ISSN NO: 2771-8840
Date of Publication: 28-09-2022

Effect of explants and auxins concentration on callus induction on peach (*Prunus persica* (L.) Batsch) micropropagation

Nazar Al Ghasheem
College of Agricultural and Marshlands, University of Thi Qar, Iraq
Corresponding author e-mail: nizar.shihan@yahoo.com,
nizar.shihan.post.am@utq.edu.iq

Abstract: Micropropagation or plant tissue culture technique is one of the most appropriate methods for plant material multiplication in order to obtain healthy and disease free planting material in mass quantity and fast time. The amis of this study the impacts of explants and auxins on callus induction on peach micropropagation. Redhaven genotype from the Field of Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania were tested. Two explants (shoots tips and nodes, 1cm length) were cultured on MS (Murashige and Skoog, 1962) basal medium plus vitamins supplemented with 30g/l sucrose, 7g/l agar and 500mg/l activated charcoal. Two auxins: Indole-3-butyric acid (IBA) in four concentrations: 0,1,3 and 5mg/l respectively and 1-Naphthaleneacetic acid (NAA) in four concentrations: 0,1,3 and 5mg/l respectively were used as plants hormones for callus induction. The growth chamber for the *in vitro* cultures had 22±2°C temperature and 70 - 80% relative humidity, with a photoperiod of 16h day light and 8h dark. The days of callus inductions; percentage of callus induction; rate of callus growth; fresh weight of callus and morphology of callus inducted were studied. The results showed a significant correlation between explants with the concentration of auxins on callus induction. The nodes explants and 5.00 mg/l NAA concentration gave the highest values in the recorded traits.

Keywords: auxins, callus induction, Indole-3-butyric acid, 1-Naphthaleneacetic acid, Redhaven

Introduction

Peach (*Prunus persica L*) a commercial fruit tree belonging to the Rosaceae family, peach was originated in China, nearly 4000 years of cultivation and currently grown commercially around the world in subtropical and tropical regions(Wang, 1985; Souza et al., 2013). Peach fruits are the fruits are popular worldwide for its delicious taste and pleasant aroma in addition to its amenability for industrial food processing and grown as ornamental plants. The peach is a fruit rich in many elements and compounds that play a major role in health (USDA, 2017).

The main chemicals in peaches are sugars, organic acids, pectic substances, tannins, vitamins and minerals. The biochemical parameters are influenced by the variety, but also by the culture technology applied (Iordănescu, 2008). Peach is a rich source of minerals such as calcium, iron, phosphorus, magnesium, copper and zinc (Kant et al., 2018). Also rich in vitamins such as vitamin A, B1, B2, B3, B6, C, E and K (USDA, 2017).

Peach is traditionally propagated by seeds to produce new varieties or to improve the characteristics of fruits and trees through breeding and improvement programs (Stănică et al., 2001). Also, peach is traditionally propagated by vegetative propagation through traditional propagation methods via grafting and cuttings (De Souza et al., 2017). In recent decades, used plant tissue culture technique to commercially produced in large numbers to producing rootstock and cultivars (Isac et al., 2010).

Tissue culture is the cultivation of cells, tissues, or organs on nutrient media under controlled conditions. Via micropropagation or tissue culture is , increases the thousands of copies of a plant can be produced in a short time disease-free and high quality (Bajaj, 1995; Bhatia et al., 2015). In this technique, different explants (cells; suspension culture; shoots; nodes; internodes; flower parts; etc.) used to produce shoots or callus. also different plant hormones are used to stimulate explant to grow and differentiate, including auxins; cytokinins; gibberellins; etc.Callus is a coherent and amorphous tissue formed when plant cells multiply in a disorganized manner (Bajaj, 1995). Callus is produced naturally from the plant body through cuts, scrapes, pricking insects, disease or stress. Callus can be initiated in vitro by placing small pieces of the

ISSN NO: 2771-8840
Date of Publication: 28-09-2022

whole plant (explants) on culture medium which contented plant hormons under sterile conditions. Recently, breeding practices in peach have been advanced by the application and development of plant tissue cultur technique on rootstock and cultivars (Martinez-Gomez et al., 2005; Felek et al., 2017 Alghasheem et al., 2018a; Mitrofanova et al., 2019). The aims of this study was for find the effects of explants and auxins on callus induction.

