RESEARCH ARTICLE **(Open Access)**

Influence of Chemical and Physiological Factors on Rooting and *in Vivo* Acclimatization of the GF-677 Peach Rootstocks Propagated Through *In Vitro* Techniques

ELEKTRA PAPAKOSTA[1*], LUMTURI XHELILAJ[2], ADHURIM LAZAJ[3], ATHINA LLAMBRO[4]

[1,2,3,4]Centre of Agricultural Tecnology Transfer (ATTC) Vlore, Shamogjin, Albania

Abstract

GF-677 hybrid peach x almond hybrid (*Prunus persica x Prunus amygdalus*) is suitable in calcareous, non-irrigated, dry and hilly soils, susceptible to Agrobacterium, having good productivity. It is propagated using grafts and *in vitro* culture. The micropropagation of the peach rootstock aims to produce clean and certified phytosanitary fruit seedlings in terms of phytosanitary and genetic safety.

The aim of this study is to optimize the micropropagation protocols of GF-677 by evaluating the influence of chemical and physiological factors on the proliferation and rooting of the rootstock. Plant materials (apical buds) were sampled from the greenhouse with clean and controlled "mother" plants. The explants were disinfected with two sterilizing agents: NaOCl 0.5 % for 20 minutes, where the plants showed a high survival rate of 75%; and $HgCl_2$ 0.03 % where explants showed a lower survival rate of 44%. The effects of phytohormones in the proliferation phase were analyzed comparing different concentrations of BAP according to the scheme: 4 variants x 4 replications (0.5 mgl $^{-1}$; 1 mg l $^{-1}$; 1.5 mgl $^{-1}$; 2 mgl $^{-1}$) with 15 plants each. The best results were obtained in the concentration of BAP 1mgl $^{-1}$ after three subcultures at the highest values of the average number of shoots 2.6 and shoot length 2.84 cm. In the proliferation phase, different nutrient media protocols were studied: DKW; MS; MS/2. The best results were recorded in the MS nutrient media (Murashige & Skoog, 1962) with the average number of shoots 5.78; average length of shoots 5.6cm; average number of leaves 8.9. The highest rooting index was achieved in MS nutrient media with average number of roots 5 and average root length 2.37 cm. After *in vivo* acclimatization of the plants, biometric parameters were measured to evaluate the grafting standard (d = 7mm) followed by grafting of the GF-677 with almond scion (Tuono) carried out. Grafting percentage resulted 70 % After budding, in the vegetative phase, were evaluated the data on the average length of shoot growth in April 40.5cm; May 71.6; June 103.2 cm and July 140.8 cm.

Keywords: Growth regulator, sterilizing agents, media MS, tuono, cytokinin, auxin

1. Introduction

Peach rootstock GF-677, peach-almond hybrid (*Prunus persica x P. amygdalus*) was selected in France in 1940 [33]. In vitro micropropagation technique allows the overcoming of small rooting difficulties for cuttings [25]. The first micropropagation studies of GF-677 from apical buds were performed by [34]. In Italy [38] performed the *in vitro* micropropagation of the peach rootstock. GF-677 is a potent rootstock [18, 19] and chlorosis tolerant [3, 30], that is added by *in vitro* techniques. The fit on the field is very good. The grafts have a rapid growth rate and may allow grafting in pedoclimatic conditions, in august-september, as the most suitable period. Placement in the soil leads to rapid development of the slightly branched, strong and well-distributed root system. The trees grafted on this rootstock are vigorous and productive [19, 22]. The GF-677 rootstock is drought tolerant and disease

*Corresponding author: Elektra Papakosta; E-mail: elektrakrist@yahoo.com
(Accepted for publication 24.12.2021)
***ISSN**: 2218-2020, © Agricultural University of Tirana*

resistant [25] and is compatible with many almond and peach cultivars [14].

Sexual reproduction of GF-677 results in the production of non-uniform seedlings. To prevent this phenomenon, asexual method with micropropagation has been applied. *In vitro* culture, the phenomenon of vitrification (glassy appearance) is often encountered, which causes a decrease in the percentage of rooting [37]. [21] have used soils with different vitamins in the rooting phase. [29] have reported the success of the effect of mio-inositol and thiamine on micropropagation of GF-677 rootstock.

The aim of our study is to optimize the *in vitro* micropropagation protocol of GF-677 rootstock in rooting and *in vivo* acclimatization in the preparation of grafted seedling as a clean and controlled product.

