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IN VITRO INFLUENCE OF CULTIVARS AND DIFFERENT CULTURE MEDIA ON VITRIFICATION AND DARKNESS ON PEACH (*Prunus persica* L. Batsch) SHOOTS MULTIPLICATION

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Abstract

Three peach varieties (Florin, Filip and Mimi), two explants (shoots-tip and nodes) were tested. The explants were cultivated on 3 medium MS, B5 and QL adding of 30 g/l sucrose and 7 g/l agar without any hormone supplements during initiation stage. 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. 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). Also QL medium give yellowish green shoots with a high resistance 95% to vitrification.

Key words*: B5, culture media, explants, MS, QL.*

INTRODUCTION

Peach (*Prunus persica* L. Batsch) was cultivation in China before 4000 years ago (Faust and Timon, 1995). Micropropagation peaches method has been used in recent years to produce seedlings with resistant and desirable qualities in large numbers (Al Ghasheem, 2018a). Micropropagation technique depends on several factors including: genetic structure; success of the sterilization process; components of the nutrient medium and added hormones; sources Carbon; blackening phenomenon and appearance of phenolic substances; vitrification phenomenon; etc. (Hammerschlag, 1986; Kubota, 2001; Kozai and Kubota, 2005). Browning was important factors for the successful plant tissue culture, phenolic leaching and contamination is one of the most common problems micropropagation. This process begins by changing and transforming the surface of the cutting plant tissues due to the oxidation of phenolic compounds to brown and thus the formation of quinine, which is considered a highly reactive substance and a toxic substance for plant tissues. (Ko et al., 2009; Taji and Williams, 1996; Xu et al., 2011a; Dayarani et al., 2013). In addition to the use of darkness and subculture, there are many methods that are used to get rid of the browning phenomenon in plant tissue culture, such as the use of activated charcoal (Weatherhead, 1979; Madhusudhanan, and Rahiman, 2000; Thomas, 2008); Antioxidants (Yari Khosroushahi, 2011; Ndakidemi, 2014); Polyphenol inhibitors (Jones and Saxena, 2013; Erland and Mahmoud, 2014; Jones et al., 2012; 2015). Vitrification or Hyperhydricity is a big problem in plants tissue culture technique which can effect on plants multiplication and culture developing (Hammerschlag, 1986). Its affects the morphological characteristics of the plant such as formed leaves and shoots (Pasqualetto, 1990). Anatomically and chemically, xylem with sclerenchyma tissues is less differentiated and lignified accompanied by a hypertrophy in the cortical and pith parenchyma. (Vieitez et al., 1986). Some researcher's divided plants into plants that are excessive in water or not, depending the vitrifaction phenomenon on the

shape of the formed leaves and shoots (Dewir et al., 2006; Tsay et al., 2006, Gribble, 1999; Casanova et al., 2008; Rojas-Martinez and Klerk, 2010) confirmed that the phenomenon of vitrification is a feature of the qualitative characteristics possessed by plants. While others confirmed that the excess water in the culture medium is the cause of the vitrification (Debergh et al., 1992; Dewir et al., 2006). While others mentioned the effect of hormones and Silicon (Si) elements added to the culture medium (Badr-Eldin et al., 2012; Sivanesan, 2014; Soundararajan, 2017) or effect of ventilation and Agar (Majada et al., 1997; Tsay et al., 2006; Badr-Eldin et al., 2012). The study aims to know the effect of the genetic factor and the period of darkness on the phenomena of browning and vitrification on peach micropropagation.

MATERIALS AND METHODS

Three peach varieties were included in the experiment: Florin, Filip and Mimi. Two types of explants (shoots and nodes) were taken at a length of 0.5-1 cm. Explants were collected from healthy trees grown at the Agricultural Research Station of the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania in 2019. For clean and sterilization of explants was carried out to get rid of dust and pathogens via washed with tap water for 30 min. Under Laminar flow cabinet, explants were treated with alcohol (70% ethanol) for several minutes (2-3), after which the alcohol was removed by washing with distilled water 3 times. The explants were treated with NaOCl (10% v/v) for 15-20 minutes for surface sterilized with continuous stirring and shaking by magnetic stirrer to increase the effectiveness of the sterile material and its spread on the surface of the explants, and then rinsed with distilled water at least three times(Alghasheem, 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 any hormone. The pH was adjusted to 5.7. The test tubes were kept in the dark (0, 1, 2, 3, 4) 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). Measurements and results were taken every day in terms of the interaction of phenolic substances and the appearance of the phenomenon of vitrification.

RESULTS AND DISCUSSIONS

Effect of variety and cultures media on tissue browning

During the study, placing the tubes in the dark had a positive effect in removing phenolic substances that cause the explants to discolour to black or brown and cause the toxicity of the cultured tissue and the low success rate of the culture. In Table 1 we were found that placing the explants in the dark for 2-3 days gave the best results, obtaining explants without phenols. Thus, they were recorded for a period of 2 days/dark (Florin 89% healthy explants, Filip 83% healthy explants and Mimi 85% healthy explants), respectively of 3 days/dark (Florin 89%, Filip 84% explants and Mimi 86% healthy explants).(Figures 1, 2 and 3)

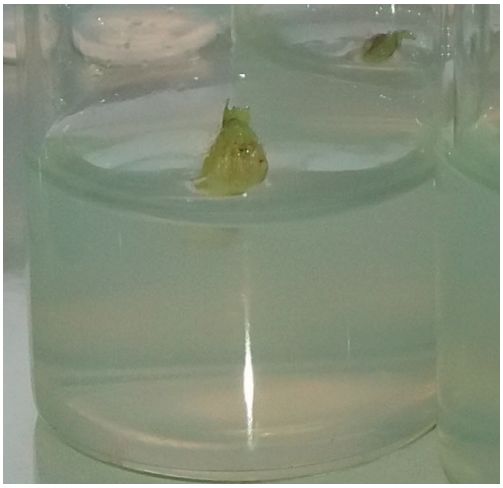

Figure 1. The appearance of phenolic substances after put the explants in culture media (Filip variety)

