**Polysomaty in Cymbidium**

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Abstract. Nuclear DNA content in various parts of Cymbidium plants was measured by flow cytometry. Two types of Cymbidium, protocorm-like body (PLB)-propagated epiphytic hybrids and rhizome-propagated terrestrial C. kanran Makino demonstrated polysomaty. Small shoots on PLBs of Cymbidium hybrids showed two peaks (2C and 4C) while PLBs showed four peaks, estimated to be 2C, 4C, 8C, and 16C. Roots and floral organs excluding ovaries of hybrids were highly polysomatic as were the rhizomes and roots of C. kanran. The patterns of polysomaty development were organ and developmental stage specific. Young leaves taken from in vitro plants are suitable material for determining the ploidy levels of Cymbidium plants by flow cytometry.

Cymbidium is among the most successful orchids as pot plants and cut flowers. Large numbers of new genotypes are released each year to meet consumer’s demands. Recent genotypes of epiphytic Cymbidium hybrids are mostly triploid or tetraploid. The basic Cymbidium genotypes of epiphytic orchids as pot plants and cut flowers. Large

Materials and Methods

Two Cymbidium hybrids [Flower Dance ‘Christmas Kiss’ (4x) and Twilight Moon ‘Day Light’ (2x)], were obtained from a commercial source (Boi-U Co., Kagawa, Japan) as both flowering plants and in vitro cultures. PLBs were cultured on medium containing half strength MS salts, 100 g L⁻¹ green banana fruit and 20 g L⁻¹ sucrose. PLBs with small shoots, leaves, pseudo bulbs and roots of in vitro young plants and leaves, flower stalks, pedicels, ovaries (premature, green), pollinia and petals of flowering plants grown in a greenhouse were collected from both genotypes. Rhizomes, roots and plantlets of C. kanran Makino were collected from rhizome cultures maintained on medium containing 3 g L⁻¹ Hypoxen (complete soluble fertilizer, 6.5 N–6 P–19 K, Hypoxen Co., Tokyo), 3 g L⁻¹ Bacto peptone, 1 mg L⁻¹ 1-naphthaleneacetic acid (NAA), 30 g L⁻¹ sucrose and 2 g L⁻¹ gellan gum (Hasegawa, 1987) at Kagawa Univ. Farm, Japan.

To isolate nuclei, 25 mm² of leaf and petal tissues, cross sections (1–2 mm thickness) of flower stalk, pedicel and green ovary, 1 mm root tips and 5 mm root tissues were prepared. Samples were collected from at least three independent plants and PLBs. Each sample tissue was chopped with a razor blade in a plastic dish with 0.5 mL of ice-cool buffer (solution A of high resolution DNA kit, Partech, PA (Partech, Münster, Germany) equipped with a mercury lamp and filter combinations (DAPI), pH 7.5 (Mishiba et al., 2000) was added to the nuclei sample. After 1 min of incubation on ice, the fluorescence of the DAPI-stained nuclei was measured using Ploidy Analyzer (Partech, Münster, Germany) and incubated on ice for 4 min. After filtration through a 20 μm nylon mesh, 2.5 mL of the staining solution containing 10 μg Tris (50 μM sodium citrate, 2 μM MgCl₂, 1% (v/v) PVP K-30 (Wako Chemicals, Tokyo), 0.1% (v/v) Triton X-100 and 2 μg mL⁻¹ 4’,6-diamidino-2-phenylindole (DAPI), pH 7.5 (Mishiba et al., 2000) was added to the nuclei sample. After 1 min of incubation on ice, the fluorescence of the nuclei was measured using Ploidy Analyzer PA (Partech, Münster, Germany) equipped with a mercury lamp and filter combinations of KG1, BG38, UG1, TK420, and GG435.

At least 8000 nuclei of three different samples were measured for each position of the plants. Data were represented as histograms showing relative nuclear DNA content of samples. The area of histogram peaks represented the number of nuclei for each ploidy level. Proportions of nuclei with different C-values were calculated and subjected to analysis of variance. The C-value of Cymbidium was determined by the measurement of the relative nuclear DNA content of pollen. Hordeum vulgare ‘Bonus’ (2C=9.41pg, Kankanpaa et al, 1996) or Triticum aestivum ‘Chikugo-izumi’ leaf tissue were used as an internal standard to obtain reproducible results.

Results

Flow cytometric analysis of pollinia resulted in two peaks (Fig. 1). One was a vegetative nucleus and another was a generative nucleus in which DNA had replicated and arrested in the G2 stage, typical of bicellular pollen (Bino et al. 1990). These two peaks representing 1C and 2C values of the Cymbidium ‘Day Light’ genome.

Small shoots (5 mm in height) produced on PLBs of ‘Day Light’ (diploid) showed two peaks, one large and one small, representing 00/01 (2C) and 02/04 (4C) stage cells, respectively. On the other hand, PLBs showed four peaks estimated to be 2C, 4C, 8C and 16C from the relative nuclear DNA contents (Fig. 1). The proportion of 2C cells was only 20% while 4C and 8C cells were 40% and 32%, respectively. No difference was observed in the ploidy distribution when PLBs were divided into upper and lower parts. Samples of PLB epidermis that were excised carefully under a binocular microscope displayed only two peaks (2C and 4C), while the inner tissue showed four peaks (2C–16C) indicating high levels of polysomaty (data not shown).

