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ScienceDirect
Scientia Horticulturae 110 (2006) 366–371
SCIENTIA HORTICULTURAE
www.elsevier.com/locate/scihorti

Short communication

Somaclonal variation in *Tricyrtis hirta* plants regenerated from 1-year-old embryogenic callus cultures

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Received 18 April 2006; received in revised form 1 July 2006; accepted 20 July 2006

Abstract

The Liliaceous perennial *Tricyrtis hirta*, sometimes called 'Japanese toad lily', has recently become popular as an ornamental for pot and garden uses. Highly embryogenic callus cultures of this plant predominately consisted of diploid cells but also contained tetraploid cells after 1 year of establishment. In the present study, plans regenerated from the 1-year-old embryogenic callus cultures were subjected to ploidy level analysis and morphological characterization following 3 years of cultivation. Among 37 plants examined, 28 kept the diploid level ($2n = 2x = 26$) but nine were tetraploid ($2n = 4x = 52$) as indicated by FCM analysis and chromosome observation. Although no morphological alterations were detected in 26 out of 28 diploid regenerants, the remaining two showed noticeable variations: both were severely dwarf and had crimped leaves and many malformed flowers. The tetraploid regenerants had several horticulturally attractive characteristics compared with the diploid controls, such as longer shoots, thicker stems, and larger flowers. Thus regeneration of tetraploid plants from 1-year-old embryogenic callus cultures offers a possibility to improve the horticultural value of *T. hirta*, although regeneration of trueness-to-type plants is essential for utilizing the cultures for micropropagation and genetic transformation.

Keywords: Japanese toad lily; Liliaceae; Morphological variation; Ornamental; Tetraploid

1. Introduction

Tricyrtis hirta, Liliaceae, sometimes called 'Japanese toad lily', is a fall-flowering, perennial native to Japan. This plant has recently become popular as an ornamental for pot and garden uses because of its beautiful foliage, erect to arching shoots with exotic starry flowers, and shade tolerant. However, no systematic breeding has yet been conducted in *T. hirta*. Recently, highly embryogenic callus cultures of *T. hirta* were established (Nakano et al., 2004), and an efficient *Agrobacterium*-mediated transformation system of this plant was developed using the established callus cultures (Adachi et al., 2005). For micropropagation and genetic transformation, it is necessary that plants regenerated from callus cultures are genetically stable without any variations. On the other hand, regenerable callus cultures may possibly be a source of somaclonal variation with potential application in plant breeding (Larkin and Scowcroft, 1981; Karp, 1995). In the present study, *T. hirta* plants regenerated from the 1-year-old embryogenic callus cultures were subjected to ploidy level analysis and morphological characterization following 3 years of cultivation in the greenhouse.

2. Materials and methods

Highly embryogenic callus cultures of *T. hirta* (Nakano et al., 2004) were maintained by monthly subculturing onto

Abbreviations: FCM, flow cytometry; MS, Murashige and Skoog; PGR, plant growth regulator; SPAD, soil and plant analyzer development

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doi:10.1016/j.scienta.2006.07.026

half-strength MS medium (1962) containing 4.5 μM 2,4-dichlorophenoxyactic acid. One year after culture establishment, relative DNA content of nuclei isolated from randomly selected 10 callus samples was measured using a flow cytometer PA (Partec, GmbH-Münster, Germany) as previously described (Saito and Nakano, 2001; Saito et al., 2003).

Embryogenic calluses from the 1-year-old cultures were transferred to half-strength MS medium lacking PGRs to induce plant regeneration. Regenerated plants were acclimatized, separately transplanted to pots (18 cm in diameter) containing a 3:1 mixture of loam soil and leaf mold, and cultivated in the greenhouse, where the night/day temperatures were 5–20/10–30 °C, respectively, depending on the season and weather. The plants were grown under about 50% shading from May to November. Each plant was transplanted to a new pot every spring, and no fertilizer was applied. Divisions, each containing one small shoot, from the mother plant of the embryogenic callus cultures were transplanted to pots and cultivated as controls under the same conditions as the regenerated plants.

