Journal of Genetic Engineering and Biotechnology (2015) **13**, 19–23

HOSTED BY

ELSEVIER

Academy of Scientific Research & Technology and National Research Center, Egypt

Journal of Genetic Engineering and Biotechnology

www.elsevier.com/locate/jgeb

ARTICLE

Optimizing culture media for *in vitro* proliferation and rooting of Tetra (*Prunus empyrean* 3) rootstock

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Received 21 August 2014; revised 18 December 2014; accepted 23 December 2014
Available online 31 January 2015

KEYWORDS

In vitro propagation;
Tetra;
Vegetative rootstock;
Rooting

Abstract The enormous demand for new rootstock genotypes in *Prunus* spp. makes us to use micropropagation as an unavoidable propagation method. Therefore, the study on micropropagation of a new semi-dwarf vegetative rootstock namely Tetra (*Prunus empyrean* 3) was carried out to develop an optimized protocol. Culture establishment using nodal segments was enhanced using WPM (woody plant medium) medium lacking growth regulators. From various shoot multiplication treatments, the highest number of shoots per explant (30.4) was found on ME (Media created specifically) medium supplemented with 0.8 mg l^{-1} BAP and 0.05 mg l^{-1} IBA. 100% *in vitro* rooting was achieved on ½ strength MS medium with 0.5 mg l^{-1} IBA, 1.6 mg l^{-1} thiamine and 150 mg l^{-1} iron sequestrene.

1. Introduction

Tetra or Empyrean® 3 (*Prunus domestica*) is a semi-dwarf (80–90% of Lovell) vegetative rootstock which is resistant to root-knot nematode, phytophthora, waterlogged, and heavy soils [18] compatible with peach, plum, almond and apricot. It is native to Istituto Sperimentale per la Frutticoltura (ISF) di Roma, Italy. It is non-suckering and is easily propagated by hardwood cutting [using a quick-dip in 2000 mg l^{-1} indol-3-butric acid (IBA)] [18]. *In vitro* propagation is also a massive propagation strategy which can be used in response to the growing request for new commercial *Prunus* spp. Micropropagation of *P. domestica* as rootstock has been dealt with first by Boxus and Quoirin [3], Quoirin et al. [20] and Zuccherelli [30]. An effective *in vitro* culture system for mature stem segments of Chinese plum (*P. salicina* Lindl. cv. 'Gulf ruby') has been established [29]. They collected 1 cm nodal explants of newly emerged shoots, sterilized and established them *in vitro*. Successful culture establishment was achieved on a woody plant medium (WPM) supplemented with 0.05–0.1 mg l^{-1} IBA, 0.5–1.0 mg l^{-1} 6-Benzylaminon purine (BAP), 30 g l^{-1} glucose, 5 g l^{-1} agar and 1.0 g l^{-1} vitamin C (VC). They obtained the highest shoot multiplication rate on WPM with 0.05–

Abbreviations: BAP, 6-benzylamino purine; DW, dry weight; FW, fresh weight; IBA, indol-3-butric acid; LS, Linsmaier and Skoog (1965); ME, media created specifically (Cos et al., 2004); MS, Murashige and Skoog (1962); WPM, woody plant medium

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Peer review under responsibility of National Research Center, Egypt.

http://dx.doi.org/10.1016/j.jgeb.2014.12.006

0.1 mg l^{-1} IBA, 0.2 mg l^{-1} BA, 0.3 mg l^{-1} kinetin (KT) and 1.0 g l^{-1} casein hydrolysate. According their results, *in vitro* shoot elongation was facilitated on WPM with 0.05 mg l^{-1} IBA, 0.3 mg l^{-1} KT/BA and 1.0 g l^{-1} casein hydrolysate. They carried out *in vitro* rooting in half strength MS (½ MS) medium supplemented with 0.2–0.5 mg l^{-1} IBA, 15 g l^{-1} sucrose and 20–40 mg l^{-1} phloroglucinol.

As well, in order to develop a protocol for micropropagation of plum (*P. domestica* L.), Ruzic and Vujovic [23] regenerated shoots from *in vitro* nodal segments of plum cultivars ČačanskaRodna and Valjevka established in Murashige and Skoog [16] (MS) medium containing 2.0 mg l^{-1} BA, 0.5 mg l^{-1} IBA, 0.1 mg l^{-1} gibberellic acid (GA_3), 7 g l^{-1} agar and 20 g l^{-1} sucrose. Regenerated shoots were multiplied in MS medium supplemented with 1.0 mg l^{-1} BA, 0.1 mg l^{-1} IBA, 0.1 mg l^{-1} GA_3, 7 g l^{-1} agar and 20 g l^{-1} sucrose. The shoot regeneration medium consisted of MS supplemented with 30 g l^{-1} sucrose and 7 g l^{-1} agar. The growth regulators used to induce shoot regeneration from plum leaves were 5 mg l^{-1} BAP and 0.5 mg l^{-1} IBA. The basal rooting medium was ½ MS with 1% sucrose, proliferation medium organics and 0.5 mg l^{-1} IBA.