Materials and Methods

Explants surface sterilization

Redhaven genotype from the Field of Faculty of Horticulture, University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania (USAMV) were tested. The explants (Shoots tips and nodes, 1cm length) from peach trees planted at the Agricultural Research Station were brought by a sharp scalpel (blade). Explants were washed under tap water using washing powder to remove dust and plankton for two hours preserved in petri dishes containing an antioxidant solution consisting of 150 mg/L citric acid + 100 mg/L ascorbic acid (Khosroushahi et al., 2011) and then placed in Refrigerate at 4°C/ 24 hours to eliminate the harmful effect of phenolic compounds. (Çördük and Aki, 2011). Explants were washed several times to remove the antioxidant solution and then first sterilized with 70% ethyl alcohol solution for five minutes and then washed with distilled water at least 3 times, then second sterilized with 10% sodium hypochlorite solution was used with added 3-4 drops of Tween-20 diffuser for 15 minutes for surface sterilization (Alghasheem et al., 2018b). Then , explants were washed with distilled water at least 3 times.

Culture media and culture conditions

Explants were cultured on MS (Murashige and Skoog, 1962) basal medium plus vitamins supplemented with 30g/l sucrose, 7g/l agar and 500mg/l activated charcoal. Two auxins were tested: Indole-3-butyric acid (IBA) in four concentrations: 0,1,3 and 5mg/l respectively and 1-Naphthaleneacetic acid (NAA) in four concentrations: 0,1,3 and 5mg/l respectively were used as plants hormones for callus induction. The pH was set at 5.7 by adding drops of HCl and NaOH 1N solution. All culture media tubes were sterilized by autoclaving at 121 °C for 20 min. After explants were cultured in culture media, they were placed in the growth chamber at 22°C, 2000-2500 lux with 80-85% relative humidity.

Data collection

The experiment was repeated 3 times, each treatment containing 20 replicates (one plant/tube) in the initiation stage and 5 replicates (three explants/can) tested in multiplication stage (callus induction). All experments were arranged in a Completely Randomized Design (CRD). Each culture duration ranged between four weeks. Data were recorded on days of callus induction; % of callus induction; rate of callus growth; fresh weight of callus (g) and morphology of callus inducted. The significance of the differences between the results was estimated by analysis of variance (ANOVA) on SPSS (2014) compared to Least Significant Difference (LSD) test at a probability level of 0.01.

Results And Discussion

Effect of explants and auxins concentrations on days of callus induction:

The results of the experiment showed that there were statistically significant differences between the experimental treatments, in table 1 the results showed that nodes explant was superior to shoots tips explant in the rate of days callus induction (15.37 and 12.63 callus/days) respectively. also the results showed that auxin (NAA) was superior to auxin (IBA) in the experimental results, where the concentration of 5 mg / l exceeded the rest of the concentrationsin the rate of days callus induction (15 days), and the concentration of 3 mg / l (NAA) came in second (18 days), While IBA auxin recorded the lowest results, the best results were at 1 mg / l concentration (18 days). The reason for the superiority of the nodes over the shoots tips in the speed of callus production may be that the nodes contain previous meristematic tissues and are easy to reactivate. Also, the reason may be due to the ratio between the concentration of the plant hormones (cytokinins and auxins) in the plant body, where the amount of cytokinin increases in the shoots tips and its percentage decreases in nodes. This result similar with a previous studies such as Chinese peony (Tian et al., 2010); Christ's thorn jujube (Ahmadi et al., 2013); coffee (Setiawan, et al., 2020)

ISSN NO: 2771-8840
Date of Publication: 28-09-2022

Table 1.

