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IN VITRO EFFECT OF CULTURE MEDIA AND GROWTH HORMONES ON THREE PEACH (*Prunus persica* L. Batsch) CULTIVARS

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Abstract

In vitro three peach cultivars (Florin, Filip and Mimi) included in this experiment, two explants (shoots-tip and nodes) were taken at 0.5-1cm length. The explants were cultivated on 3 medium MS, B5 and QL adding of 30 g/l sucrose and 7 g/l with tested different concentrations of plant hormones BAP and NAA in 12 variants. The aim of this study was to test the effect of culture medium and growth hormones on some peach cultivars. 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) medium gave the lowest value. 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, while adding just BAP to all media in the experiment gave shoots.

Key words: *BAP, culture media, explants, NAA, shoots.*

INTRODUCTION

In recent years, the technique of plant tissue culture has been widely used in the production of plants in large quantities, good characteristics, pathogens free and in regeneration, propagation and *in vitro* preservation of rare and economically important plants (Altman and Hasegawa, 2012a; Bhatia, 2015). *In vitro* technique propagation is growthing and maintaining and developing any plant parts (cells, tissues or organs) in a culture medium in suitable containers under controlled environmental conditions (Debergh and Read, 1991; Sharma et al., 2015). Cultures media should generally contain: Macronutrients, micronutrients components, vitamins, carbon sources, unspecified organic supplements, growth hormones and antioxidants. The quantities of nutrients supported in cultures media must be compatible to promote growth during culture period (Altman and Hasegawa, 2012b; Datta, 2019). Currently, there are many culture media used in tissue culture technique depending on the purpose of propagation, such as Gautheret (1939); White (1943); Murashige and Skoog MS (1962); Linsmaier and Skoog LS (1965); Gamborg B5 (1968); Nitsch and Nitsch NN (1969); Quoirin and Lepoivre QL (1977). Most experiments used the Murashige and Skoog (1962) medium without modification, while the experiments focused on the effect of plant growth regulators. There are many studies in effect of cultures medium such as: Shoot- tips and axillary buds in MS and B5 on Penta Rootstock (Balapour et al., 2020); shoot tips and axillary buds in MS, WPM and DKW on GF677 rootstock (Hamidi and Rezagholy, 2016); axillary buds in MS, LS, B5, N6 and QL on *Salvia guaranitica* Benth (Echeverrigaray et al., 2010); callus induction in MS, B5 and WPM on *Crataeva tapia* L. (Sharma et al., 2017). Despite extensive studies in other species, *in vitro* multiplication of peach (*Prunus persica* L. Batsch) is still finite. Peach has been successfully regenerated *in vitro* from immature cotyledons (Mante et al., 1989; Pooler and Scorza, 1995); cell suspension cultures (Schiavone and Wisniewski, 1990; Bhansali et al.,1991); callus induction (Hammerschlag et al., 1985; Declerck and Korban, 1996; Pérez-Jiménez et al,. 2012); shoot-tip and leaf (Cordts et al., 1987; Al ghasheem et al., 2018a). The aim

of this study was to test the effect of culture medium and growth hormones on some peach cultivars of Romania via micropropagation technique.

MATERIALS AND METHODS

Peach varieties (Florin, Filip and Mimi) were included in the experiment. Shoots and nodes explants (0.5-1 cm) were collected from healthy trees grown in the agricultural research station of Faculty of Horticulture / University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania in 2019. All explants were washed with washing liqued to remove plankton and dust under tap water to 30 min. For primary sterilization, the explants were immersing in 70% ethanol with constant stirring to 2-3 minutes, after which the alcohol was removed by washing with distilled water 3 times under the laminar flow cabinet. The explants surface were sterilized with NaOCl (10% v/v) with constant stirring by magnetic stirrer for 15-20 minutes, and then rinsed with distilled water at least three times (Al ghasheem et al., 2018b). The explants were cultured on 3 cultures media MS (Murashige and Skoog, 1962), B5 (Gamborg, 1968) and QL (Quoirin and Lepoivre, 1977) with 30 g/l sucrose and 7 g/l agar without hormones supplements during the initiation stage. In the multiplication stage, 12 variants were tested (Table 1).

