлено, что содержание терпеноидных соединений варьировало от 4,0 до 8,6 мг/дм³.

У сортов винограда, не обладающих мускатным ароматом, но также с ароматическими характеристиками, находят эти же терпены, но в значительно более низких концентрациях, порядка 0,2 мг/дм³, но в этом случае терпены также ответственны за специфический аромат винограда. У сортов с менее выраженным ароматом (Совиньон, Мюскадель) присутствуют эти же самые терпены, но в концентрациях 0,05 мг/дм³. Во всех сортах, ягоды которых не имеют выраженной ароматической характеристики, эти терпеновые производные присутствуют как следы [4].

Ранее проведенные исследования показали, что синтез эфирных масел в ягоде винограда происходит до определенного момента, затем он прекращается, и абсолютное количество эфирных масел начинает снижаться. Наивысшая концентрация ароматобразующих веществ наблюдается в период технологической зрелости, а в дальнейшем их содержание в ягодах понижается [3].

Наличие ароматобразующих веществ в ягодах винограда является высоко ценным свойством для сортов всех направлений использования – потребления в свежем виде и переработки на вино и соки. Однако качественный и количественный состав ароматобразующих соединений сортов и форм винограда в связи с их происхождением и