Effect of explants and auxins concentrations on days callus induction after sixth week from culture

Auxin concentration	Days of callus inductions		Means
	Shoots tips	Nodes	
IBA			
0.00	0.00	0.00	0.00
1.00	14.00	**18.00**	16.00
3.00	18.00	23.00	20.50
5.00	20.00	25.00	22.50
NAA			
0.00	0.00	0.00	0.00
1.00	19.00	24.00	21.50
3.00	17.00	18.00	17.50
5.00	**13.00**	**15.00**	14.00
Means	12.63	15.37	
LSD	Explant=3.11	Auxin=2.79	

Effect of explants and auxins concentrations on percentage of callus induction:

The results of the experiment (Table 2) showed that there were statistically significant differences between the experimental treatments, the results showed that nodes explant was superior to shoots tips explant in the percentage of callus induction (67.00 % and 26.25 % callus/explant) respectively. also the results showed that auxin (NAA) was superior to auxin (IBA) in the experimental results, where the concentration of 5 mg / l exceeded the rest of the concentrations in the percentage of callus induction (75.00 %), and the concentration of 3 mg / l (NAA) came in second (69.50 %), while IBA auxin recorded the lowest results, the best results were at 1 mg / l concentration (67.00 %). The reason for the decrease in its percentage callus induction in the shoots tips may be due to the rapid death of the shoots tips due to the secretion of phenolic substances that are produced from the plant body when wounds occur. Because shoots tips are thin explant that are easily affected by new environmental conditions in the culture media as well as callus formation depends on several factors, including the type and age of explant; nutrients in the culture media; elements of environment and growth regulators (hormonal and non-hormonal) that may work cooperatively in the regeneration of the callus. (Skoog and Miller, 1957; Arumugam et al., 2009). This is result similar with a previous studies on Gotu Kola (Rahayu et al., 2016); Pineapple (Salihan and Yusuf, 2020).

Table 2.

Effect of explants and auxins concentrations on percentage of callus induction after sixth week from culture

Auxin concentration	percentage of callus induction		Means
	Shoots tips	Nodes	
IBA			
0.00	0.00	0.00	0.00
1.00	**34.00**	**100.00**	67.00
3.00	29.00	70.00	49.50
5.00	25.00	62.00	43.50
NAA			
0.00	0.00	0.00	0.00
1.00	33.00	82.00	57.50
3.00	39.00	100.00	69.50
5.00	**50.00**	**100.00**	75.00
Means	26.25	67.00	

ISSN NO: 2771-8840
Date of Publication: 28-09-2022

Effect of explants and auxins concentrations on fresh weight of callus induction:

The results in our study (Table 3) confirmed that there is a statistical significance between the explants and plant hormones on the fresh weight of the callus formed. the results showed that nodes explant was superior to shoots tips explant in the percentage of callus induction (1.91 and 0.30) respectively. also the results showed that auxin (NAA) was superior to auxin (IBA) in the experimental results, where the concentration of 5 mg / l exceeded the rest of the concentrations in the percentage of callus induction (2.89 g/ explant), and the concentration of 3 mg / l (NAA) came in second (2.20 g/ explant), while IBA auxin recorded the lowest results, the best results were at 1 mg / l concentration (1.63 g/ explant). An auxin when added in low concentrations in the culture media helps in the start of cell division and elongation. (Ali et al 2016). addition of auxins to arrested cells after absence of auxin leads to induction of cell division. (Inze and De Veylder 2006). This is what the researchers confirmed that high auxin concentrations lead to inhibition of cell growth and may stop them from dividing and expanding, the reason for the low rates of callus weight when a auxin is high in the culture media may be attributed to the role of auxin in stimulating the production of ethylene which reduces the rate of cell division and thus affects the growth of callus as the industrial auxin releases ethylene by about double. These results similar findings of the researchers (Samsudeen et al., 2000; Paul, and Shylaja, 2010; El-Nabarawy et al., 2015; Ali et al., 2016).

