Available online at www.sciencedirect.com
ScienceDirect
Scientia Horticulturae 113 (2007) 129–133
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Somatic embryogenesis and plant regeneration in *Psidium guajava* L. cv. Banarasi local

Manoj K. Rai, N. Akhtar, V.S. Jaiswal *

Lab of Morphogenesis, Department of Botany, Banaras Hindu University, Varanasi 221005, Uttar Pradesh, India

Received 7 October 2006; received in revised form 20 February 2007; accepted 20 February 2007

Abstract

A protocol for plant regeneration by somatic embryogenesis was developed in guava cv. Banarasi local by using immature zygotic embryo explants. Best induction of somatic embryogenesis was achieved from 10-week-old zygotic embryos on MS medium supplemented with 2,4-D (4.52 μM) and 5% sucrose. Maximum number of somatic embryos was produced when zygotic embryo explants were transferred to growth regulator free full strength MS basal medium after 8 days treatment with 2,4-D. Full strength MS basal medium containing 5% sucrose was most favorable for maturation of somatic embryos. Highest frequency of conversion and normal plantlet production were recorded from elongated torpedo stages of somatic embryos on half strength MS medium containing 3% sucrose. Over 90% of rooted shoots survived acclimatization.

Keywords: Plant growth regulators; Plant regeneration; *Psidium guajava*; Somatic embryogenesis; Zygotic embryos

1. Introduction

Guava (*Psidium guajava* L.) belongs to the family Myrtaceae and is an important fruit crop of the tropics and subtropics. It is facing major agronomic and horticultural problems in view of its susceptibility to many pathogens, stress sensitivity, low fruit growth, short shelf life, etc. Various biotechnological approaches may be effective practical solutions for such problems. Recently, developments of genetic transformation technology and efficient systems of controlling morphogenesis from cells and tissues have been shown to have great advantage in crop improvement. The difficulty in regenerating many tropical fruit tree species from elite or mature phase selections is one of the most serious problems for applying gene transfer technologies to these plants (Gomez-Lim and Litz, 2004). There is need for development of an efficient regeneration system in guava. During the past years, considerable efforts have been made for *in vitro* plant regeneration of this important fruit species via somatic embryogenesis (Chandra et al., 2004; Jaiswal and Jaiswal, 2005).

Somatic embryogenesis provides an ideal experimental process for investigation of plant differentiation as well as the large-scale production of plants (Litz and Gray, 1992). Regeneration of plants through somatic embryogenesis has been a preferred method for genetic transformation in woody perennials because somatic embryogenesis leads to the formation of bipolar structures, possessing both a shoot and a root meristem and somatic embryos have always assumed to have a single cell origin and have closed vascular system (Litz and Gray, 1992). Chandra et al. (2004) have reported indirect embryogenesis in guava from mesocarp explants. But they did not mention that how many somatic embryos induced per explant or which stage of somatic embryo undergoes till germinating phase, as this is very important for applying genetic engineering tools.

In the present paper, we report the direct somatic embryogenesis in guava cv. Banarasi local from immature zygotic embryo explant answering the above questions. The present embryogenesis system may be used for high frequency regeneration of guava, and as the embryogenesis is the direct, the system also has implications for genetic manipulations and propagation of genetically modified plants.

Abbreviations: 2,4-D, 2,4dichlorophenoxyacetic acid; MS, Murashige and Skoog (1962)

* Corresponding author. Tel.: +91 542 2575387; fax: +91 542 2368174.
E-mail addresses: vsj@bhu.ac.in, umavsj@gmail.com (V.S. Jaiswal).

0304-4238/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.scienta.2007.02.010

2. Material and methods

2.1. Explant material, surface sterilization, media and culture condition

To ensure self-pollination, unopened flowers were bagged before anthesis. Zygotic embryos were dissected out of seeds by collecting immature fruits from a 15–20-year-old guava (cv. Banarasi local) tree growing in the Botanical Garden, Department of Botany, B.H.U., Varanasi, India. The seeds were isolated from immature fruits and washed in running tap water for 10 min and then treated with 1% (v/v) cetrimide (a detergent and antiseptic; ICI, India) with 2–3 drops of Tween-20 (surfactant; HiMedia, India) for about 5–15 min. Surface sterilization was carried out with 0.05% mercuric chloride (disinfectant; Merck, India) for 3–5 min after a brief rinse in 70% ethanol under aseptic condition. It was finally rinsed thrice with sterile distilled water. Zygotic embryos were dissected out of seeds and cultured on MS medium supplemented with 2,4-D (0–9.05 μM) for the induction of somatic embryogenesis. The pH of the medium was adjusted to 5.8 ± 0.02 prior to autoclaving for 15 min at 121 °C temperature and 1.1 kg cm^{-2} pressure. Cultures were maintained at 25 ± 2 °C with 16/8 (light/dark) photoperiod at a photon flux of 50–70 μmol m^{-2} s^{-1} from white fluorescent tubes.