2. Material and Methods

2.1. Plant materials; 2.2 Inoculation;
2.3. Propagation; 2.4. Rooting;
2.5 Acclimatization

In the greenhouse of the "mother" plants of the nuclear fruit rootstocks in Experimental Base of QTTB Vlora, in May 2019 was taken plant material - apical buds (1.5-2cm) in new branches. The explants were rinsed with running water 2-3 times, immersed in 70 % ethyl alcohol for 10 " and disinfected with two sterilizing agents: NaOCl 0.5 % and $HgCl_2$ 0.03 % for 20 minutes. It was then rinsed twice with sterilized distilled water. In the profiling phase, different concentrations of BAP cytokinin were analyzed according to the scheme: 4 variants x 4 replications (0.5 mgl^{-1}; 1 mgl^{-1}; 1.5 mgl^{-1}; 2 mgl^{-1}) with 15 plants each. The effect of salt concentration on the proliferation and rooting of shoots as a test of nutrient media was also studied: DKW [10]; MS and MS/2 [23]. Data were obtained after three subcultures. Plant cultures went through these stages: inoculation, proliferation, rooting, acclimatization. The growing conditions of the crops in the vegetative room of the plants were with controlled parameters: photoperiod 16 hours/ light and 8 hours/darkness, temperature 24 ± 1° C, phosphorescent lighting 3500 lux. Plants after *in vitro* rooting were transferred to *in vivo* acclimatization process. After 1 year of *in vivo* growth of plants, biometric measurements were made in the evaluation of the grafting standard (d = 7mm) and then the grafting of GF-677 rootstock with almond scion (Tuono) was performed in September 2020. After opening the buds, after grafting, in the vegetation period (April-May-June-July) of 2021, the data on the average growth length of the shoots of standard seedlings were evaluated.

Biometric indicators in the study: Proliferation stage: number and length of shoots;

Rooting stage: number and length of roots; Acclimatization stage: trunk diameter and shoot length; Visual indicators

Statistical analysis. Data processing for all experimental tests was done by analysis of variance ($P < 0.05$) Test (ANOVA). Data are presented as mean accompanied by standard error and standard deviation. Mean comparisons were made using the Tukey-Kramer test. Statistical analysis was performed with the statistical program JMP version 16.0.

3. Results and Discussions

3.1. Disinfection with sterilizing agents

After receiving the explants, they were disinfected, after being cut to the right size. The reaction of the plant explant in the inoculation terrain according to sterilizing agents: NaOCl sodium hypochlorite 0.5 % and tested $HgCl_2$ mercury chloride 0.03 % was analyzed and evaluated in the percentage (%) of survival as well as percentage of GF-677 explants infection. The survival of the explants is conditioned by the chemical agents used in the sterilization, the time of their action, the period when the explants were taken, the type of explant and the growing conditions.

Explants - apical buds inoculated in the universal planting nutrient medium MS [23] after sterilization with NaOCl 0.5 % for 20', after 4 weeks in the vegetative growth chamber, gave a high survival rate 75 %. The occurrence of necrosis was shown by 5% of explants and fungal contamination 20 %. (graph. 1).

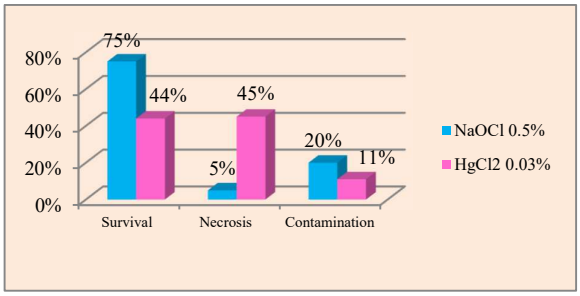


Figure 1. Survival rate of explants according to sterilizing agents

Sterilization of buds explants with mercury chloride $HgCl_2$ 0.03 % for 20', resulted in a lower survival rate of 44 %. The necrosis rate was high 45 % and fungal contamination 11 %. These results conclude with similar works [1].

