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Optimizing shoot regeneration and transient expression factors for *Agrobacterium tumefaciens* transformation of sour cherry (*Prunus cerasus* L.) cultivar Montmorency

Guo-Qing Song, Kenneth C. Sink *

Plant Transformation Center, Department of Horticulture, Michigan State University, East Lansing, MI 48824, USA

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Abstract

An efficient, adventitious shoot regeneration protocol was devised, and transient expression studies were carried out to enable *Agrobacterium*-mediated stable transformation of sour cherry (*Prunus cerasus* L.) cultivar Montmorency. Leaves, from in vitro stock cultures, with the petiole removed and four partial cuts made transversely and equidistant through the midrib area were found to be the optimum explant type. A 24 h liquid TDZ-pretreatment (0.05, 0.10 or 0.25 mg/l) in MS medium [Murashige, T., Skoog, F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Plant Physiol. 15, 473–497.] of leaf explants stimulated shoot formation upon subsequent culture on QL medium [Quoirin, M., Lepoivre, P., 1977. Improved media for in vitro culture of *Prunus* sp. Acta Hort. 78, 437–442.] supplemented with 3.0 mg/l BAP and 0.5 mg/l NAA. A frequency of 38.9–54.4% of the explants produced at least one shoot with the maximum mean number of shoots, 4.5 per explant with the 0.10 mg/l TDZ pretreatment. The shoot regeneration scheme was subsequently linked with inoculation with *Agrobacterium tumefaciens* strains EHA105, GV3101 or LBA4404, each harboring the binary plasmid pBISN1. PBISN1 contains an intron interrupted ß-glucuronidase (GUS) gene (*gus*A) under control of the chimeric super promoter

Abbreviations: AS, acetosyringone; BAP, 6-benzylaminopurine; *gus*A, ß-glucuronidase gene; Cx, cefotaxime sodium salt; GUS, ß-glucuronidase; IBA, indole-3-butyric acid; MS, Murashige and Skoog; NAA, α-naphthaleneacetic acid; *npt*II, neomycin phosphotransferase gene; QL, Quoirin and Lepoivre; RM, regeneration medium; TDZ, thidiazuron; WPM, Lloyd and McCown; YEB, Vervliet et al. (1975)

* Corresponding author. Tel.: +1 517 355 5191x384; fax: +1 517 432 8825.
E-mail address: sink@msu.edu (K.C. Sink).

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$(Aocs)_3$AmasPmas. Blue stained leaf cells were observed after co-cultivation with all three strains. Co-cultivation for 4 days with 19.6 mg/l acetosyringone (AS) and assay by GUS indicated over 90% of the leaf explants were infected with an average 7.5–8.8 blue foci per explant. No differences were observed in regard to *A. tumefaciens* strain used.
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Keywords: *Agrobacterium*; Fruit crops; GUS; Rooting; Thidiazuron; Tissue culture

1. Introduction

Breeding of tetraploid sour cherry (*Prunus cerasus* L.) is a difficult and time-consuming process due to heterozygosity, time interval between generations, asexual propagation, and length of field evaluations. Thus, gene transformation provides an attractive means to incorporate single or multiple genes into existing cultivars. However, sour and sweet cherry are still among the plant species that yet have been reported transformed by *Agrobacterium tumefaciens* due principally to the lack of efficient shoot regeneration and transformation system(s). However, progress has been made as several reports are devoted to the development of shoot regeneration systems for sweet (Mante et al., 1989; Yang and Schmidt, 1992) and sour cherries (Mante et al., 1989; Dolgov and Firsov, 1999; Tang et al., 2000). Plant regeneration from leaf explants of sour cherry at low frequencies was reported for only a few genotypes (Dolgov and Firsov, 1999; Tang et al., 2002). Shoot regeneration of Montmorency only has been reported using cotyledon explants at a frequency of 58% on MS (Murashige and Skoog, 1962) supplemented with 1.6 mg/l thidiazuron (TDZ) and 0.5 mg/l indole-3-butyric acid (IBA) (Mante et al., 1989). However, the genetic integrity of Montmorency would be compromised by the use of self-pollinated offspring.

To date, transformed plants of cherry rootstock Colt (*P. avium* L. × *P. pseudocerasus*) containing the three genes, *rol* A, B, and C of the non-disarmed *A. rhizogenes* pRi1855 T-DNA were produced and exhibited enhanced rooting capacity, shortened internodes, and wrinkled leaves (Gutièrrez-Pesce et al., 1998). *A. tumefaciens*-mediated transformation of sour cherry has progressed only to the transient expression stage. Dolgov and Firsov (1999) obtained callus lines resistant to hygromycin after infection of leaf explants of six sour cherry cultivars with several strains. Using a sour–sweet hybrid and inoculation with EHA105:p35SGUSINT, 3/58 regenerants grew on hygromycin medium and had GUS activity. No molecular studies were made on the regenerates. Numerous factors influence transient expression including *Agrobacterium* strain, plasmid type, time of inoculation and/or co-cultivation, and temperature and environmental conditions at various stages.

Further regeneration and transient expression studies appeared warranted for sour cherry. Especially the cultivar Montmorency as it is the economically most important sour cherry in Michigan. Thus, in the present studies, a two-step regeneration method starting with TDZ liquid pretreatment was evaluated for improvement of adventitious shoot regeneration from leaf explants of Montmorency. Subsequently, the optimum regeneration protocol was linked to transient expression studies of *A. tumefaciens* strains, use of acetosyringone, and co-cultivation time to provide guidelines for stable transformations.

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