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IN VITRO IMPACT OF CONCENTRATION AND ADDITION METHODS OF PLANT HORMONS ON PEACH (*Prunus persica* L. Batsch) MICROGRAFTING

Nazar AL GHASHEEM[2], Florin STĂNICĂ[1], Adrian George PETICILĂ[1], Oana VENAT[1], Ana BUTCARU[1], Imad Al-SUWAID[1], Basim HASAN[2], Ihasn AZEB[2], Kawther ALGHASHEEM[2]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest, 59 Marasti Blvd, District 1, Bucharest, Romania
[2]College of Agricultural and Marshlands, University of Thi- Qar, Iraq

Corresponding author emails: nizar.shihan@yahoo.com, nizar.shihan.post.am@utq.edu.iq

Abstract

Florin peach variety and Myrobalan 29C rootstock were included in the experiment. For the preparation of graft and rootstock, two methods were tested for this operation: 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 and II, take the explants resulting from the initiation step and grow these explants into culture media tubes containing NAA and IBA 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: I, Micrografting before rooting and II, Micrografting after root formation. 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 shoots.

Key words: *explants, culture media, node, rootstock , Myrobalan 29C.*

INTRODUCTION

Grafting is the process of attaching a part of a desired plant to another plant that has some qualities that resist biotic and abiotic stresses. The first is called (scion) graft, while the second is rootstock. Used by the Chinese since 1560 B.C. (Melnyk and Meyerowitz, 2015; Kumari et al., 2015). Grafting is one of the methods of propagation in trees, we resort to grafting to propagate species and varieties with good specifications, high productivity, and diseases free (Murashige et al., 1972), which cannot be propagated by cuttings, Layering or other methods of vegetative propagation. The compatibility between rootstock and scion plays a major role in the success of the propagation process by grafting (Canan et al., 2006; Goldschmidt, 2014; Warschefsky et al., 2016). Grafting is a common method in the production of compound plants, and it depends on the growing season. The failure of the grafting process means waiting for the next growing season, and this means a loss of time, effort and money. To get rid of this problem, the technique of micrografting was used on *in vitro*, which is conducted carefully and under controlled conditions of temperature, humidity, lighting and free of pathogens (Murashige et al., 1972).

Micrografting technology is used to produce seedlings free of viral, bacterial and fungal infections and according to market needs (Navarro, 1981). The first successful micrografting process was carried out by Navarro et al., (1975) studies to obtain citrus seedlings free of pathogens, especially viruses.

Today, *in vitro* micrografting technology is widely used in the production of many types of fruit trees, especially citrus trees that are free of viral infection (Navarro, 1981).

This method is based on that the apical meristem does not contain viruses for the growth of meristem cells in the apical buds faster than the reproduction of viruses and that the grafting process is applied under controlled

conditions and cultures. (DeLange, 1978). Many methods and techniques have been developed that help the success of the micrografting process on *in vitro* (for fixation of the grafting area), like filter paper bridge by Huang and Millikan (1980); elastic bands by Jonard et al. (1983); translucent silicon tubes by Gebhardt and Goldbach (1988); tubes, nylon strips, aluminum foil tubes, double-layer aluminum foil devices and absorbent paper by Obeidy and Smith (1991). The aim of the study is to know the methods of micrografting of peach seedlings and the effect of concentration and method of adding auxins on the rooting process of seedlings used in the micrografting process.

MATERIALS AND METHODS

The study was conducted at the micropropagation laboratory, Centre for Studies of Food and Agricultural Products Quality, USAMV of Bucharest, Romania. On Peach (*Prunus persica* L.), Florin peach variety and Myrobalan 29C rootstock were included in the experiment. Nodes explants were taken at 0.5-1 cm length. All explants were washed by tap water to 30 min. Primary sterilization was done by put the explants in 70% ethanol to 2-3 minutes, after which the alcohol was removed by washing with distilled water 3 times under the sterile hood. The explants were sterilized on the surface with NaOCI (10% v/v) for 15-20 minutes, and then rinsed with distilled water at least three times (AL Ghasheem et al., 2018).