GF-677 explants were not isolated from adult field trees but were taken to the screen house from clean and controlled "mother" plants and sterilized with strong reagents such as $HgCl_2$ 0.3 % for 20 min. as a toxic substance, damages plant cells by displaying the phenomenon of necrosis that reduces their survival rate and causes their death. [31] treated the buds of *Prunus avium* L. with NaOCl solution 0.35 % for 15 min. and 70 % ethanol for 30 sec. Sterilization of the explants with NaOCl 0.5 % was more effective compared to $HgCl_2$ 0.03%. [32] has shown different sterilization schemes of stone fruits explants with varying degrees of contamination. However treatments with NaOCl in stone fruits from (0.5-0.8 %) are more effective in controlling the degree of contamination [16].

3.2. Effect of phytohormones on shoot proliferation

The effects of phytohormones in the MS terrain proliferation phase were analyzed by comparison different concentrations of BAP (0.5 mg l^{-1}; 1 mgl^{-1}; 1.5 mgl^{-1}; 2 mgl^{-1}) with 15 plants each. Cytokines in the case of bud explants inhibit apical dominance and stimulate the development of lateral buds [35]. The cytokinin BAP is combined with the endogenous auxin ANA (0.1 mgl^{-1}) with the effect on callus formation, increase in stem length. The best BAP concentration resulted (1mgl^{-1}) after two subcultures, which gave the highest values of the average number of shoots 2.6 and the average length of shoots 2.8 cm. By changing the concentrations of BAP more than (1 mgl^{-1}) the negative influence reflected in biometric indicators has been evidenced. Increasing the concentration to higher values decreases the level of GF-677 proliferation. This is caused due to hight cytoninin concentrations stimulate formation of small buds wich can not further develop.

The data of our evidence were statistically processed by means of analysis of variance (ANOVA), Tukey-Kramer test on the veracity of the evidence.

Table 1. The diagram of boxplots (variances, standard deviation and the mean) for the number of shoots for explants

Concentrations BAP (mg/l)	**Average**	**Groups test Tukey-Kramer**
Control	0.54	c
0.5 mgl^{-1}	1.86	ab
1 mgl^{-1}	2.6	a*
1.5 mgl^{-1}	1.73	ab
2 mgl^{-1}	1.45	b
Average	1.63	

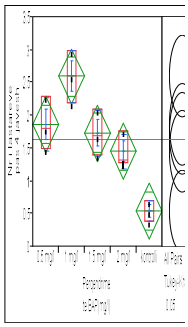
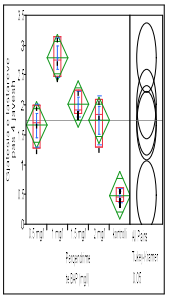


Table 2. The diagram of boxplots (variances, standard deviation and the mean) for the length of shoots for explants

Concentrations BAP (mg/l)	**Average**	**Groups test Tukey-Kramer**
Control	0.49	c
0.5 mgl^{-1}	1.66	ab
1 mgl^{-1}	2.8	a*
1.5 mgl^{-1}	2.02	ab
2 mgl^{-1}	1.75	b
Average	1.74	



Analysis of variance (ANOVA) on estimating the number and average length of shoots in the test according to BAP concentrations in the proliferation phase (Tab. 1, 2) shows that we have statistically proven differences for the level of authenticity (P = 0.05) according to the Tukey-Kramer test. Comparisons of means for the variants studied for the smallest proven difference Lsd = 3.29108 for @ = 0.05 list the treatments in different classes represented by different letters. Cytokines stimulate cell division, lateral buds formation, promoting shoots proliferation [9]. Influence of cytokinins in tissue and organ cultures depend on nutrient medium, species and age of explant [36].

Buds proliferation and elongation are stimulated by the combination of cytokinin BAP with auxin ANA 0.1 mgl^{-1}. [28, 5].

The interaction between the nutrient medium and BAP has a significance of $P \leq 0.05$ for the number of shoots per explant. After 4 weeks for the mean values of the number te sprouts 2.6 and shoot length 2.8 cm achieved high results in BAP concentrations $1mgl^{-1}$. This result indicates that there is a positive correlation between BAP concentration and number of shoots at a given concentration 1 mgl^{-1}. At concentrations of BAP higher than $1mgl^{-1}$ there is a decrease in the number of shoots. The reason may be the reducing effect of high concentrations of BAP, which may cause callus formation in tissue cultures [7]. Similar results [17, 24, 34] have been reported in the micropropagation of GF-677 buds in MS nutrient medium with 1 mgl^{-1} BAP.