Explants incubation in the dark decreases browning of the culture medium caused by exudation of phenolic by explants (Nehra et al., 1989; Rugini, 1992; Bhatia and Ashwath, 2005; Xu et al., 2011b). When the plant is cut many enzymes such as polyphenol oxidase,

Table 1. Effect of darkness on phenolic compounds that cause the oxidation of tissue of plant cultured in 3 culture media

Variety	Number of explants	Days														
		0			1			2			3			4		
		% Dead	% growth	Notiţe	% Dead	% growth	Notes	% Dead	% growth	Notes	% Dead	% growth	Notes	% Dead	% growth	Notes
Florin	30	26	74	green to brown green	14	86	Green to yellowish green	11	89	Gree n	11	89	Green	10	90	Green to yellowish green
Filip	30	34	66	green to brown green	20	80	Green to yellowish green	17	83	Gree n	16	84	Green	16	84	Green to yellowish green
Mimi	30	31	69	green to brown green	19	81	Green to yellowish green	15	85	Gree n	14	86	Green	15	85	Green to yellowish green

superoxide dismutase and peroxidase are released into the affected part of the plant. These enzymes work to maintain plant tissues by catalysing various reactions for the purpose of eliminating reactive oxygen and thus healing the plant (Titov et al., 2006).

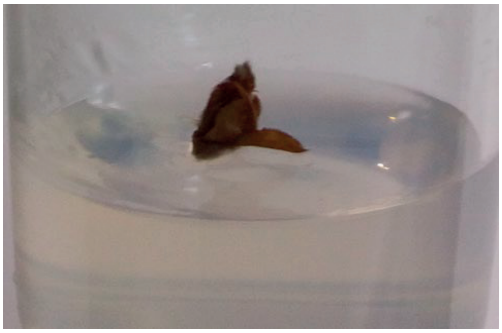

Figure 2. Loss of explant after one week from culture due to reactions of phenolic substances (Florin variety)

In this process, many compounds are produced, including "melanin", which is a pigment characterized by a dark colour and causes the colour of the media as well as the explants to turn brown, and an increase in this substance leads to stopping or impeding the growth of the plant and may lead to its death (Banerjee et al., 1996; Murata et al., 2001; Wu and Lin, 2002; Aquino-Bolaños and Mercado-Silva, 2004; Yari Khosroushahi, 2011).

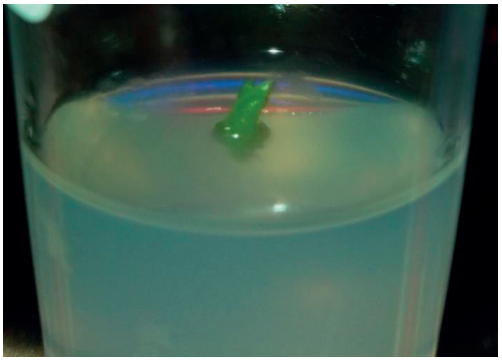

Figure 3. Shoot Mimi variety in QL medium (vitrification resistance of 95%)

Effect of variety and cultures media on vitrification

The study showed that the variety and culture medium affect the resistance to vitrification. The QL medium gives the shoots a high vitrification resistance of 95% (Mimi variety) compared to the MS and B5 media **(**Table 2**).** The study results was similar. Vieitez et al. (1986) studies when using Heller's macronutrient formula with MS medium, Heller's macronutrient recorded the highest vitrification resistance. Morphological abnormalities may be associated with decreased chlorophyll and lignin and increased tissue moisture**.** Vitrification can lead to leaf deformation and bud necrosis or loss the dominance in apical shoots (Cassells and Curry, 2001; Machado et al., 2014). Plants are characterized by abnormal growth and buds, stems and leaves are easy to break; their leaves are also characterized by shrunken or thin and shiny and slow growth and may eventually die(Figure 4) Which leads to a decrease in the vital processes inside the plant from carbon building and the formation of enzymes necessary for the plant and the lack of ionic and phenolic content (Phan and Letouze, 1983; Kevers and Gaspar, 1986; Bottcher et al., 1988; Perry et al., 1999; Frank et al., 2004).

Table 2. Effect of varieties and culture media on resistance of shoots to vitrification

V / M	Florin %	Filip %	Mimi %
MS	45	65	85
B5	70	50	80
QL	85	80	95

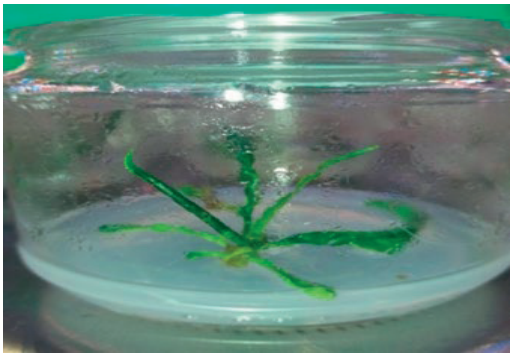

Figure 4. Shoots in MS medium, Filip variety with vitrification state

CONCLUSIONS

In our study we were found that the placement of the cultures tubes in the dark has a positive effect in the disposal of phenolic materials. Results presented that placing the explants in

dark for 2-3 days was the best result to explants phenolic-free, Also QL culture medium gave a high resistance 95% to vitrification.

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