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ESTABLISHING APPROPRIATE PARAMETARS FOR ROOTING OF MICROPROPAGATED PEAR ROOTSTOCK OHF X 333 *(PYRUS COMMUNIS L.)*

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Abstract
The study was carried out in period September 2016 – April 2017, in laboratory for in vitro propagation of Fruit Growing Institute – Plovdiv. These rootstocks were obtained by hybridization of pear varieties of Old Home X Farmigdale in the American state of Oregon. Micropropagated plants *in vitro* were rooted on B (Dimanov, 1987) nutrient media in two different forms (solid and liquid with perlite) with different concentration of plants grow up regulators- auxins (IAA and IBA). The results show that the micropropagated plants of this rootstock are highly dependent from the nutrient media composition and concentration of grow up regulators for rooting. Plants with roots was produced 15 – 20 days after cultivated on nutrient media for rooting, but results were reported 30 days after cultivation date . According from forms (solid and liquid) and plants regulators –auxins (IBA and IAA), percent of the rooted plants it is different.

Keywords: pear rootstocks, rooting, micropropagated, *in vitro*.

Introduction

The pear is valuable fruit crop in our and international market. In the last years the cultivated areas with this culture are significantly reduced. One of the real reasons are Fire blight caused by the enterobacterium *Erwinia amylowora* and incompatible of same pears cultivars with quince (*Cidonia oblonga)* rootstock. By biotehnological methods can be produce a pear rootstoks, resistant to *Erwinia amylovora*, nematodes and *Phytophtora cactorum*. Tolerant of drought, heavy and calcareous soils and low temperatures and suitable with all pear kinds. Theese rootstocks were obtinated by hibridization of pear varieties of Old Home x Farmigdale in American state Oregon. Some clones of OH × F are difficult to propagate, that is why they are propagated in vitro. Also they are free from viruses, with quaranteed varietal autenticity.

Material and methods

The attempts are conducted in laboratory for in vitro propagation of the Fruit-Growing Institute-Plovdiv in period September 2016-April 2017. The scope of this study is to explore the micropropagated rootstocks, for risogenesis on different variants B (solid and liquid with perlite) nutrient media. The Perlite was accepted as a component of the examined nutritive media that means easier manipulation with micro-cuttings from the practical point of view. The chemical compositon of the nutrient media is combination of few basic ones medias for tissue cultures: macronutrients in ½ MS (Murashige and Skoog 1962), micronutrients in H (Heller, 1953), is removed AlCl3 and vitamines in MW (Morel and Wetmor 1951). For rooting were used two plant grow regulators (IBA and IAA) in different concentration (0,5; 1,0; 1,5) mg/l. Like a carbohidrated sourse used 20g/l sucrose. The PH was adjusted to 5,7 ± 0,1 for all media prior to autoclaving at 121°C for 20 min and 1 atm. In solid nutrient media includet 6g/l agar-agar. The plants are placed in a growth - chamber at 22 ± 2 °C and photoperiod 16/8 light/dark. The study was carried out in glass jars with a volume of 600 ml (solid media) and 150 ml (liquid with perlite) (Fig.5). Each container contained 100 ml, respectively 40 ml of culture medium, on which 10 shoot tips with a length of 15 mm and 6 unfolded leafs were set.

Table 1. Rhizogenesis nutrient media with different PGRs concentration and forms

Nutrient media	PGR	Concentration (mg/l)
B` solid	IAA	0,50
B` liquid	IAA	0,50
B1 solid	IAA	1,00
B1 liquid	IAA	1,00
B2 solid	IAA	1,50
B2 liquid	IAA	1,50
B3 solid	IBA	0,50
B3 liquid	IBA	0,50
B4 solid	IBA	1,00
B4 liquid	IBA	1,00
B5 solid	IBA	1,50
B5 liquid	IBA	1,50

The results were reported 30 days after cultivation date. After propagated, the next step is rooting of the plants. Initially, the culture media B (Dimanov, 1987) was customized for Solanaceae sp, but in our case, depending from the forms (solid or liquid with perlite) and from PGRs concentration (Table 1) this media is suitable for OHFx333 rootstock.

Results and discussion

Rhizogenesis induction in vitro and the rate at which the progress goes, it is essential for overall development of plants. The source nutrient media, showed that the rooting of the micro - propagated plants are heavily dependent from PGRs and the shape of the nutrient medium (liquid with perlite and solid); (Fig. 6). The results presents in Fig.1 shows that the most favorable for the rooting were obtained on solid culture media as opposed to liquid. The highest percentage of rooting we received for the plants cultivated on B` solid nutrient media ≈97% and B2 solid nutrient media ≈ 93%. Both of them are with same PGR (IAA), but in different concentration B` 0, 5 mg/l IAA, and B2 - 1, 5 mg/l IAA. In the other variants of media the percentage of rooted plants is smaller (Fig.1).

