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Research Article

Effect of Polyvinylpyrrolidone (PVP) on Meristem Establishment and *In-vitro* Organogenesis of Iranian Pear (*Pyrus glabra*)

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ABSTRACT

Iranian pear (*Pyrus glabra*) is one of the most important specie for breeding programs and rootstock production. Propagation of plants by the tissue culture method is very common in many plant species including fruit crops like pear. This paper describes the procedure for the efficient regeneration of plants from meristem cultures of *Pyrus* and focused on the browning problem in the Iranian pear (*Pyrus glabra*), by the application of polyvinilpyrolidine (PVP) and suggest the best ways to overcome the problem. In order to study the effect of polyvinilpyrolidine (PVP) on meristem viability and browning, one year mature shoot of *Pyrus glabra* was collected during growing season of 2011 from tree in Sardasht (West Azerbaijan Province, Iran) and three level of PVP (0, 100 and 200 mgl^{-1}) were added to culture medium. The experiment was arranged as complete plots on the basis of completely randomized design with three replications. The result showed that mersitem survival was significantly affected by concentration of PVP. Maximum meristem surveying in 1 day and 28 days after culture has been seen in medium which supplemented by 200 mgl^{-1} of PVP. Based the result of the present study, strict control of minimizing phenol pollution in the whole culture process is important and effective.

Key words: Iranian Pear, *In-vitro* Organogenesis, Meristem Culture, Polyvinylpyrrolidone, *Pyrus glabra*

INTRODUCTION

Pear is one of the oldest fruit crops widely grown in temperate and sub-tropical regions of the world and is one of the most important temperate fruit crops. It belongs to the *Rosaceae* family and *Pomoideae* subfamily (Jackson, 2003; Jules and James, 1996). Out of the 22 species of Pyrus, 16 originated from Asia. Mainland China is the center of origin of most Asian pears (Nee *et al*., 2002; Wang, 1996). Based on Khatamzas (1994) there are 12 *Pyrus* species in Iran and *Pyrus glabra* is one of the most important species in Iran.

Propagation of plants by the tissue culture method is very common in many plant species including fruit crops like pear. However, sometimes during *in vitro* culture of some plant species, the media will become brown and the explants unable to grow further and eventually die. Some explants leach some phenolic substances or secondary metabolites from cut surfaces, which oxidize later and turn the media brown and is toxic to the explants (Aliyu, 2005). Browning of media and explants is common especially from tree species and mature tissues from the woody species. Although browning of medium and also explants is a serious problem of many plant species for tissue culture, it is an important phenomenon in plants. According to Chen *et al*. (1997), polyphenol has some regulatory effects on the growth and development of plants, disease resistance, and induction of gene expression, signal transduction, biological nitrogen fixation and UV ray absorption. The problem of browning during tissue culture was reported in many plant species including fruit trees like cashew (Aliyu, 2005), litchi (Chandra and Padaria, 1999), guava (Meghwal *et al*., 2000), banana, avocado (Castro *et al*., 1995) pear (Gao *et al*., 2003). Within the plant species, different varieties differ greatly with each other in terms of the browning problem. This phenomenon is very common in both Asian as well as in the European pears. Success or failure of tissue culture largely depends on browning of the culture medium especially in fruit trees like pears.

The technique of meristem culture has been successfully employed to eliminate viral pathogens from a wide range of plant species. Meristem culture of rootstocks and cultivars in Malus and Vitis has been

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performed by many researchers (Abbot *et al*., 1976). However, there are few reports of meristem culture in *Pyrus* especially *P.serotina* (Bhojwani *et al*., 1984). This paper describes the procedure for the efficient regeneration of plants from meristem cultures of *Pyrus* and focused on the browning problem in the Iranian pear (*Pyrus glabra*), by the application of polyvinilpyrolidine (PVP) and suggest the best ways to overcome the problem.