Table 1. Scheme of treatments for concentration of hormones added to three culture media

Treatments	Media	BAP mg/l	NAA mg/l
T1	1/2MS	1.00	0.50
T2	1/2MS	5.00	0.50
T3	1/2B5	1.00	0.50
T4	1/2B5	5.00	0.50
T5	1/2QL	1.00	0.50
T6	1/2QL	5.00	0.50
T7	1/2MS	1.00	0.00
T8	1/2MS	5.00	0.00
T9	1/2B5	1.00	0.00
T10	1/2B5	5.00	0.00
T11	1/2QL	1.00	0.00
T12	1/2QL	5.00	0.00

The pH was adjusted to 5.7 by using drops from solution of NaOH and HCl. All cultures media were placed in tubes culture and sterilized by autoclaving at 121°C for 20 min. Growth chamber were controled at 22°C, 2000-2500 lux with relative humidity of 80-85%. The test tubes were kept in the dark (2-3 days) to get rid of the oxidation process of phenolic substances resulting from the cutting of plant tissues. The experiment was repeated twice, each treatment containing 30 replicates (one explant) in the initiation stage and 5 replicates (three explants) tested in multiplication. All experiments were arranged in a completely randomized design. The duration of the culture varied between four and eight weeks, depending on the individual experiment. The data were recorded on number shoots formed, shoots length (cm) and leaves number/shoot. The significance of the differences between the results was estimated by analysis of variance (ANOVA) on the SPSS (2005) program compared to the standard error means of the mean difference and lowest difference (LSD) test at probability level 0.05.

RESULTS AND DISCUSSIONS

In the initiation stage, the results showed that there were responses of all varieties to the tissue culture technique, the results showed that there was growth in all varieties on all cultures media used in the experiment.

MS culture medium gave highest average of shoots for all the varieties used (Florin 3.33 cm, Filip 2.61 cm and Mimi 2.59 cm), while B5 culture medium gave lower value (Florin 2.15 cm, Filip 1.84 cm and Mimi 2.01 cm). (Tables 2, 3 and Figures 1, 2). MS has a higher concentration of nitrogen and phosphorus compared to B5 and QL.

Nitrogen plays an important role in the construction of the plant through enzymes that stimulate the absorption of nitrates which is used in the manufacture of plant tissues (Mashayekhi, 2000). Our results are similar to what the researchers Bell and Reed (2002) reached when they planted 20 pear cultivars in (MS, LP and DKW) media. In the multiplication stage, the results showed that there were significant differences at the level of 0.05.

All varieties shoots formed depending on the amount of phosphorus in the culture medium and the concentration of plant hormones were added. The results showed that there is an effect of genotypes and cultures media. Culture media

MS and QL showed the highest average shoots number formed in 5 mg/l BAP: medium MS (Florin 4.20 shoots / explant, Filip 3.60 shoots / explant and Mimi 4.00 shoots / explant) and QL culture medium (Florin 4.40 shoots / explant, Filip 2.40 shoots / explant and Mimi 4.00 shoots / explant), while B5 culture medium gave the lowest value (Tables 4 and 5).

The study also showed that the shoots growth on QL culture medium have a yellowish-green colour may be due to the concentration of the element nitrogen compared to the MS and B5 media (Figure 3).