2.2. Induction, development, maturation and germination of somatic embryos

Initial experiments were performed by culturing the zygotic embryos on 3% sucrose and 2.26 μM 2,4-D supplemented full strength MS medium (induction medium), to evaluate and standardize the physiological age of zygotic embryos and maximum period of treatment of zygotic embryos with 2,4-D. To study the effect of physiological age of zygotic embryos on induction of somatic embryogenesis, zygotic embryos were dissected out of seeds by collecting immature fruits of different physiological age (7–14 weeks of anthesis) and cultured on induction medium. To evaluate the effect of maximum period of treatment of zygotic embryos with 2,4-D, zygotic embryos were cultured on induction medium either continuously for 60 days (continuous treatment) or treated with 2,4-D for 2-, 4-, 6-, 8-, 10-, 12-, 14-, 18- and 28-days (pulse treatment).

To study the interactive effect of 2,4-D and sucrose on the induction of somatic embryogenesis, zygotic embryos (10-week-old) were cultured on MS medium containing 2,4-D (0–9.05 μM) as well as different concentrations of sucrose (0–20%). After a pulse treatment with 2,4-D for 8 days, explants were transferred to full strength MS medium devoid of 2,4-D. To study maturation and germination, 8-week-old somatic embryos of various developmental stages were transferred to full or half strength MS medium containing various sucrose levels (1, 3 and 5%).

2.3. Transfer of plants to soil

Well-developed plantlets regenerated from somatic embryos were transferred to plastic pots containing a mixture of sand and soil in 3:1 ratio. Plantlets were covered with polyethylene bag to maintain a high humidity and irrigated with tap water. Pots with plantlets were kept at 25 ± 1 °C in artificial light (irradiance of 30 μmol m^{-2} s^{-1}) provided by white fluorescent tubes for 2–3 weeks and then the pots were transferred to sunlight, initially for a short time and gradually, the time was increased. After 3–4 months, plants were transferred to garden soil in clay pots and kept in sunlight.

2.4. Experimental design and data analysis

For the above experiments, 24 explants were used for each treatment and each experiment was repeated thrice. The frequency of embryogenesis was calculated as the percentage of cultures showing at least one somatic embryo and intensity of embryogenesis was calculated as the mean number of somatic embryos produced per responsive explant in a particular treatment. The number of somatic embryos of different stages formed from each responding explant was counted under a stereo-microscope (Nikon, SMZ-2T, Japan). The mean, standard error and one-way ANOVA were calculated using SPSS (version 10) software. The mean separations were carried out using Duncan's multiple range tests (Duncan, 1955) and significance was determined at $P < 0.05$.

3. Results

3.1. Somatic embryogenesis induction

3.1.1. Effect of physiological age of zygotic embryos on the induction of somatic embryogenesis

Eight- to 14-week-old zygotic embryos were able to produce somatic embryos on induction medium (3% sucrose and 2.26 μM 2,4-D supplemented full strength MS medium). Among different age of zygotic embryos, 10-week-old zygotic embryos (Fig. 2A) induced somatic embryo formation at highest frequency and intensity on induction medium (Fig. 1). Seven-week-old zygotic embryos were unable to undergo somatic embryogenesis on the induction medium.

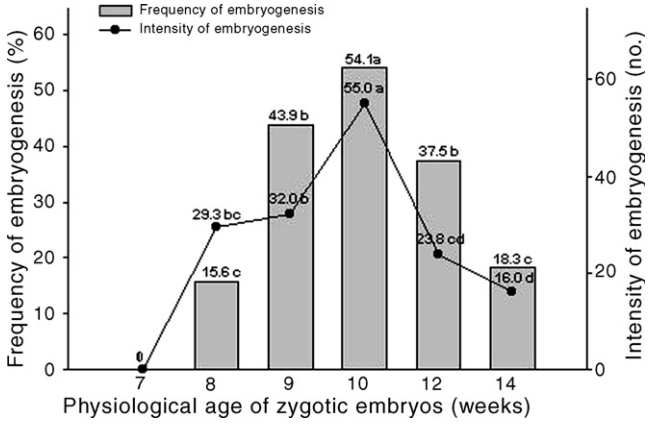

Fig. 1. Effect of physiological age of zygotic embryos on the induction of somatic embryogenesis (frequency and intensity of embryogenesis) of guava cv. Banarasi local. Mean values sharing the same *letter* do not differ significantly ($P < 0.05$) according to Duncan's multiple range test.