3.3. Influence of salt concentration on GF-677 proliferation

In the subculture stage [13], shoots in the DKW terrain turned yellow, showing the phenomenon of necrosis and cases of tissue oxidation, as a result of the high concentration of salts in the terrain. In the stage of plant proliferation in the DKW terrain, the phenomenon of vitrification (glass appearance) of plants was observed, such as weak crops, with necrotic tops, large leaves, lack of juvenility, yellowed and arched plants [6]. Other authors [4] report good results in rooting *Juglans regia* L. in DKW nutrient medium.

Table 3. The diagram of boxplots (variances, standard deviation and the mean) for the number of shoots for explants

Culture medium	**Average**	**Groups test Tukey - Kramer**
DKW	5.375	a*
MS	5.775	a
MS/2	3.35	b
Average	4.833 333	

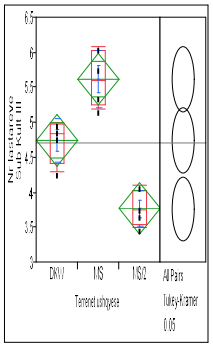


Table 4. The diagram of boxplots (variances, standard deviation and the mean) for the length of shoots for explants

Culture medium	**Average**	**Groups test Tukey Kramer**
DKW	4.5	a
MS	5.6	a*
MS/2	4.9	a
Average	5.0	

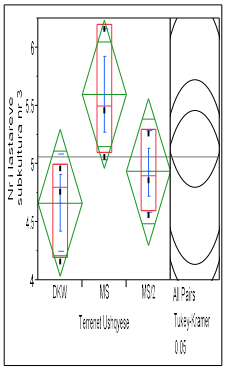
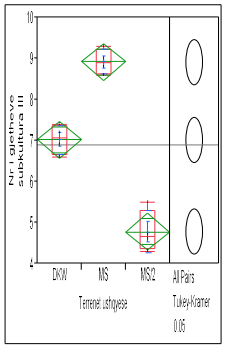


Table 5. The diagram of boxplots (variances, standard deviation and the mean) for the leveas for explants

Culture medium	**Average**	**Groups test Tukey Kramer**
DKW	7.025	a*
MS	8.925	a
MS/2	4.775	b
Average	6.908 333	



Analysis of variance (ANOVA) on the estimation of the number of shoots in the test according to nutrient medium in subculture three stage (table 3, 4, 5) shows that we have statistically proven differences for the level of authenticity P = 0.05 according to the Tukey-Kramer test. Analyzing the data, the best results of *in vitro* culture of GF-677 explants in the proliferation phase after three subcultures (fig. 1a; 1b), were obtained in MS nutrient medium. Regarding the average length of the shoots (tab. 4) there are no significant differences between the terrains MS/2 and DKW. Good results in GF-677 proliferation were also obtained in MS/ 2 terrain, with half of the salt concentration [8].

MS terrain [23] rated best: average number of shoots 5.78; average length 5.6 cm; average number of leaves 8.9, shoots are elongated, slender and intensely green in color, young shoots developed normally and without the presence of necrosis. These results are consistent with similar work on GF-677 [1, 27].

This better increase in MS terrain is due to the higher content of N_2 (nitrogen 1471 m l^{-1}) and K (potassium 781 mgl^{-1}) in the terrain.

[31] reported successful micropropagation of *Prunus avium* in MS terrain. [27], have reported successful micropropagation of *Prunus avium* buds in MS terrain, combined with BAP.

Other authors [15, 20] have reported optimal results in micropropagation of the genus *Prunus* by cultivating buds explants in MS terrain. [17] refer to good results in proliferation and rooting in KNOP terrain with 1mgl^{-1} BAP for proliferation and 0.3 mgl^{-1} NAA for rooting. [2] reported good results in the addition of GF-677 in the study of the concentration of constituent components of the nutrient medium

3.4. GF-677 rooting phase by nutrient medium

After the proliferation phase it was passed to the rooting phase of the seedlings using a rooting terrain respectively according to the three proliferation terrain protocols (MS, MS/2, DKW), in the presence of IBA hormone 0.2 mg l^{-1}. The rooting phase (20 days) resulted in significant changes for the three tested terrains, in numerical and morphological terms. (fig. 1 c)

a.Proliferation b. Subculture c. Rooting

Figure 2. (a, b, c). Micropropagation of GF-677 rootstock

The data of our evidence were statistically processed by means of analysis of variance (ANOVA), Tukey-Kramer test on the veracity of the evidence