MEDIA AND CULTURE CONDITIONS

Explants were grown on 1/2 MS (Murashige and Skoog, 1962) medium supplemented with 14 g/l Sucrose, 3.5 g/l Agar, pH adjusted to 5.7 was filled into test tubes and sterilized by autoclaving at 121°C for 20 min. The parameters of the growth chamber were maintained at 22°C, 2000-2500 lux with a relative humidity of 80-85% for each treatment.

Graft and rootstock preparation

Two methods were tested for this operation:

Method I: immersion of the explants resulting from the initiation stage in solutions containing hormones that stimulate rooting at NAA and IBA concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l, respectively. Separated for 5 minutes, then cultured explants in tubes of culture medium containing GA3 1 mg/l and then placed the tubes of culture medium in the incubation room.

Method II: The explants resulting from the initiation stage were taken and cultured in tubes of culture medium containing GA3 1 g/l with NAA and IBA rooting hormones in concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l respectively, then separately placed the tubes of culture medium in the incubation chamber.

Application of micro-grafting

Two methods were tested for this operation:

Method I: Micrografting was performed before root formation. The explants resulting from the initiation stage were taken, the shoots were removed from the node on the rootstock, after which the micro-grafting was performed by placing the graft (node containing 1 shoot) on the rootstock and then the explant was placed in test tubes with medium containing GA3 1 g/l and hormones that stimulate the rooting of NAA and IBA in concentrations (0.00 control, 0.01, 0.50, 1.00 and 2.00) mg/l respectively, then separated the culture medium tubes were placed in the growth chamber.

Method II: Micrografting was performed after root formation. The explants were transplanted into perlite and sand containers (1: 1) for the purpose of the acclimatization process, and then transferred to the greenhouse.

STATISTICAL ANALYSIS

A completely randomized design (CRD) was used in experiment with three repetitions, 10 replicates per treatment were used with 1 shoot/tube. After the shoots were incubated in the rooting medium for 8 weeks, data on the number of roots per shoot and the average root length (cm) were recorded. Significance of differences between the results was estimated by Analysis of Variance (ANOVA) on SPSS version 14 (SPSS 2005) program with the means compared with LSD test at < 0.05.

RESULTS AND DISCUSSIONS

Effect of IBA and NAA auxins on roots number formed

The results (Table 1) showed that contamination and no roots were formed for the grafts (Florin) in all the treatments used in the experiment, while the rootstock Myrobalan 29C was resistant to contamination and managed to form roots.

Table 1. Percentage of rooting and contamination on Florin variety and Myrobalan 29C rootstock.

Variety	% of contaminants	% of rooting
Florin	40.92%	0.00%
Myrobalan 29C rootstock	13.11%	81.56%

In addition, the hormone NAA showed a tendency to induce callus or bud death in high concentrations. It was confirmed by Swistowska and Kozak (2004) that the addition of NAA to the media leads to the formation of callus at the base of shoots in the tissue culture of *Columnea hirta*, while the hormone IBA has a tendency to take root. Analysis of variance (Tables 2 and 3) revealed that the treatment had highly significant effect on mean roots number and roots length. The results showed that the hormone IBA determined the formation of 7.00 roots/explant, higher than the average number of roots formed compared to the hormone NAA (3.66 root/explant).

Figure 1. Appearance of roots in Myrobalan 29C rootstock (0.50 mg/l IBA) by method of explants submerging in solutions containing hormones. Data were taken after 4 weeks of culture.

The results showed that the use of the method of immersing explants in solutions containing hormones is superior to the variant in which hormones were added in the culture medium, the highest root rate was recorded at the hormone IBA (7.00 roots/explant in method I compared to 5.66 roots/explant in method II) and the hormone NAA (3.66 roots/explants in method I compared to 3.00 roots/explant in Method II).

The study showed that the concentration (control 0.00 without hormone) did not show any root in the rootstock Myrobalan 29C compared to other concentrations; the concentration of 0.50 mg/l IBA gave the best results in root formation. The results showed a clear effect of the addition of auxins to the culture media compared to the hormone-free culture medium.

