SUMMARY

PhD thesis:

EFFECT OF EXPLANTS, GROWTH HORMONES AND CULTURE MEDIA ON MICROPROPAGATION OF PEACH VARIETIES

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PhD thesis **Effect of explants, growth hormones and culture media on micropropagation of peach varieties** is structured in four experiments, completed by the Introduction, Summary, Conclusions and recommendations, references and annexes. The study was conducted at the Tissue Culture Laboratory of the Faculty of Horticulture and Micropropagation Laboratory of the Reserch Center for Studies of Food and Agricultural Products Quality, University of Agronomic Sciences and Veterinary Medicine Bucharest (USAMV), Mărăşti Blvd., no. 59, 011464, Bucharest, Romania. (https://www.usamv.ro/index.php/en/) between 2016 and 2019 on peach (*Prunus persica* L.).

The main research objectives were: General research objective (Use of *in vitro* culture techniques for the propagation of peach varieties) with Specific objectives (Study of different types of peach explants for *in vitro* culture initiation; Study of optimal hormonal balance in different peach micropropagation phases; testing of culture media in different phases of peach micropropagation; Developing a technology for *in vitro* cultures multiplication of peach varieties)..

The justification was that, multiplying peach varieties *in vitro* would open a new era. The advantages of this method are, among other things, the rapid multiplication and introduction of new varieties in production, propagation of virus-free material, uniform planting material, etc. The study included 55 tables and 110figures and graphs.

The first experiment included two explants: shoot tips and nodes in primary sterilization by using ethanol 70% for 2-3 min (C_2H_5OH), and four sterilizing agents, 18 different variants were tested: sodium hypochlorite (NaOCl) in three concentrations: 5%, 10% and 15% for 5 and 10 minutes; hydrogen peroxide (H_2O_2) in two concentrations: 5% and 10% for 10 and 20 minutes; captan (50%) WP fungicide in four concentrations: 1%, 2%, 3% and 4% for 5 minutes and boric acid (BH_3O_3) in two concentrations: 1% and 2% for 5 and 10 minutes. The various sterilization protocols were tested for the successful establishment of the *in vitro* culture of peach tissue culture. Our results showed that during sterilization they were dependent on sterilization factors, exposure time and type of explants used for micropropagation.

It is recommended to use this study among the different sterilization variants, sodium hypochlorite being the most effective treatment with a survival rate of 50% at (15% concentration for 5 minutes) and a survival rate of 60% (at 10% concentration time 10 minutes). Also Hydrogen peroxide (H_2O_2) is recommended at 10% concentration for 20 minutes; Captan 50% in 4% concentration for 5 minutes; Boric acid (BH_3O_3) in 2% concentration for 10 minutes recommended for use in initial sterilization.

In the second experiment the aim of the study was to test the ability of some Romanian peach varieties (*Prunus persica* (L.) Batsch) to be propagated *in vitro* and then to be cultivated on their own roots. The peach varieties micropropagation is not a common practice, while most of the peach rootstocks are nowadays produced *in vitro*. Ten peach varieties were included in the experiment: three dwarf varieties (Valerica, Cecilia, Dan) a standard controll - Redhaven and six commercial cultivars (Raluca, Monica, Florin, Filip, Mimi and Catherine sel. 1). Two explants (shoots-tip and nodes at 0.5-1cm length) were taken from trees planted in the field. The explants were cultivated on MS medium consisting without any hormone supplements during initiation stage and add benzyl aminopurine (BAP) on four

variants with 0, 1, 5 and, respectively, 10 mg/l was used as cytokinine during multiplication stage (T1=0 mg/l (control); T2=1 mg/l; T3=5 mg/l; T4=10 mg/l).

The results showed there were significant differences between the varieties tested in the sterilization and initiation stage. There was a highly variable response to commercial varieties and a small response from the dwarf varieties. In the initiation stage, Florin and Filip varieties showed the highest percentage of buds growing (68%) for each one, while Cecilia and Catherine sel. 1 varieties showed lowest percentage of buds growing (12%, respectively 20%). Also, in the initiation stage, Florin and Monica varieties showed the highest shoot length (4.78 cm, respectively 3.94 cm), and Florin, Valerica and Dan varieties showed highest leaves number formed (8.00 leaf/explant, 6.20 leaf/explant, 6.20 leaf/explant) respectively. While Dan and Cecilia varieties showed lowest shoots length (1.59 cm, 1.67 cm) and Valerica variety showed lowest leaves number formed (2.40 leaf/explant).