Table 6. The diagram of boxplots (variances, standard deviation and the mean) for roots of number for explants

Culture medium	**Average**	**Groups test Tukey - Kramer**
DKW	4.15	a*
MS	5	a
MS/2	2.625	b
Average	3.925	

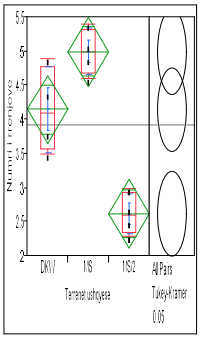
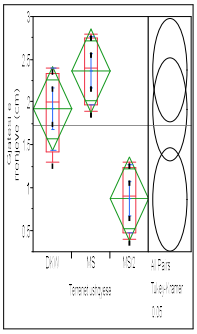


Table 7. The diagram of boxplots (variances, standard deviation and the mean) for the roots length for explants

Culture medium	**Average**	**Groups test Tukey - Kramer**
DKW	1.925	a*
MS	2.375	a
MS/2	0.875	b
Average	1.725	



MS terrain [23] is most effective in rooting GF-677: average number of roots 5, average root length 2.37 cm, with normally developed plants, elongated leaves, medium size, green color [1]. MS/2- terrain halved in macro-microsalts. Plants cultivated in this nutrient medium had normal performance, with leaves with medium development, number of roots 2.62 and root length 0.87 cm. [8, 12] have reported that reducing the concentration of salts in the MS terrain, increases the percentage of rooting and stimulates root elongation. The effect of reducing salts on rooting is explained by the reduction in the percentage of nitrogen [11].

DKW terrain [10] it resulted in inefficient soil for micropropagation of GF-677, plants with large and

yellowed leaves, bent shoots, with the presence of necrosis and vitrification [4], average number of roots 4.15 and root length 1.92 cm. According to the studied variants (tab. 6, 7), the average values of the number of roots according to different terrains MS, MS/2, DKW; have had variability.

3.5. Acclimatization phase of GF-677 rootstock

After the rooting phase, the plants were transferred to *in vivo* acclimatization as a delicate process for the seedlings, which adapted to the heterotrophic type of feeding, poor lighting, switching to the acclimatization greenhouse, in vivo autotrophic conditions, high lighting and a strong hydric stress. The acclimatization index resulted 83% for GF-677 plants. Rooted plants with short roots resulted in higher survival than those with long roots that gave a lower survival. After staying about (40 days) in the acclimatization greenhouse with hormonal and antiparasitic treatments, the plants (rootstocks) were shaded for growth (fig. 2 a).

a.Acclimatization phase b. Grafting phase

Figure 3. (a, b, c). Acclimatization and grafting phase

After a year of in vivo plants growth , the biometric measurements were made in the evaluation of the grafting standard (d = 7mm) and then the grafting of GF-677 rootstocks (20 plants) with almond scion (Tuono) in september 2020. Grafting percentage resulted to be 70% and is influenced by the grafting technique (fig. 2 b) after bud's sprout. In the vegetative period of 2021, the data of the average growth length of the shoots of standard seedlings were evaluated and resulted (40.5 cm) in April; (71.6 cm) in May; (103.2 cm) in June and (140.8 cm) in July (graph.2; fig. 2 c).

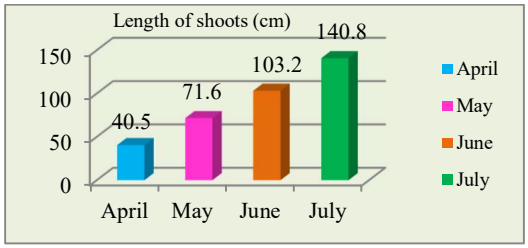


Figure 4. Mean length of shoots per months (April-May- June- July 2021)

These standard seedlings will be planted in the field and the study on the plant growth indicators will continue and the method of in vitro propagation will be compared with the traditional propagation.