These are similar to what were confirmed by (Miri, 2018) studies to obtain from testing different concentrations of three auxins (IAA, IBA and NAA) on rooting apple roots stock M.9 and M.26 (*Malus pumila* Mill.) on *in vitro*, he was found that the treatment was (0.5+0.5 mg/l IAA+IBA and 0.1 mg/l NAA) respectively, gave the highest results. Also, similar studies by Ali et al. (2009) when different concentrations of auxin (NAA and IBA) were used on olive shoots, he was found that IBA auxin concentration (1.5 mg/l) gave the best results.

Auxins are necessary for the formation of roots, plants need it in small quantities the auxin indole-3-acetic acid (IAA) is producing at apex of the buds and is transferred to the base of the buds (Muday and DeLong, 2001; Casson and Lindsey, 2003). Auxins activate the synthesis of RNA, and mRNA provides energy through its activity, which in turn is associated with the processes of oxidation of nutrients and the formation of enzymes necessary for growth (Rabechault et al., 1976). Studies have confirmed that genes that contribute to the construction of auxin are expressed within the roots (root apex) and auxin, is turn contributes to the induction and growth of roots (Leung et al., 2005 and Peterson et al., 2009).

Figure 2. Emergence of root from callus in Myrobalan 29C rootstock, 2.00 mg/l NAA by placing the explants into a culture medium containing hormones. Data were taken after 6 weeks of culture

Effect of IBA and NAA auxins on the resulting root length

The results showed that the use of the method of immersing explants in solutions containing hormones is superior to the method in which hormones were added to the culture medium. The highest average root length was recorded with the hormone IBA (9.07 cm root length/ explants in method I compared to 6.36 cm root length/explants in method II) and the hormone NAA (5.50 cm root length/explants in method I compared to 5.40 cm root length/explants in Method II).

Figure 3. Appearance of roots on Myrobalan 29C rootstock, 0.01 mg/l NAA by method of explants submerging in solutions containing hormones. Data were taken after 8 weeks of culture

The results showed that the hormone IBA leads to better results (9.07 cm root length/explants) compared to the hormone NAA (5.50 cm root length/explants). The concentration of 0.50 mg/l IBA gave the best results. Our results are similar to studies (Dabski and Parzymies, 2007) on *Hebe buchananii* (Hook) and *Hebe canterburiensis* (JBArmstr.) 'Prostra, when using different concentrations of auxins (IAA, IBA and NAA), IAA with IBA 2.5 and 5.0 mg/l treatments gave the highest average roots length.

Auxins effect has been confirmed in several studies by effect of IBA on root length such as (Ma et al., 1998; Leonardi et al., 2001; Swamy et al., 2002; Wawrosch et al., 2003; Dabski and Divya et al., 2008; Bhatt and Chouhan, 2012; Ullah et al., 2013).

Micrografting methods

Method I: grafting by placing the graft on the rootstock before root formation at the rootstock: The results showed the success of callus in the micrografting area, but it was observed that the roots were not formed by rootstocks, despite the passage of 8 weeks from the micrografting process.

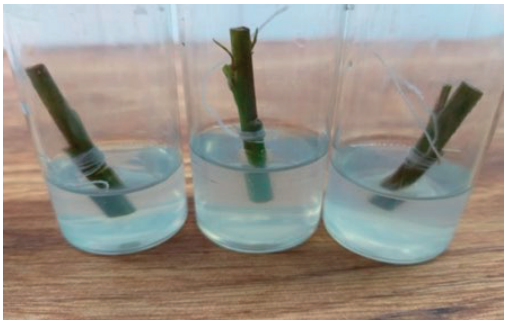

Figure 4. Grafting by placing graft (Florin) on Myrobalan 29C rootstock before root formation on rootstock (Method I). Data were taken after 4 days of grafting

It was also found that most of the shoots stopped growing or withered after 4 weeks of micrografting, perhaps the reason for the stunted growth is due to the consumption of carbohydrates stored in the buds (Table 4).