MATERIALS AND METHODS

One year mature shoot of *Pyrus glabra* was collected during growing season of 2011 from tree in Sardasht (West Azerbaijan Province, Iran). Wood was cut into 10 - 15 cm long sections, the basal ends (2 cm) were immersed in tap water in 500 ml beakers, and kept at room temperature (in the laboratory) at 24 + 2°C day/l8 + 2°C night for 3 weeks until buds broke and new growth was initiated. Bud scales were removed, and buds were surface sterilized in 5% Clorox (5.25% sodium hypochlorite) plus 0.1% Tween-20 (surfactant) for 10 min and then rinsed three times with sterile distilled water (for 5 min each time) under a laminar air-flow cabinet. Shoot tips (0.5-0.7 mm) were excised aseptically under a binocular microscope and inoculated in 9 cm petri plates (two shoot tips per plate) on l/2 strength MS (Murashige and Skoog, 1962) The medium was supplemented with 0.5 mg 1-l benzyl adenine (BA) and 0.1 mg I-' napthaleneacetic acid (NAA), 30 g 1-l sucrose and 8.0 g 1-i Difco Bacto agar (Difco Laboratories, USA). Plates were kept in the dark for 2 days, then moved to a growth chamber (22 f 2"C), under a 16 h light (photosynthetic photon flux 40-60 p,mol m-2 s-I)/8 h dark photoperiod. In order to study the effect of polyvinilpyrolidine (PVP) on meristem viability and browning three level of PVP (0, 100 and 200 mgl^{-1}) has been add to culture medium. Shoot tips were initially transferred every 3 days, three times to minimize phenolic accumulation. Two weeks after establishment, shoot tips were transferred to 40 ml solid medium supplemented with 1.0 mg l- ' BA and 0.1 mg 1-l NAA, and transferred to fresh media six times at 7 week interval before starting experimentation.

The experiment was arranged as complete plots on the basis of completely randomized design with three replications. Analysis of variance (ANOVA) carried out with SPSS v.17 software. The significance of the differences among treatments was tested by applying a one-way ANOVA, at a confidence level of 95%.

RESULTS AND DISCUSSION

In-vitro organogenesis of *Pyrus glabra* from meristme culture was shown in figure 1. Successful in vitro establishment of wild pear was achieved in this study. The result showed that mersitem survival was significantly affected by concentration of PVP (Fig 2 and 3). Supplementation of medium by PVP not only after 24 hours (Fig 2), but also one month after sowing increased their surviving meaningfully with relating to the control. After one day of culture, the maximum meristem surveying was shown in 200mg/L PVP. Maximum meristem surveying in 4 weeks after culture has been seen in medium which supplemented by 200 mgl^{-1} of PVP (fig 3). According to Mager and Harel (1979), in the normal tissue, no browning happens, because Polyphenol Oxidase (PPO) and phenolic compounds are separated by a membrane structure and if the structure were to be broken browning happens. Likewise, according to Ju *et al*.

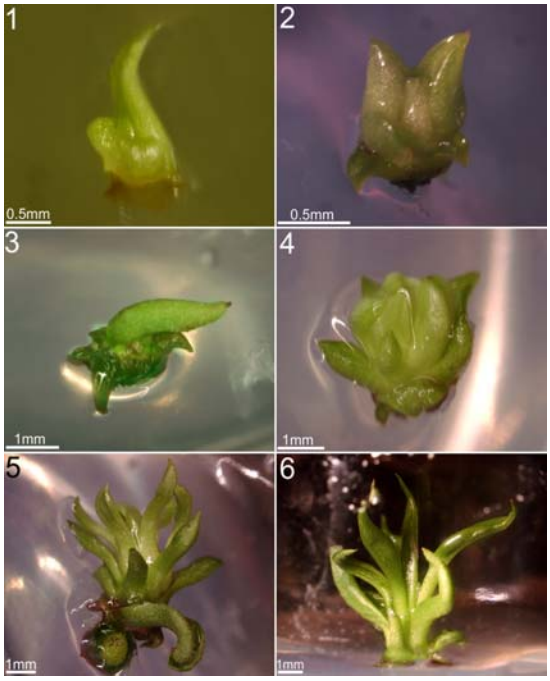

Fig. 1: Evolution of regenerated Pyrus glabra leafs from meristem culture during 6 weeks.