Table 3.
Effect of explants and auxins concentrations on fresh weight of callus after sixth week from culture

Auxin concentration	Fresh weight of callus (g)		Means
	Shoots tip	Nods	
IBA			
0.00	0.00	0.00	0.00
1.00	0.34	**2.91**	1.63
3.00	0.31	1.10	0.71
5.00	0.27	0.53	0.40
NAA			
0.00	0.00	0.00	0.00
1.00	0.41	1.63	1.02
3.00	0.44	3.95	2.20
5.00	**0.63**	**5.15**	2.89
Means	0.30	1.91	
LSD	Explant=0.091	Auxin=0.073	

Effect of explants and auxins concentrations on appearance of callus induction:

The results in our study (Table 4) showed that the formed callus had different colors, shapes and sizes depending on the type of explant, type and concentration of auxin used. The results showed that the node explant gave the best results (+++, creamy with brown to ++++, creamy with brown and green) compared to the callus formed from the shoot tip, which was characterized as weak in growth.(++, Brown). The results also showed that NAA auxin (3 and 5 mg/l concentration) gave good growth compared to IBA auxin. The reason for the appearance of the brown colour in the callus is due to the secretion of phenolic substances when the plant body is injured; It contains several toxic compounds which eventually lead to cell necrosis (Banerjee et al., 1996). This is due to the activity of various browning phenomenon enzymes. Such as polyphenol oxidase (Murata et al., 2001) and peroxidase (Wu and Lin, 2002). Otherwise the continued appearance of the brown colour in the developed callus may be due to the presence of sucrose in the culture media (Kumar et al., 2015).

ISSN NO: 2771-8840
Date of Publication: 28-09-2022

Table 4.
Effect of explants and auxins concentrations on appearance of callus after sixth week from culture.

Auxin concentration		Appearance of callus			
		Shoots tips	Callus morphology	Nodes	Callus morphology
IBA	0.00	+	No growth	+	No growth
	1.00	++	Brown	++++	Creamy with brown and green
	3.00	++	Brown	+++	Creamy with brown
	5.00	++	brown	+++	Creamy with brown
NAA	0.00	+	No growth	+	No growth
	1.00	++	Brown	+++	Creamy with brown
	3.00	++	Brown	++++	Creamy with brown and green
	5.00	+++	Creamy with brown	++++	Creamy with brown and green and shoots stimulated

Notes:+=No growth, ++Bad, +++ = Enough, ++++ = Good

A **B** **C**

Figure 1. Fig A=shoot tip explant (5 mg/l NAA), fig B=node explant (1mg/l IBA), fig C=noge explant (5mg/l NAA)

Conclusion

Our results were confirm that the explant type has an effect on stimulating callus in peach explant planted in culture media, the nodes explant gave the highest values in the recorded traits. Also, our results were confirm that the auxins type has an effect on stimulating callus in peach explant planted in culture media, the NAA auxin (5 mg/l) gave the highest values in the recorded traits.

Acknowledgements

I would like to express my deepest appreciation and special thanks to supervisors and colleagues who helped in the completion of this research in the College of Horticulture /UNIVERSITY OF AGRONOMIC SCIENCES AND VETERINARY, BUCHAREST, ROMANIA.

References

1.Ahmadi, E., Nasr, S. M. H. and Jalilvand, H. 2013. Callus induction and plant regeneration from node explants of *Ziziphus spina- christi*, Journal of Agricultural Engineering and Biotechnology, Vol. 1 Iss. 1, Pp. 9-16.

ISSN NO: 2771-8840
Date of Publication: 28-09-2022

2.Alghasheem, N., Stănică, F., Peticilă, A.G. and Venat O. 2018a. *In vitro* effect of genotype, growth season and cytokinines on peach varieties (*Prunus persica* L. Batsch) propagation, Scientific Papers. Series B, Horticulture. Vol. LXII, 2018, Pp: 217-226.