Figure 1. Shoots of Florin variety growing in the MS media after 8 weeks of multiplication stage

Table 2. Effect of variety and culture medium on average shoots length formed (cm) at initiation stage on three peach varieties. Data were taken after 4 weeks of culture

V / M	Florin	Filip	Mimi	Total
MS	**3.33 ± 0.10**	**2.61 ± 0.25**	**2.59 ±0.13**	2.71 ± 0.12
B5	2.15 ± 0.19	1.84 ± 0.06	2.01 ± 0.16	2.15 ± 0.10
QL	2.59 ± 0.18	2.01 ± 0.16	2.25 ± 0.16	2.28 ± 0.09
Total	2.84 ± 0.10	2.00 ± 0.09	2.30 ± 0.09	

Table 3. Effect of variety and culture medium on average leaves number formed/explants in initiation stage on three peach varieties. Data were taken after 4 weeks of culture

V / M	Florin	Filip	Mimi	Total
MS	4.50 ± 0.23	5.30 ± 0.27	4.10 ± 0.18	4.63 ± 0.20
B5	**5.80 ± 0.29**	5.00 ± 0.28	**5.10 ± 0.26**	5.30 ± 0.23
QL	4.70 ± 0.25	**5.80 ± 0.29**	4.80 ± 0.25	5.10 ± 0.20
Total	5.00 ± 0.28	5.36 ± 0.27	4.66 ± 0.19	

Table 4. Effect of variety, BAP and culture medium on average shoots grown number at multiplication stage on three peach varieties. Data were taken after 8 weeks of culture

Varieties	Treatments	Mean shoots number formed		
		MS	B5	QL
Florin	T7	2.16 ± 0.092	1.10 ± 0.089	2.95 ± 0.120
	T8	**4.20 ± 0.181**	**1.80 ± 0.136**	**4.40 ± 0.158**
Filip	T9	1.74 ± 0.082	1.35 ± 0.078	1.81 ± 0.086
	T10	**3.60 ± 0.128**	1.40 ± 0.118	**2.40 ± 0.136**
Mimi	T11	2.01 ± 0.081	1.79 ± 0.092	2.09 ± 0.084
	T12	**4.00 ± 0.136**	2.20 ± 0.128	**4.00 ± 0.137**

Table 5. Effect of variety, BAP and culture medium on average shoots length formed (cm) in multiplication stage on three peach varieties. Data were taken after 8 weeks of culture

Varieties	Treatments	Mean shoots length formed		
		MS	B5	QL
Florin	T7	2.19 ± 0.04	1.12 ± 0.04	**2.59 ± 0.04**
	T8	1.98 ± 0.03	1.37 ± 0.03	1.84 ± 0.02
Filip	T9	**2.63 ± 0.04**	1.55 ± 0.04	2.56 ± 0.04
	T10	1.12 ± 0.04	1.15 ± 0.04	1.09 ± 0.04
Mimi	T11	2.15 ± 0.04	**1.63 ± 0.04**	2.18 ± 0.04
	T12	1.89 ± 0.03	1.10 ± 0.05	1.91 ± 0.03

Figure 2. Shoots of Filip variety growing in the B5 media after 8 weeks at multiplication stage

These results are similar to those studied (Nowakowska et al., 2019) on two groups of culture medium (MS and WPM) where they were used on explants of *Daphne mezereum* L. and the superiority of MS medium over WPM medium was found. Also, our results are similar to those studied by Aviles et al., (2009) on 4 groups of culture media (MS, BTM, DKW and WPM) on the callus formed in the common walnut (*Juglans regia* L.) where the BTM medium was superior on all cultures media. According to Garton et al., (1984), there is effect of clonal origin or genotype of the plant material on organogenesis while Thakur et al., (2011) suggested that the growth of the plant depends on the components of the nutrient medium used in the plant tissue culture technique.