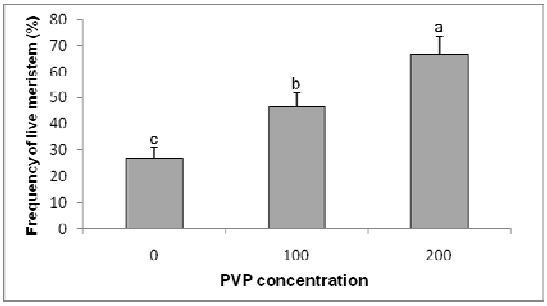

Fig. 2: Meristem viability after 1 day. Values carrying different letters are significantly different at P ≤0.05.

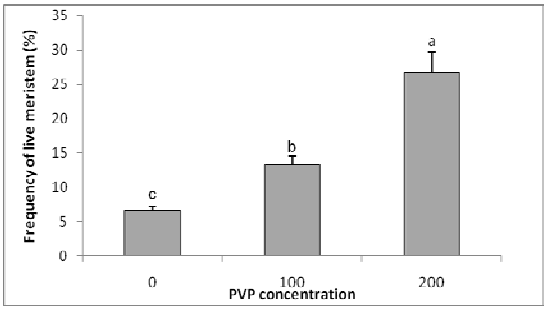

Fig. 3: Meristem viability after 28 days. Values carrying different letters are significantly different at P≤0.05.

(1988), in the normal cells, polyphenolic compounds are in vacuoles, while the PPO is located in the cytoplasm and if compartmentation were to be broken, browning will happen. Application PVP was not significant effect on the process of leaf initiation and organogenesis. The rate of leaf initiation in explants taken from both kinds of branches, initially decreased and then increased gradually.

Pear has high content of polyphenol in the apical meristem tissue. When they were dissected, the polyphenols were oxygenated under catalysis of Polyphenol Oxidase (PPO) and became brown quinines. These oxides could restrain the normal physiological metabolism in the tissues and inhibit the absorption of nutrition by the stem tip explants. Finally, the tissue became brown and dead. Phenol pollution is very common in the tissue culture of pear (Poydal *et al*. 2008). One of the critical point to ensure successful meristem culture of pear is to prevent phenol pollution. The result of this study showed that application of PVP to medium could be control phenol pollution. Based the result of the present study, strict control of minimizing phenol pollution in the whole culture process is important and effective.

REFERENCES

Abbot A and E Whiteley, 1979. Culture of *Malus* tissues in vitro. I Multi-plication of apple plants from isolated shoot apices. Sci Hort, 4: 183-189.

Aliyu OM, 2005. Application of tissue culture to Cashew (*Anacardium occidentale*) breeding: an appraisal. Afr J Biotechnol, 4: 1485-1489.

Bhojwani SS, K Mullins and D Cohen. 1984. In vitro propagation of *Pyrus jynfolia.* Sci Hortic, 23: 247-254.

Castro M, E Oyanedel, R Cautı´n .1995. In vitro shoot proliferation in Avocado (Persea americana) induced by CPPU. Proceedings of the World Avocado Congress III: 223-226.

Chandra R and JC Padaria, 1999. Litchi shoot bud culture for micro propagation. J Appl Hortic, 1: 38-40.

Jackson JE, 2003. Biology of Apples and Pears. Cambridge: Cambridge University Press, 22

Jules J, James NM, 1996. Pears in fruit breeding. Tree and Tropical Fruits, pp: 441-514.

Khatamsaz M, 1994. Rosacea Family. Flora of Iran. Pub. Iranian research organization of forests and pastures.

Meghwal PR, Sharma HC, Singh SK, 2000. Effect of surface sterilizing agents on in vitro culture establishment of guava (*Psidium guajava*). J Appl Hortic, 2: 94-95

Murashige T and F Skoog, 1962. A revised medium for rapid growth and bio assays with tobacco tissue cultures. Physiol Plant, 15: 473-497.

Ju Z, 1987. The effects of PPO and its substrates on tissue browning of four pear cultivars. J Laiyang Agri Coll, 4: 42–47.

Nee CC, CH Tsai and DD Anstine, 2002. Asian pear germplasm future trends and current research in the industry. Acta Hortic, 587: 61-69.

Poudal BK, Y Zhang and DU Guoqiang, 2008. Adventitious shoot regeneration from the leaves of some pear varieties (*Pyrus spp.*) grown in vitro. Front Agri China, 2: 82-92.

Wang YL, 1996. Chinese Pears. Beijing: China Agricultural Scientech Press, 1.