To our knowledge, this is the first comprehensive compiled study on the micropropagation of a new important *Prunus* rootstock viz. Tetra. The aim of the present investigation was to optimize *in vitro* propagation technique for Tetra (*P. domestica*) rootstock, develop a practical protocol for its commercial propagation. As well as study the effect of the chelated form of the iron salt of ethylene diamine di-o-hydroxyphenyl acetic acid (Fe-EDDHA) (6% Fe) on *in vitro* rooting of the Tetra rootstock.

2. Material and methods

2.1. Plant material sterilization and culture conditions

15–20 cm length shoots were cut of pot plants maintained in glasshouse of the Horticultural Department of Faculty of Agriculture, Tarbiat Modares University, Tehran, Iran during September 2011 and transferred to laboratory.

Nodal segments (3 cm) were excised and were surface sterilized by agitating for 15 min in a solution containing 2 drops of Tween-20 in 100 ml of water and then washed under running tap water for 30 min. Explants were sterilized with 0.1% mercury chloride for 4 min. Later, they were washed twice with double distillated water containing 700 mg l^{-1} citric acid.

All explants were cultured on different types of media and transferred to growth room with light intensity of 2500–3000 lux, photoperiod of 16/8 h light/dark, relative humidity of 45% and constant temperature of 25 ± 1 °C.

2.2. Culture establishment

McCarthy glasses were used for culture establishment stage of explants. Woody Plant Medium (WPM) containing 7 g l^{-1} agar and 30 g l^{-1} sucrose (pH of all media was adjusted to 5.75 before autoclaving) was used to establish the explants and after 4 weeks, explants were transferred to shootlet proliferation media.

2.3. Shootlet proliferation

WPM and media created specifically [6] (Cos) containing 3 concentrations of BAP (0.4, 0.8 and 1.2 mg l^{-1}) and 30 g l^{-1} sucrose and 7.5 g l^{-1} agar were compared. Number and length of shoots were recorded after 4 weeks.

In the second experiment of proliferation optimization, the effect of different concentrations of IBA (0, 0.05, 0.1, 0.15 and 0.2 mg l^{-1}) in combination with 0.8 mg l^{-1} BAP in Cos medium was assessed.

2.4. Shoot elongation

The proliferated shoots were elongated on Cos medium supplemented with different concentrations of Naphthalene acetic acid (NAA) (0, 0.01 and 0.1 mg l^{-1}) and sucrose (20 and 30 g l^{-1}) and length of shoots was measured.

2.5. Rooting

In vitro shoots (2–3 cm) were transferred to ½ MS, LS [11] and Cos media supplemented with 20 g l^{-1} sucrose, 7.5 g l^{-1} agar, and different concentrations of IBA (0.5, 1, 1.5 and 2 mg l^{-1}).

All treatments were maintained in the dark for 1 week and then they were transferred to the light. Each treatment included 5 replications, each replicate included 2 explants. Rooting percentage, root number and root length (cm) were recorded after 40 days.

In order to optimize the rooting conditions, the effect of different combinations of Fe-EDDHA (100, 150 and 200 mg l^{-1}) and thiamine (0, 1.6, 2.8 and 4 mg l^{-1}) was considered.

2.6. Statistical analysis

The experiment was carried out based on completely randomized design (CRD) with factorial arrangement and 5 replications per treatment. Statistical analysis of the data was carried out using SPSS 18 software and obtained means were compared using Duncan's Multiple Range Test ($p \leqslant 0.05$).

3. Results and discussion

3.1. Proliferation

Based on the results obtained, the interaction effect of culture media and BAP concentration (Table 1) on both number and length of shoots were significant. The effect of Cos medium containing 0.4 mg l^{-1} BAP on number of shoot (5.6) was significantly more than the effect of the same medium containing 0.8 mg l^{-1} BAP (3.6) and WPM medium containing 0.4 mg l^{-1} BAP. In this respect, there were no significant differences among treatments.

Shoot length mean in Cos containing 0.8 mg l^{-1} BAP (2.03 cm) was significantly higher than ones in the rest treatments except WPM containing 0.4 mg l^{-1} BAP (1.99 cm). So it can be realized that in Cos medium enhancing shoot number and length will be achieved by 0.4 and 0.8 mg l^{-1} BAP, respectively. But in case of WPM medium it is suggested that in general, both 0.4 and 1.2 mg l^{-1} BAP concentrations could be