Figure 3. Shoots of Mimi variety (yellowish-green color) growing in the QL media after 8 weeks at multiplication stage

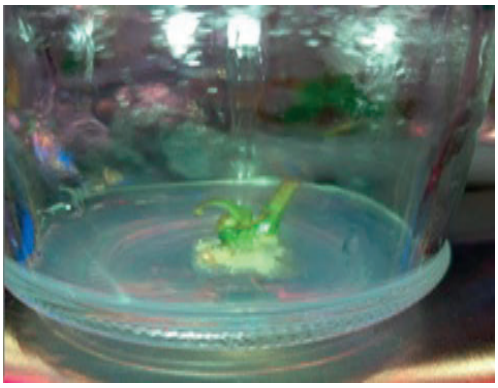

Figure 4. Effect of interaction between BAP and NAA in callus production from shoots growing in B5 culture media after 8 weeks of culture, Filip T1 (1/2 B5 + 1.00 mg/l BAP + 0.50 mg/l NAA)

Many studies reported that the amount of shoots formed was related to the phosphorus amounts absorbed by the explants (Pierik, 1990) confirmed that the amounts of nutrients in the culture medium have an important impact on the morphogenic response, also even possible to exclude the effect of hormones in plants by changing the compositional concentration of the culture medium (Ramage and Williams, 2002). In our study, we were found that there is a relationship between BAP and NAA, when were addition of both hormones led to the appearance of callus tissue (the highest amount of callus Florin 44% callus / explant in T1 (1/2 MS + 1.00 BAP mg / l + 0.50 NAA mg / l), while were adding only BAP to all media in the experiment give shoots formed (Table 6 and Figure 4). We were found that the BAP concentration of 5 mg / l led to the formation of the highest amount of shoots number formed compared to the concentration of 1 mg / l BAP. Auxin and cytokinins are plant hormones that play a very important role in controlling the plant growth process. These hormones have been used extensively in plant tissue culture technology. There are relationship between the concentration of auxins and cytokinins in the culture medium on growth nature and specialization of explants grown in culture media. We were found that increasing the ratio of auxin to cytokinins makes this medium a catalyst for the formation of root mass of plant parts, while increasing the ratio of cytokinin to auxin makes the culture medium ready to stimulate plant shoots to grow and form new

shoots or callus. The results of our study are similar to previous studies that were confirmed by Gentile et al., (2002) on *Prunus species* when different concentrations of BAP were used, which led to good results in peach genotypes used in experiments.

Table 6. Effect of auxin and cytokinin interaction (BAP and NAA) on shoots growth and callus formed in 3 culture media

V / T	Florin			Filip			Mimi		
	Callus %	**Shoots %**	**Dead %**	**Callus %**	**Shoots %**	**Dead %**	**Callus %**	**Shoots %**	**Dead %**
T1	**44**	18	38	22	37	41	**38**	34	28
T2	25	34	41	13	32	55	29	42	29
T3	42	27	31	**27**	43	30	37	22	41
T4	37	48	15	19	25	56	23	37	40
T5	21	36	43	11	27	62	19	32	49
T6	16	28	**56**	06	34	60	12	26	**62**
T7	00	78	22	00	65	35	00	32	24
T8	00	77	23	00	**82**	18	00	26	39
T9	00	**89**	11	00	49	51	00	67	33
T10	00	69	31	00	67	33	00	**70**	30
T11	00	76	33	00	44	**66**	00	55	45
T12	00	66	34	00	70	30	00	68	32

CONCLUSIONS

The study confirmed that MS culture media showed the highest average shoots number formed in 5 mg / l BAP: (MS) medium (Florin 4.20 shoots / explant, Filip 3.60 shoots / explant and Mimi 4.00 shoots / explant) respectively, while (B5) medium gave the lowest value (Florin 1.80 shoots/ explant, Filip 1.40 shoots/ explant and Mimi 2.20 shoots / explant). Also found that there is a relationship between BAP and NAA, when addition of both hormones led to the appearance of callus tissue (the highest amount of callus in Florin 44% callus / explants in T1 (1/2 MS + 1.00 mg / l BAP + 0.50 mg /l NAA)), while adding only BAP to all cultures media were got shoots formed . Also, we were found that the BAP concentration of 5 mg / l led to the formation of the highest number of shoots compared to the concentration of 1 mg / l BAP.

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