Three years after cultivation in the greenhouse, ploidy level, number of shoots per plant, shoot pattern (erect or decumbent), mean shoot length and stem diameter of the longest three shoots, mean number of nodes per shoot of the longest three shoots, mean leaf length and width of randomly selected nine leaves (three leaves each from the longest three shoots), total number of flowers per plant, mean number of flowers per shoot of the longest three shoots, mean flower length and diameter of randomly selected 3 flowers were recorded from 37 regenerated plants and five division-derived plants. Mean SPAD value of randomly selected nine leaves was measured using a chlorophyll meter (SPAD-502; Fujiwara Scientific Co., Tokyo, Japan). This value expresses relative amounts of chlorophyll in leaves by measuring transmittances at red (650 nm) and near-infrared (940 nm) wavelength regions. SPAD value has a high correlation with chlorophyll concentration on a leaf area basis, and is commonly used to predict chlorophyll concentration in many plant species (Campbell et al., 1990; Koike et al., 2003). According to the morphology, 37 regenerated plants were classified into three types, Types I–III. Ploidy level was determined by FCM analysis of leaf tissues as described above for embryogenic callus cultures. In order to verify the ploidy level, chromosome observation in root tip cells was carried out according to Fukui (1996).

3. Results

The histogram from FCM analysis of the 1-year-old embryogenic callus cultures had two peaks, each corresponding to 2C and 4C DNA contents, indicating that the cultures consisted of diploid and tetraploid cells (Fig. 1A). On average, the cultures contained 75% of diploid cells and 25% of tetraploid cells. FCM histogram of the mother plant used for callus induction had a single 2C peak (data not shown). The 1-year-old embryogenic callus cultures still kept high ability to produce somatic embryos, and more than 500 somatic embryos were obtained from 0.5 g fresh weight of embryogenic calluses after 1 month of transfer to PGR-free medium. Following transfer to fresh PGR-free medium, almost all of the somatic embryos developed into plantlets. Randomly selected 40 plantlets were acclimatized, transplanted to pots and cultivated in the greenhouse in September. Although three plants died probably due to cold injury in the first winter, the remaining 37 plants grew well and more than 80% of them produced a few flowers in the next autumn.

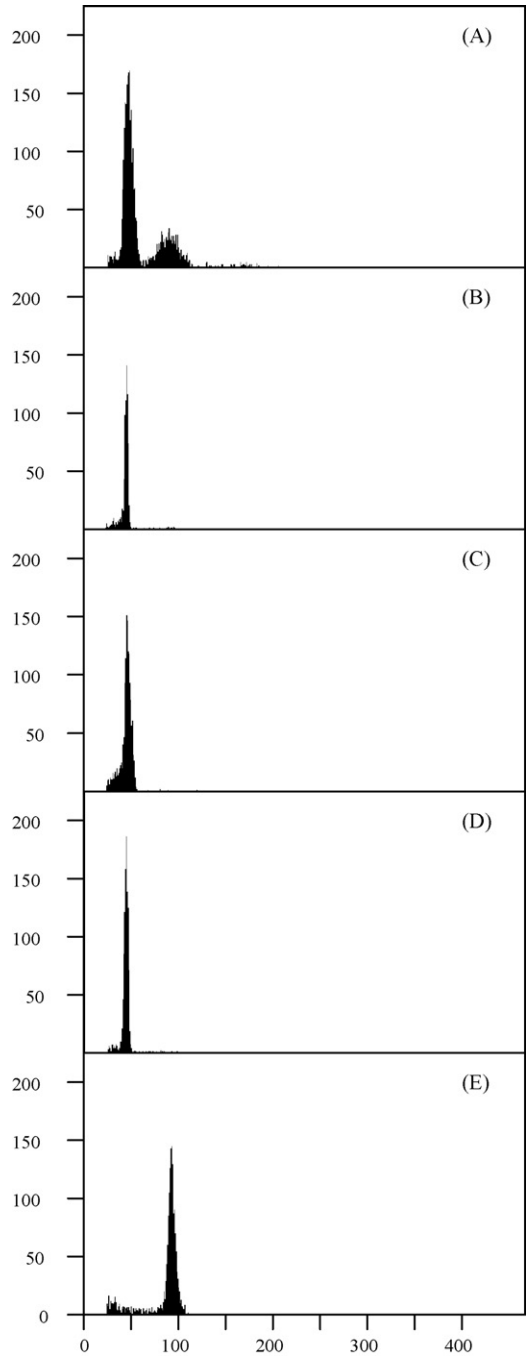

Fig. 1. Histograms from FCM analysis of nuclear DNA content of 1-year-old embryogenic callus cultures and plants regenerated from the cultures of *Tricyrtis hirta*. DNA content is expressed as the relative fluorescence intensity (RFI) on the horizontal axis, and the number of nuclei on the vertical axis. Typical histograms of the 1-year-old embryogenic callus cultures (A) and plants of the control (B), and Type I (C), Type II (D) and Type III (E) regenerants are shown.