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Regeneration of adventitious shoots from mature stored cotyledons of Japanese plum (*Prunus salicina* Lind1)

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ABSTRACT

Adventitious shoot regeneration from mature stored cotyledons of Japanese plum (*Prunus salicina* Lind1) was achieved *in vitro*. The influences of the presence and absence of the light, different concentrations of thidiazuron (TDZ) and benzyladenine (BA) in the culture media, TDZ pretreatments and different basal salts on shoot regeneration were evaluated. TDZ was more effective in inducing shoot regeneration from mature stored cotyledons than BA. Dark incubation significantly increased the regeneration frequencies. Quoirin/Lepoivre (QL) basal salts stimulated shoot regeneration more than woody plant (WPM) or B5 salts did. The frequency of adventitious shoot formation varied among the varieties and the regeneration ability appeared to be genotype depended. The frequency of regeneration under the optimum tested conditions for 'Bruce', 'Shiro', 'Redheart', 'Gladstone' and 'Early Golden' cotyledons were 66.7%, 46.7%, 43.3%, 26.7% and 6.7%, respectively.

1. Introduction

Adventitious shoot regeneration is a prerequisite for any plant improvement programme aimed at improving plants by *in vitro* techniques and genetic engineering. Plant biotechnology and genetic engineering technologies are particularly useful for fruit trees since these technologies have the potential to reduce the time needed for traditional breeding (Petri and Burgos, 2005). Attempts to improve fruit trees by genetic engineering depend largely on availability of a reliable regeneration protocol.

There are a number of reports on regeneration of adventitious shoots in *Prunus* species using different explants. Regeneration has been achieved from leaves of almond (*Prunus dulcis* Mill.) (Miguel et al., 1996), apricot (*Prunus armeniaca*) (Pérez-Tornero et al., 2000; Burgos and Alburquerque, 2003), black cherry (*Prunus serotina* Ehrh) (Hammatt and Grant, 1998), sour cherry (*Prunus cerasus* L.) (Dolgov and Firsov, 1999; Tang et al., 2002; Song and Sink, 2005), and sweet cherry (*Prunus avium* L.) (Tang et al., 2002; Bhagwat and Lane, 2004; Matt and Jehle, 2005). Regeneration of adventitious shoots has also been reported from immature cotyledons of apricot (Lane and Cossio, 1986), sweet cherry (Lane and Cossio, 1986), peach (*Prunus persica*) (Mante et al., 1989; Wu et al., 2005; Yan and Zhou, 2002), sour cherry (Mante et al., 1989; Tang et al., 2000) and almond (Ainsley et al., 2001). In addition, regeneration using mature cotyledons has been reported for peach (Pooler and Scorza, 1995), sweet cherry (Canli and Tian, 2008) and ornamental cherries (*Prunus* spp.) (Hokanson and Pooler, 2000).

Japanese plum (*Prunus salicina* Lind1), a close relative of European plum (*Prunus domestica* L.), is an important *Prunus* fruit species and is widely grown across the world (Okie and Ramming, 1999; Bellini et al., 2002; Kaufmane et al., 2002). Adventitious shoot regeneration in European plum has been reported from leaves (Bassi and Cossio, 1991, 1994; Nowak and Miczynski, 1997; Nowak et al., 2004), cotyledons (Mante et al., 1989) and hypocotyls (Mante et al., 1991), and it has been routinely transformed (Mante et al., 1991; Camara Machado et al., 1994; Yancheva et al., 2002). There are only two reports of *in vitro* regeneration of Japanese plum using hypocotyls explants (Tian et al., 2007b; Urtubia et al., 2008), but the reported regeneration frequencies are very low. Transformation has also recently been reported for Japanese plum (Urtubia et al., 2008), however, the efficiency was very low due mainly to the low frequency of regeneration in this species (Urtubia et al., 2008). Improvement of transformation efficiency is important

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Abbreviations: BA, 6-benzylaminopurine; B5, Gamborg; IBA, indolebutyric acid; MS, Murashige and Skoog; NAA, 1-naphthalene-acetic acid; QL, Quoirin and Lepoivre; TDZ, 1-phenyl-3-(1,2,3-thiadiazol-5-yl)urea,thidiazuron; WPM, Lloyd and McCown.

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for genetic engineering of this species, which necessitates further regeneration experiments for Japanese plum.

In the present study, shoot regeneration from stored mature cotyledons of Japanese plum was achieved for the first time. This regeneration protocol may be used to improve the transformation efficiency in Japanese plum.

2. Materials and methods

2.1. Plant material and explant preparation

Mature plum fruits were collected from trees of cv. 'Shiro', 'Early Golden', 'Gladstone', 'Red heart', 'Bruce' and 'Cocheo' in Vineland Station, Ontario, Canada. After the removal of the fruit flesh, endocarps were washed with tap water, disinfected with a 0.05% sodium hypochlorite solution and rinsed under running tap water 3–5 times. The seeds were dried on a lab bench for 3–4 days at room temperature (20–25 °C) and stored in plastic mesh bags at 4 °C.