Figure 5. Method I, Florin's micrografting on Myrobalan 29C rootstock. Data were taken after 4 weeks of grafting

Figure 7. Shoots of Myrobalan 29C rootstock after 4 weeks of culture

Method II: Grafting by placing the graft on the rootstock after root formation at the rootstock:
The results showed the success of the grafting process in this way, compared to the first method. The resulting shoots were placed in plastic containers containing previously sterilized perlite and sand for the purpose of the adaptation process.
Our results are similar to studies by Tangolar et al., (2003) the on micro grafting in two grape varieties (Early Cardinal and Yalova incisi) grafted on four rootstocks (Dogridge, Salt Creek, 1613 C and 41 B).
Micrografting has been used successfully in many studies such as: Apples (Huang and Millikan, 1980); pistachio (Abousalim and Mantell, 1992; Onay, 2002); cloves (Mneney and Mantell, 2001); grapes (Tangolar et al., 2003); avocado (Raharjo and Litz, 2003; 2005); apricots (Piagnani et al., 2006); cherry (Amiri, 2006; 2007); walnut (Wang et al., 2010); almonds (Yıldırım et al., 2013; Isıkalan et al., 2011).

Figure 8. Method II, grafting prepares, by placement of Florin graft on Myrobalan 29C rootstock after roots formation on Myrobalan 29C rootstocks.

Figure 6. Shoots that have formed have ceased to grow or have wilted - Method I, Florin on Myrobalan 29C. Data were taken after 6 weeks of grafting

Figure 9. Grafting process by placement Florin graft on Myrobalan 29C rootstock (Method II)

Table 2. Effect of IBA and NAA auxins on roots number formed/explant at rooting phase on the Myrobalan 29C rootstock. Data were taken after 8 weeks of culture

Hormons \ Concentrations		0.00	0.01	0.50	1.00	2.00	Mean
NAA	I	***	***	***	**3.66±0.28**	3.00±0.18	1.33±0.08
	II	***	**3.00±0.33**	***	***	***	0.60±0.07
IBA	I	***	4.00±0.46	**7.00±0.85**	4.66±0.21	***	3.06±0.18
	II	***	3.33±0.23	**5.66±0.68**	4.33±0.20	***	2.66±0.28
Mean		***	2.58±0.49	3.16±0.10	3.16±0.66	0.75±0.41	

***shoots did not produce roots.

Table 3. Effect of IBA and NAA auxins on roots length formed/explant (cm) at root stage on the Myrobalan 29C rootstock. Data were taken after 8 weeks of culture

Hormons \ Concentrations		0.00	0.01	0.50	1.00	2.00	Mean
NAA	I	***	***	***	4.22±0.39	**5.50**	1.92±0.10
	II	***	**5.40±0.57**	***	***	***	1.08±0.19
IBA	I	***	7.06±0.66	**9.07±0.78**	6.70±0.58	***	4.57±0.46
	II	***	5.30±0.52	**6.36±0.54**	4.43±0.33	***	3.22±0.32
Mean		***	4.44±0.33	3.85±0.23	3.83±0.37	1.35±0.11	

***Shoots did not produce roots.

Table 4. Percentage of callus formed at the graft area and rooting on Florin variety and Myrobalan 29C rootstock

Micrografting	% of callus formed at the graft area	% of Root formation	Grafting status
Method I	66.21%	0:00 %	Shoots stopped growing or withered after 4 weeks of micrografting.
Method II	73:55%	69:00%	Grafting success

Figure 10. Acclimatization stage, shoots resulting was placed in plastic pots covered with glass caps, Florin graft on Myrobalan 29C rootstock. Data were taken 1 week after grafting

CONCLUSIONS

The study demonstrated the success of the method of micro-grafting multiplication of peach varieties resulting from tissue culture technology and can be used in the production of a large number of seedlings without pathogens.

These results also show the role of IBA in rooting explants that gave the highest rate of number of roots formed (7.00 root/explant) and the longest root length (9.07 cm root/explant length) at all studied concentrations compared to the same NAA concentrations. Also, the method of immersing explants 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 hormones to culture media.

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