3.Alghasheem, N., Stănică, F., Peticilă, A.G. and Venat, O. 2018b. *In vitro* effect of various sterilization techniques on peach (*Prunus persica* L. Batsch) explants, Scientific Papers. Series B, Horticulture. Vol. LXII, 2018, Pp:227-234.

4.Ali, A. M. A., El-Nour, M. E. M. and Yagi, S. M. 2016. Callus induction, direct and indirect organogenesis of ginger (*Zingiber officinale Rosc*.). Afr. J. Biotechnol., 15(38): 2106-2114.

5.Arumugam, S., Chu, F. H., Wang, S. Y. and Chang, S. T.2009. *In vitro* plant regeneration from immature leaflets derived callus of *Acacia confusa* Merr via organogenesis. Plant Biochem Biotechnol, 18 (2), 197-201.

6.Bajaj, Y.P. 1995. Cryopreservation of plant cell, tissue, and organ culture for the conservation of germplasm and biodiversity. Springer, Pp. 3–28.

7.Banerjee,. S, Upadhyay, N., Kukreja, A.K., Ahuja, P.S., Kumar, S., Saha, G.C., Sharma, R. and Chattopadhyay, S. 1996. Taxanes from *in vitro* cultures of the *Himalayan yew Taxus wallichiana*. Planta Medica, 62(4), 329-331.

8.Bhatia, S., Sharma. K., Dahiya, R. and Bera, T. 2015. Modern applications of plant biotechnology in pharmaceutical sciences.Pp 209–230.

9.Çördük, N. and Aki, C. 2011. Inhibition of browning problem during micropropagation of *Sideritis trojana* bornm., an endemic medicinal herb of Turkey. Romanian Biotechnological Letters 16: 6760-6765.

10.De Souza, A., Wulff, M., Camargo, S., Pereira, R., de Souza, E. and Pasa, M.2017. Does propagation method affect the field performance of peach trees? Semin. Ciências Agrárias, 38, 2815–2821.

11.El-Nabarawy, M. A., El-Kafafi, S. H., Hamza, M. A. and Omar, M. A. 2015. The effect of some factors on stimulating the growth and production of active substances in Zingiber officinale callus cultures. Ann Agric. Sci., 60:1-9.

12.Felek W., Mekibib, F. and Admassu, B. 2017. Micropropagation of peach(*Prunus persica*. cv. Garnem) African Journal of Biotechnology. Vol. 16(10), pp. 490-498.

13.Inze, D. and De Veylder, L. 2006. Cell cycle regulation in plant development. Annual Review of Genetics. 40:77–105.

14.Isac, V., Coman, T., Marinescu, L., Isac, M., Teodorescu, A., Popescu, A., Coman, M. and Plopa, C. 2010. Achievements and trends in the use of tissue culture for the mass propagation of fruit plants and germplasm preservation at the research institute for fruit growing, Pitesti, Romania, Romanian Biotechnological Letters, vol. 15, Pp. 92–101.

15.Kant, R., Kumar, R. and Shukla, A. 2018. A review on peach (*Prunus persica*): an asset of medicinal phytochemicals. International Journal for Re-search in Applied Science & Engineering Technology 6(1): 2186-2200. https://doi.org/10.22214/ijraset.2018.1342.

16.Khosroushahi, A. Y., Naderi-Manesh, H. and Simonsen, H. T. 2011. Effect of antioxidants and carbohydrates in callus cultures of *Taxus brevifolia*: Evaluation of Browning, callus growth, total phenolics and paclitaxel production. Bioimpacts. 1(1): 37–45.

17.Kumar, G.P., Subiramani, S., Govindarajan, S., Sadasivam, V., Manickam, V., Mogilicherla, K., Thiruppathi, S.K. and Narayanasamy, J. 2015. Evaluation of different carbon sources for high frequency callus culture with reduced phenolic secretion in cotton (*Gossypium hirsutum* L.) cv. SVPR-2. Biotechnol Rep (Amst). 7, 72–80.