The results showed highest percentage of contamination on nods cultivated in cultures tubes, and Valerica and Dan varieties were recorded highest percentage of contamination. Also, in initiation stage there was a statistically significant disparity between shoots length and leave number formed (total average shoots length 2.95 cm per shoots tip explant, total average shoots length 2.47 cm per nods explant and total average leaves number formed 4.76 leaf per shoots tip explant, total average leaves number formed 3.89 leaf per nods explant, in multiplication stage only 4 varieties with control succeeded in giving the average number of shoots formed by adding BAP to MS (Florin 8.00 shoots/explant, Filip 7.40 shoots/explant, Mimi 5.40 shoots/explant and Redhaven 4.00 shoots/explant) respectively, while the 4 varieties (Valerica, Cecilia, Raluca and Monica) were lost after two weeks of testing.

Also Dan and Catherine sel. 1 varieties did not produce acceptable results. Also, there are significant differences between T2, T3, T4, and T1 (control) treatments for all studied criteria, T3 (5 mg/l BAP) (2.86 shoots/explant) treatment was superior on other treatments in terms of total average number of shoots formed per explant T1 (1.00 shoots/explant); T2(1.58 shoots/explant); T4(1.00 shoots/explant), this confirms that the presence of hormones in the culture media is the main factor in the multiplication of peach varieties and the importance of adding to culture media.

The third experiment included three peach varieties (Florin, Filip and Mimi). Two explants (shoots-tip and nodes) were taken at 0.5-1cm length. The explants were cultivated on 3 medium MS (Moorashige and Skoog, 1962), B5 (Gamborg, 1968) and QL (Quoirin and Lepoivre, 1977) adding of 30 g/ l sucrose and 7 g/ l agar without any hormone supplements during initiation stage. In multiplication stage were tested different concentrations of plant hormons BAP and NAA in 12 variants. The cultures tubes were kept in the dark (0, 1, 2, 3, 4) days to get rid of the process of oxidation of phenolic materials resulting from cutting plant tissues. It was found during this study that the placement of the tubes of culture in the dark has a positive effect in the disposal of phenolic materials that cause discoloration of explants in black or brown and cause the toxicity of the cultivated tissue and low success rate of establishing culture.

The results presented that placing the explants in dark for 2 - 3 days was the best result to explants phenolic-free, in 2 days/dark (Florin 89% explant growing, Filip 83% explant growing and Mimi 85% explant growing), in 3 days/dark (Florin 89% explant growing, Filip 84% explant growing and Mimi 86% explant growing). In initiation stage the results showed that there was growth of all varieties on all the culture media used in the experiment. MS culture medium gave the highest mean longitudinal of shoots for all varieties used MS (Florin 3.33cm, Filip 2.61 cm and Mimi 2.59 cm) respectively, while the B5 culture medium gave the lowest value (Florin 2.15 cm, Filip 1.84 cm and Mimi 2.01 cm) respectively.

In multiplication stage the results showed that there were significant differences at 0.05 level.

All cultivars were given shoots depending on the amount of phosphorus in the culture medium and the concentration of the added plants hormones. Cultures media MS and QL showed the highest mean number of shoots at 5 mg/l BAP in (MS) medium (Florin 4.20 shoots/ explant, Filip 3.60 shoots/ explant and Mimi 4.00 shoots/ explant respectively), and QL medium (Florin 4.40 shoots/ explant, Filip 2.40 shoots/ explant and Mimi 4.00 shoots/ explant) respectively, while the B5 culture medium gave the lowest value (Florin 1.80 shoots/ explant, Filip 1.40 shoots/ explant and Mimi 2.20 shoots/ explant) respectively.

Moreover, we were found that there is a relationship between BAP and NAA, since the addition of both hormones has led to the emergence of callus tissue (the highest callus formation were recorded in Florin variety 44 % callus/explant in T1 (1/2 MS +1.00 BAP mg/ l + 0.50 NAA mg/l), while adding just BAP to all media in the experiment gave shoots. Also QL medium give yellowish green shoots wiht a high resistance 95% to vitrification is a physiological disorder that affects the proliferation of tissue based on the cultivation of many plant species.

In fourth experiment, Florin peach variety and Myrabalan 29C rootstock were included in the experiment. Explants (node contains 1 bud) were taken at a length of 0.5-1 cm. For the preparation of graft and rootstock, two methods were tested for this operation: Method I: submerging the explants resulting from the initiation step in solutions containing NAA and IBA in concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l, respectively; Method II: take the explants resulting from the initiation step and grow these explants into culture media tubes containing NAA and AIB in concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l respectively. For the application of micrografting, two methods were tested for this operation: Method I: Micrografting before rooting; Method II: Micrografting after root formation.

The study demonstrated the success of the micro-grafting method of peach varieties resulting from tissue culture technology and it can be used to produce a large number of pathogen-free seedlings. Also, these results show the role of IBA in rooting the explants that gave the highest rate of root number (7.00 root/explant) and root length (9.07 cm root length/explant) at all concentrations studied with the same NAA concentrations. Also, the method of submerging explants (immersion in solutions containing rooting hormones and then planting them in hormone-free culture media) was superior to the number and length of roots produced by the method of adding rooting hormones to culture media.