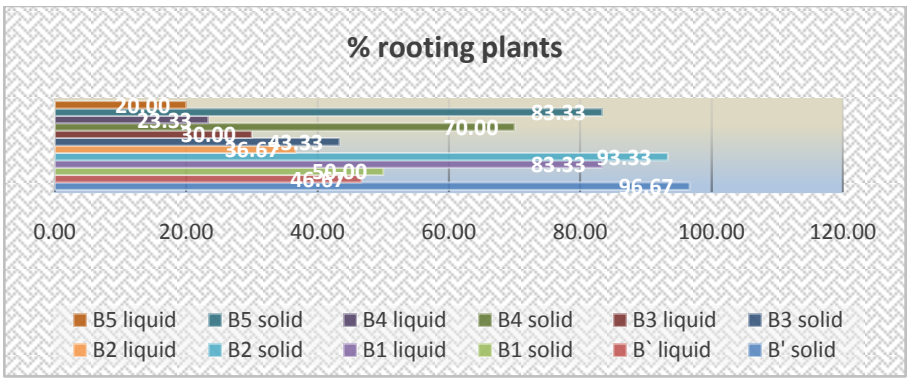

Figure 1. Rooting plants on different B media (%)

Taking into account the results, plants with average number of roots we have received again on B` solid and B2 solid media. The smallest average number of roots were obtained on B5 liquid media, with 1,5 mg/l IBA. (Fig.2) The next indicator, average length of the roots is showed on Fig.3. The induction of rhizogenesis and root system development to different degrees is reported for all media variants after 30 days of cultivation. Greatest average length of the roots is for plants rooted on B1 liquid media. Similar results were obtained on nutrient media B2 solid. The forms and PGR concentration are the differences between both of mediums. B1 liquid with perlite– 1, 00 mg/l IAA and B2 solid- 1, 5 mg/l IAA. Rooted plants with shortest roots were obtained on B4 liquid with perlite and B5 liquid with perlite media.

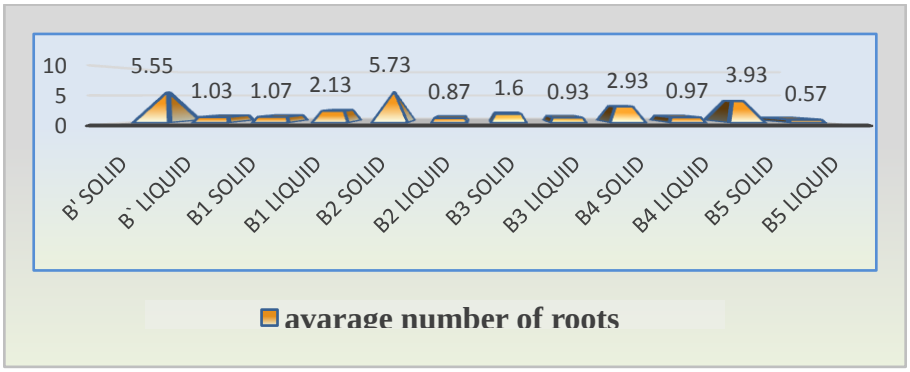

Figure 2. Average number of roots cultivated on variants B media, differences between solid and liquid with perlite and kind of PGRs and their concentration

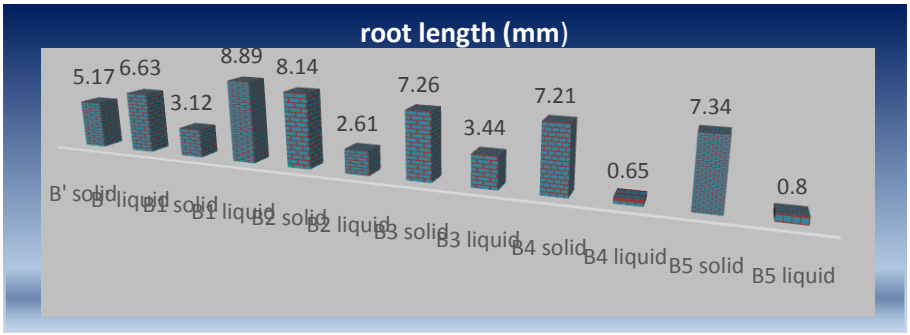

Figure 3. Average root length cultivated on variants B media, differences between solid and liquid with perlite and kind of PGRs and their concentration

The average length of roots is smaller than 1mm. After multiplication, the plants were set on rooting culture medium, with shoot length 15mm. For 30 days, shoots had grown up with different height. Fig.5. In attempt, highest shoot growth was measured on B4 solid and B5 solid. The both of them include the same composition. The only difference is concentration of PGR IBA. B4 solid – 1, 00 mg/l IBA and B5 solid – 1, 5 mg/l IBA. Rootstock with lowest shoots were obtained in B` liquid with perlite culture media.