After endocarps were cracked, seeds were surface sterilized in 0.525% sodium hypochlorite (10% commercial bleach) for 15 min and rinsed three times with sterile distilled water. After disinfection, seeds were imbibed overnight in sterile distilled water. Under a dissecting microscope, the seed coat was removed and the embryonic axis was separated from the cotyledons. Small slices from cotyledons were removed with the tip of a scalpel on both sides of the point where the embryonic axis was attached. The cotyledons were then cut into two equal segments transversely. Proximal segments were placed on shoot induction medium adaxial sides down (Fig. 1A). Since the morphogenic capacity elevated significantly from the distal parts towards proximal parts of the cotyledons in other species (Lane and Cossio, 1986; Mante et al., 1989; Tang et al., 2000), distal parts of the cotyledons were not used in the current study. Each treatment was replicated three times and each replicate contained ten cotyledon explants.

2.2. Thidiazuron (TDZ) pre-soaks

Explants that received TDZ pretreatments after disinfection were imbibed for 24 h in sterile distilled water containing different levels of TDZ (0, 0.23, 2.3 and 4.6 μM TDZ).

2.3. Shoot induction medium and growth conditions

The shoot induction medium was basal salts as described at Quoirin and Lepoivre (1977) (QL) containing vitamins (555 μM myo-inositol, 1.2 μM thiamine HCl, 1.4 μM nicotinic acid, 2.4 μM pyridoxine HCl), 2.5 μM indolebutyric acid (IBA), 25 g l^{-1} sucrose, and 7 g l^{-1} Bactoagar. The pH of the medium was adjusted to 5.6 before autoclaving at 121 °C and 106 kPa for 30 min. TDZ or 6-benzylaminopurine (BA) at different concentrations (3.75, 7.5, 11.75, 15 or 30 μM TDZ and 4.4, 8.8, 17.6 or

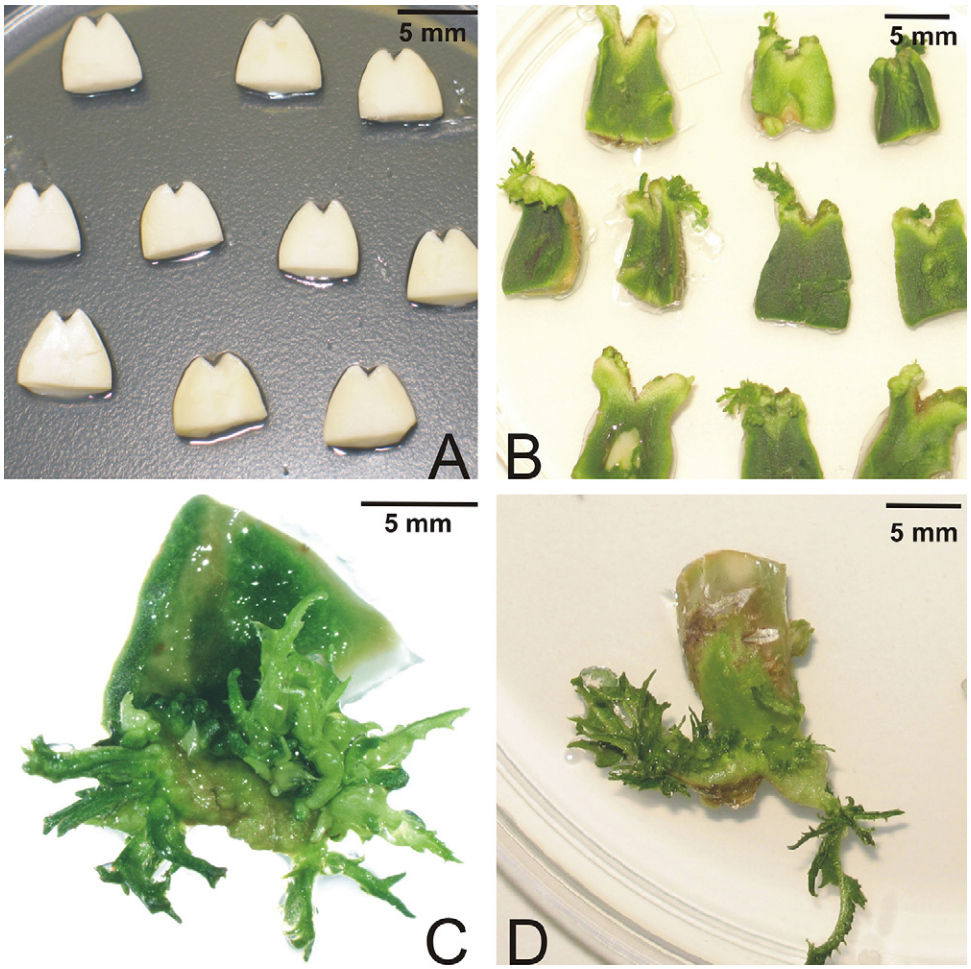

Fig. 1. Regeneration of adventitious shoots from stored mature cotyledons of Japanese Plum (*Prunus Salicina* Lind1) *in vitro* (A) 1-day-old cotyledon explants; (B) adventitious shoots on explants that were incubated in dark of cv. 'Bruce' on the 18th day; (C and D) close ups of the adventitious shoots from cotyledon explants after 18 days of culture initiation.