4. Conclusions:

- The stages of proliferation the highest number and length of shoots 2.6 and 2.84 cm was recorded in MS culture medium supplemented with BAP at 1 mgl^{-1} ;
- MS terrain (Murashige & Skoog, 1962) rated best: average number of shoots 5.78; length of shoots 5.6 cm; number of leaves 8.9;
- In the rooting phase, the highest rooting index 76 % was recorded in MS medium supplemented with IBA at 0.2 mgl^{-1}, the highest number and length of roots 5 and 2.36 cm);
- Acclimatization phase: index resulted 83 %. Grafting percentage resulted to be 70% after bud's sprout. Average growth length of the shoots of standard seedlings were evaluated and resulted 40.5 cm in April, 71.6 cm in May, 103.2 cm in June and 140.8 cm in July;
- Optimisation of this micropropagation protocol for mass production of GF-677 rootstock, enhance the possibilities *in vivo* acclimatization in the preparation of grafted seedling as a clean and controlled product for further development of almond plantations in our country

5. Literature

1. Ahmad T., Rahman, H. U., Ahmad. C. H., Laghari, M. H. **Effect of culture media and growth regulators on micropropagation of peach rootstock GF- 677**. Pak. J. Bot., 2003, 35(3): 331-338.
2. Antonopoulou, C., Dimassi, K., Therios, I., Chatzissavvidis, C. **The influence of radiation quality on the in vitro rooting and nutrient**

concentration of peach rootstock GF 677. Biologia plantarum., 2004, 48, 549 -553.

3. Bindro A.S. **Inducing resistance to iron-deficiency chlorosis in peach-plants through the use of suitable rootstock.** J.of Research., 1975, 14: 38-40.
4. Caboni E., Delia G., La Starza S. **La radicazione *in vitro* di noce (*Juglans Regia* L.)** Convegno Nazionale "L'Innovazione nel Vivaismo Ortoflorofrutticolo" Italus Hortus. 2003. Vol 10. nr.4.
5. Damiano, C., Monticelli, S., A Fratarelli. **Allestimento di una collezione *in vitro* di pesco di interesse storico"** VI Convegno Nazionale sulla Peschicoltura meridionale. 2008, P.60.
6. Damiano C., Catanero E., Giovinazzi J., Frattarelli A., Caboni E. **La micropropagazione del nocciolo.** Frutticoltura., 2004, No. 12: 53-56.
7. De Olivera M.L.P., Costa M.G.C., Silva C.V., Otoni W.C. **Growth regulators, culture media and antibiotics in the *in vitro* shoot regeneration from mature tissue of Citrus cultivars.** Pesquisa Aropecuaria Brasiliera., 2010, V45, p.654-660
8. Dimassi-Theriou. K. ***In vitro* rooting of rootstock GF-677 (*Prunus persica x P. amigadalus)*.** In Sciences Annals. Aristotle Univ. of Thessaloniki, 1995, pp.31-35.
9. Dobranszki, J., Teixeira da Silva, J.A. **Micropropagation of apple - A review.** Biotechnology Advances., 2010, 28: 462-488.
10. Driver J.A., Kuniyuki A.H. ***In vitro* propagation of paradox walnut rootstock.** *Hort. Sci.,*1984, 19:507-709.
11. Driver J.A., Suttle G.R. **Nursery handling of propagles**. In: Cell and Tissue Culture in Forestry. (Bonga. J.M & Durzan D.J. eds), 1987, pp. 320-335. Netherlands.
12. Fotopoulos S., Sotiropoulos T.E. ***In vitro* rooting of PR 204/84 rootstock (*Prunus persica x P. amigdalus*) as influenced by mineral concentration of the culture medium exposure to darkness for a period**. Agronomy Research., 2005, 3. 3-8.
13. Grant N.J., Hammat N. **Increased root and shoot production during micropropagation of cherry and apple rootstock: effect of subculture frequency**. Tree Physiol., 1999, 19:899-903.
14. Grassely C. **New French stone fruit rootstock**. *Fruit Varieties Jornal*, 1987, 41: 65-67.
15. Gurel S., Gulsen Y. **The effect of IBA and BAP on *in vitro* shoot production of almond (*Amygdalus communis* L.).** Tr.J. of Botany., 1998, 22:375-379.
16. Hu C.J., Wang P. J. **Meristem, shoot tip and bud cultures**. Ne: *Handbook of plant cell culture,* 1983, Vol. 1: 177-187.
17. Kamali K., Majidi E., Zarghami R. **Micropropagation of GF-677 rootstocks *(Prunus amygdalus x P. persica):*** CIHEAM Cahiers Options Méditerranéennes; 2001, n. 56, p. 175- 177.
18. Loreti F., Morini S., Grilli A. **Rooting response of PSB_2 and GF-677 rootstock cuttings**. Acta Horticulturae., 1985, 173: 261-269.
19. Maragoni B., Antonelli M., Scudellari D., Liverani A. **The behavior of cv 'Redhaven' on different rootstok**. Acta Hort*.,* 1985, 17: 94.
20. Marino F., Magnaini E., Battistini S., Righetti B. **Effect of hormone and main carbone Energy sources on *in vitro* propagation of apricot (*Prunus armeniaca* L.) cvs. San Castrese and Portici**. Acta Horticulturae., 1989, 293:355-362.
21. Miller, G**.** A., Coston, D, C., Denny, E. G., Romeo, M.E. ***In vitro* propagation of nemaguard peach rootstock.** *Hort. Sci*., 1982, 17: 94.
22. Minguzzi A. **Rootstcok effect on peach replanting**; A ten years trial. Acta Hort., 1989, 254: 357-361.
23. Murashige, T. & Skoog, F. **A revised medium for rapid growth and bioassay of tabacco tissue cultures.** Phsiol Plant., 1962, 15: 473-497.
24. Rahim N.M. **Micropropagation of GF 677 rootstock**. Jounal of Agricultural Science. 2012, Vol 4. No.5.
25. Rom C.R., Carlson R.F. **Rootstocks for fruit crops**. John Wiley and Sons., 1987.
26. Ruzic, D., Rosati, P., Marino, G.1984. **Effeto dei fitoregolatori nella micropropagazione**