In the first time, the medium B has been developed for tobacco rooting (Dimanov, 1987), later is successfully used for growth and development of varieties of potatoes, (Dimitrova and Dimanov 2002), representatives of the family *Solanaceaea sp*. (Dimanov et all, 2013), chrysanthemum (Dimanov et al. 2001) ,and grape (Roychev et al. 2002). Other research showed a successfully plant rooting in vitro from OHF x333 rootstock on MS medium with ¼ MS macro-nutrient, 1.00mg/l IBA and ¼ MS macronutrient, 1, 00 mg/l IAA (Kornova and Popov. 2014), where rooting is more than 90%, independently from the PGRs. Liquid B media was successfully used for potatoes in vitro rooting (Dimitrova and Dimanov 2002), but is not applicable for studied plants. Our results showed that apart MS basic media, for the pear rootstock OHFx333 can be used the other with different chemical composition, like B media.

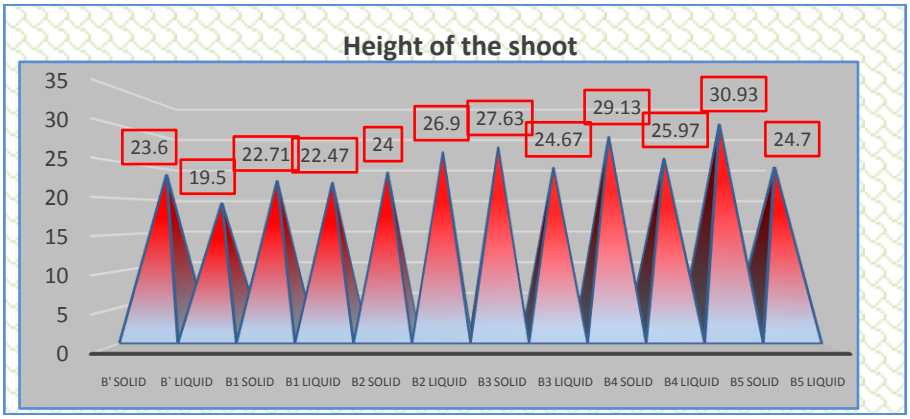

Figure 4. Average height of shoot (mm) on B nutrient media with differences PGR and forms (solid and liquid with perlite)

Fig.5. OHF x 333 rootstock in liquid with perlite and solid nutrient medium

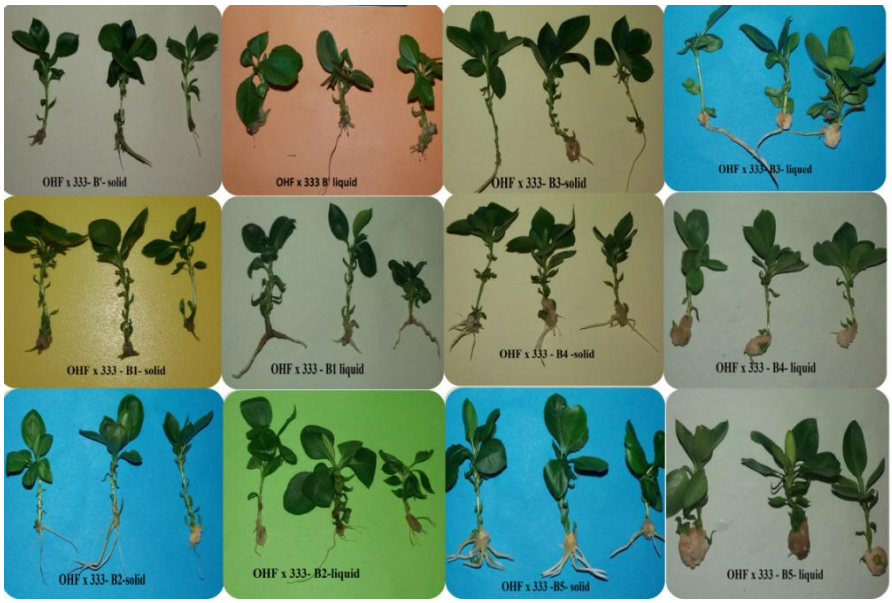

Figure 6. The rooting of the micro - propagated plants are heavily dependent from PGRs and the shape of the nutrient medium (liquid with perlite and solid)

Conclusions

Successfully rooted micro-propagated plants of OHFx333 pear rootstock. Better results were achieved on solid nutrient medium than liquid. The highest percentage of rooting we received for

the plants cultivated on B` solid and B2 solid nutrient media. Greatest average length of the roots is for plants rooted on B1 liquid media. Highest shoot growth was received on B4 solid and B5 solid

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