18.Martínez-Gómez, P., Sánchez, P.R., Rubio, M., Dicenta, F., Gradziel, T.M. and Sozzi, G.O. 2005. Application of recent biotechnologies to prunus tree crop genetic improvement. Ciencia e Investigación Agraria 2005, 32, 73–96, http://dx.doi.org/10.7764/rcia.v32i2.308.

19.Mitrofanova, I.V., Smykov, A.V., Mitrofanova, O.V., Lesnikova-Sedoshenko, N.P., Chirkov, S.N., and Zhdanova, I.V. 2019. Using *in vitro* embryo culture for obtaining new breeding forms of peach. IV Balkan Symposium on Fruit Growing, 1289: 159-166.

20.Murata, M., Nishimura, M., Murai, N., Haruta, M., Homma, S. and Itoh, Y. 2001. A transgenic apple callus showing reduced polyphenol oxidase activity and lower browning potential. Bioscience Biotechnology and Biochemistry, 65(2), 383-388.

ISSN NO: 2771-8840
Date of Publication: 28-09-2022

21.Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. physiologia plantrum. 15 : 460-497.
22.Paul, R. and Shylaja, M. R. 2010. Indirect organogenesis in ginger. Indian Journal of Horticulture, 67(4):513-517.
23.Rahayu, S.; Roostika, I.; Bermawie, N. 2016. The effect of types and concentrations of auxins on callus induction of *Centella asiatica*. Nusant. Biosci.(8) 283–287.
24.Setiawan, R. B. Rahmah, M., Trisnia, H., Chaniago, I., Syukriani, L., Yunita, R.and Jamsari, J. 2020. Embryogenic callus induction of coffee (*Coffea arabica* L.) on several plant growth regulator concentration and incubation temperature. IOP Conf. Ser.: Earth Environ. Sci. **497** 012012.
25.Samsudeen, K., Nirmal Babu, K., Divakaran, M. and Ravindran, P.N. 2000. Plant regeneration from anther derived callus cultures of ginger (*Zingiber officinale Rosc*.). Journal of Horticultural Science and Biotechnology 75(4):447-450.
26.Skoog, F. and Miller, C.O. 1957. Chemical regulation of growth and organ formation in plant tissue cultures *in vitro*. Symp. Soc. Exp. Biol. 11, 118–131.
27.Salihan, A.N. and Yusuf, N.A. 2020. High frequency callus induction and proliferation of MD2 pineapple (*Ananas comosus*). Food Research 4(S5):115-123.DOI: 10.26656/fr.2017.4(S5).016.
28.Souza,F.B.M., Alvarenga, Â. A., Pio, R., Gonçalves, E. D. and Patto, L. S. 2013. Fruit production and quality of selections and cultivars of peach trees in Serra da Mantiqueira, Brazil. Bragantia, v.72, p.133-139.
29.SPSS, Inc. (2005). SPSS Base 14.0 for Windows User's Guide. SPSS Inc., Chicago, IL.
30.Stănică, F., Dumitraşcu, M., Davidescu, V., Madjar, R. and Peticilă, A. 2001. Înmulţirea plantelor horticole lemnoase. Ceres Press, Bucharest.
31.**Tian, M., Dane, F., Woods, F.M. and Sibley, J.L. 2010.** Comparison of shoot induction ability of different explants in herbaceous peony (*Paeonia lactiflora* Pall.) Daike" Sci Hort., vol. 123, Pp. 385–389.
32.USDA., 2017. United States Department of Agriculture, Foreign Agricultural Service, Fresh Peach and Nectarine 2016/17.
33.Wang, Y.L. 1985. Peach growing and germplasm in China. Acta Hort. 173:51-55.
34.Wu, J and Lin, L . 2002. Ultrasound-induced stress responses of *Panax ginseng* cells: enzymatic browning and phenolics production. Biotechnology Progress, 18(4), 862-866.