Proportions of cells with each ploidy in different organs are summarized in Fig. 2. Leaves of in vitro plantlets (0.5 cm in height) and small shoots on PLBs had >90% and 80% of 2C cells, respectively, while the rest were 4C. The basal part of the plantlet, which develops into a pseudobulb, had 2C to 8C cells. Root tips (1 mm in length) of in vitro plantlets had 2C to 16C cells. Roots taken from 1 to 3 cm above the root tip had no 2C cells. Proportions of higher ploidy cells (8C and 16C) were significantly increased (P = 0.01) as the distance from the root tip increased.

‘Christmas Kiss’ (4x) showed the same developmental pattern of polysomaty as ‘Day Light’ if 4C–32C in the tetraploid is considered to correspond to 2C–16C in the diploid. ‘Christmas Kiss’ had a higher percentage of cells with lower ploidy levels than ‘Day Light’ (Fig. 2).

Flower stalks, pedicels and petals of ‘Day Light’ showed polysomaty having 2C to 16C cells (Fig. 3). On the other hand, ovaries had a significantly larger proportion (P = 0.01) of 2C cells. ‘Christmas Kiss’ showed a similar pattern of polysomaty development.

Rhizomes of C. kanran also showed polysomaty while shoots regenerated from rhizomes had no polysomaty (Fig. 4). Older parts of the rhizome consisted of cells with

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significantly higher \((P = 0.01)\) ploidy levels in comparison with younger parts. Roots of \(C.\) kanran had polysomy as observed in other \(Cymbidium\) hybrids (data not shown).

**Discussion**

Endoreduplication is cyclic DNA synthesis without cell division, leading to the existence of cells with different ploidy levels in a tissue or organ, which is termed polysomy. Despite the appearance of polysomy in a wide range of plant species (Cavallini et al., 1988; De Rocher et al., 1990; Galbraith et al., 1991; Gilissen et al., 1993; Mishiba and Mii 2000; Pijnacker et al., 1989; Smulders et al., 1995), the function of polysomy is not well understood. It has been claimed that polysomy is related to nuclear genome size (De Rocher et al., 1990), interaction between nuclear and organelle genomes (Galbraith et al., 1991), cellular dimension (Melaragno et al., 1993), and auxin-induced cell elongation (Gilissen et al., 1993).

In this study both diploid and tetraploid genotypes showed a similar developmental pattern of polysomy. The results showed that the patterns of polysomy development were organ and developmental stage specific, agreeing with previous reports in various species (Galbraith et al., 1991; Gilissen et al., 1993; Smulders et al., 1994). The proportion of lower ploidy cells in the tetraploid genotype ‘Christmas Kiss’ was much higher than that in the diploid genotype ‘Day Light’ as also reported in tomato (Smulders et al., 1994) and \(Portulaca\) (Mishiba and Mii, 2000).

Genome size of diploid \(Cymbidium\) ‘Day Light’ estimated from the genome size of \(H.\) vulgare ‘Bonus’ \((2C=9.41pg,\) Kankanpää et al., 1996) was 16.5 pg/2C. The estimated DNA value from DAPI-stained samples depends on the base composition of the plant because DAPI binds preferentially to AT-rich regions of DNA (Dolezel et al. 1998). The limitation of DAPI-stained samples in this experiment must be taken into account to compare the value of genome size estimated by PI stain.
But the obtained 2C value of ‘Day Light’ still does not support the idea that the occurrence of polysomaty relates to small genome size (De Rocher et al., 1990).

Shoots regenerated from PLBs were 2C/4C as expected whereas PLBs showed more frequent polysomaty. This means that a meristematic center of PLBs remains euploid. In the propagation of epiphytic Cymbidium hybrids, PLBs are cut into small pieces and each piece produces new protocorms on an appropriate medium. PLBs start to produce shoots if the cutting is postponed. New PLBs regenerate from epidermal cells, not from parenchyma cells inside of PLBs (Kim and Kako, 1984). In our study, epidermal tissue of PLBs showed only 2C and 4C peaks. Fujii et al. (1999) also reported that most cells in the outer tissue of Cymbidium PLBs had a DNA content of 2C while Nagl et al. (1972) observed that DNA amplification was restricted to the green assimilating parenchyma cells near the epidermis. Maintenance of euploidy in epidermal cells may be a reason why change of ploidy mis. Maintenance of euploidy in epidermal cells inside of PLBs (Kim and Kako, 1984). This is considerably different from that in Phalaenopsis and Dendrobium which showed a high level of polysomaty in mature leaves. Floral organs of Cymbidium consisted of higher ploidy cells except for the ovary. Development of polysomaty in floral organs, especially the perianth, has been reported in Portulaca (Mishiba and Mii, 2000).

The present results suggest that shoot regeneration arises from euploid cells and that endoreduplication occurs in cells without a potential for shoot regeneration. In practice flow cytometry is a very useful tool to determine the ploidy level of plant materials. The present study showed that leaves from in vitro plants are suitable materials for flow cytometric analysis in Cymbidium as described in Dendrobium (Jones and Kuehnle, 1998).

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