dell' ibrido pesco x mandorlo GF-677. *Riv.* Ortoflorofrutticultura., 1984, *It.* 68: 413-422.

27. Ružić V., Vujović T.I**. The effects of cytokinin types and their concentration on *in vitro* multiplication of sweet cherry cv. Lapins (*Prunus avium* L.).** Hort. Sci. (Prague)., 2008, 35, (1): 12-21.

28. Scarpa G.M., Milia M., Satta M. **The influence of Growth Regulators on Proliferation and Rooting of *in vitro* Propagated Myrtle.** Plant Cell, Tissue & Organ Culture, 2000, 62: 175-179.

29. Sepahvand S., Ebadi A., Kamali K.,Ghaemmaghami S.A. **Effect of Myo-Inositol and Thiamine on Micropropagation of GF-677.** Journal of Agricultural Science., 2012, Vol.4. N. 2.

30. Syrgiannidis G. **Control or iron chlorosis and replant diseases in peach by using the GF-677 rootstock**. Acta Hort., 1985, 173: 383-388.

31. Shatnawi M.A., Shibli R., Qrunfleh I.,Bataeineh K., Obeidat M. ***In vitro* propagation and cryopreservation of *Prunus avium* using vitrification and encapsulation dehydration methods.** Journal of Food Agriculture & Environment., 2007, Vol.5 (2): 204-208.

32. Skirvin R.M. **Stone fruit.** *In: Hand Book of Plant Cell Culture, Vol III.*(Eds): P.V. Ammirato, D.A Evans, W.R. Sharp & Y.Yarnado, Macmillia Publishing Company.N.Y., 1984, pp. 402-452.

33. Stylianides D.C., Syrgianidis G. D., Almaliotis D. **The peach rootstocks; a review of bibliography with relative observations in Greece**. Agriculture Technology., 1988, 12: 34-69.

34. Tabachnik L., Kester D. E. **Shoot culture for almond and almond peach hybrid clones *in vitro***. Hort Science., 1977, 12(6), 545-547.

35. Taiz L., Zeiger E. **Plant Physiology**. Fourth Edition ., 2006, (Chapter 5,19, 20, 21, 26.)

36. Thorpe T. Stasolla C., Yeung E.C. De Klerk., G.J., Roberts A., George E.F. **Plant Growth Regulators II: Cytokinins, their Analogues and Antagonists.** In: George, E.F., Hall M.A. De Klerk, G.J. Plant Propagation by Tissue Culture third Ed Vol.1, Springer, 2008, pp. 115-173.

37. Tsipouridis K., Thomidis T. **Methods to improve *the vitro* culture of GF-677 (peach x almond) rootstock**. New Zealand J. Crop *Hort. Sci*, 2003,31: 361-364.

38. Zuccherelli G. **Metodologie nella moltiplicazione industriale *in vitro* dei portinnesti clonali del pesco GF -677**., 1979. pp. 147-154.