Induction of polyploidy plants through colchicine treatments in balloon vine (*Cardiospermum halicacabum*)©

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Abstract

The balloon vine (*Cardiospermum halicacabum*) is a popular plant with balloon-like fruits, but there are no cultivars. Polyploidization could be used to breed new cultivars. Here, we tried to breed polyploid balloon vines for improved horticultural value. Polyploidization was induced by immersing germinated seeds in colchicine solution. The frequency of tetraploids depended on concentration and treatment time. No tetraploids were obtained from seedlings treated at 1 mM. Some tetraploids were obtained from seedlings treated at 10 mM for 24, 36, or 48 h. All were diploid–tetraploid chimeras. Some chimeras had bigger leaves and fruits than diploids, but others showed no difference. Tetraploid seeds were obtained from chimeras and their progenies. From tetraploid seeds treated with 10 mM colchicine for 24 or 48 h, five survived, including one tetraploid–octoploid chimera. It had thicker leaves and grew more slowly than diploids and tetraploids.

INTRODUCTION

The balloon vine (*Cardiospermum halicacabum* Linn.) is a vine plant in the Sapindaceae. It is popular in horticulture for its balloon-like fruits and heat tolerance, but there are no cultivars. The *Cardiospermum* genus comprises 16 known species with pantropical distribution (Ferrucci, 2000). There is no report of interspecific crosses within the genus for improving horticultural value. Polyploidization is a valuable technique for breeding. *Cardiospermum halicacabum* is diploid (x=11, $2n$=22) (Sugiura, 1931). Many other species in the *Cardiospermum* genus are also diploid, and only *C. bahianum* is known as polyploid ($2n$=$4x$=36) (Urdampilleta et al., 2013). Polyploidization could be useful for breeding new and valuable cultivars of balloon vine. Here, we bred tetraploids and octoploids by polyploidization for improved horticultural value.

MATERIALS AND METHODS

Balloon vine seeds were soaked in 95% sulfuric acid (Wako Pure Chemical Industries, Ltd, Japan) for 1 h and then germinated on wet filter paper in a glass Petri dish at 25°C in constant light. Polyploidization was carried out by immersing germinated seeds in colchicine (Wako) solution containing 10% (v/v) dimethyl sulfoxide (Nacalai Tesque, Inc., Japan). To induce tetraploid plants, we treated diploid seeds with 1 or 10 mM colchicine for 12, 24, 36, or 48 h (Table 1). The treated seeds were sown in a 1:2 (v/v) mixture of perlite and BM2 culture soil (Berger Peat Moss Ltd., Canada), and then grown in a glasshouse under natural day length. To induce octoploid plants, we treated tetraploid seeds with 10 mM colchicine for 24, 48, or 72 h. Tetraploid seeds were obtained from diploid–tetraploid chimeras and their tetraploid progenies. Ploidy level was determined from young leaves with a PA flow cytometer (Partec GmbH, Germany) according to the manufacturer's instructions. Signals with a relative fluorescence intensity of <20 were ignored as they were mostly noise.

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Table 1. Effects of colchicine treatment on induction of polyploids in diploid balloon vine.

Colchicine conc.	1 mM			10 mM		
Duration (h)	24	48	12	24	36	48
No. of seeds treated	59	20	35	59	23	20
No. of survivals	44	13	16	25	7	10
No. of diploids	44	13	16	19	6	8
No. of tetraploids	0	0	0	6	1	2
Survival ratio (%)	74.6	65.0	45.7	42.4	30.4	50.0
Polyploidization ratio (%)	0	0	0	10.2	4.3	10.0

Polyploidization ratio (%) = No. of tetraploids / No. of seeds examined × 100.

RESULTS AND DISCUSSION

Two hundred and sixteen (216) diploid seeds were treated with colchicine solution to induce tetraploid plants and ploidy level of 115 surviving plants were determined with a PA flow cytometer. Untreated (diploid) plants showed a single peak relative fluorescence intensity of about 100 (Figure 1A). Some surviving plants showed two peaks of about 100 and 200 (Figure 1B). Flow cytometric measurement of young leaves can show two peaks (Galbraith et al., 1983), due to rapidly dividing G2 and M phase cells, in contrast to the single peak of G1 phase cells. Peak of the M phase cells should be lower than the G1 peak. In our results, cell counts of each peak were nearly the same, so these plants were deemed to be chimeric plants with both diploid and tetraploid cells. Treatment with 10 mM colchicine for 12 h or 1 mM for 24 or 48 h produced no tetraploid plants. Treatment with 10 mM for 24 h produced six diploid-tetraploid chimeric plants (polyploidization ratio of 10.2%), treatment for 36 h produced one plant (4.3%), and treatment for 48 h produced two plants (10.0%) (Table 1). As colchicine is a toxic chemical that prevents cell division by inhibiting mitosis (Taylor, 1965), the optimum concentration for polyploid induction reduces survival. Survival after 1 mM treatment was relatively high; it is possible that 1 mM was not high enough for polyploid induction.

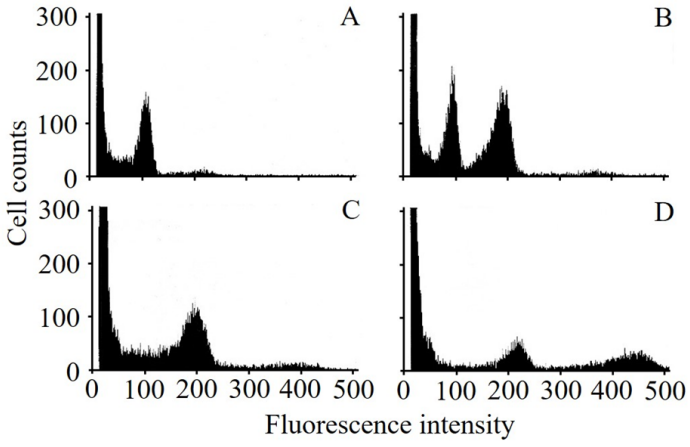

Figure 1. Flow cytometric histograms of balloon vines with different ploidy levels: (A) diploid, (B) diploid–tetraploid chimera, (C) tetraploid, (D) tetraploid–octoploid chimera.

We compared the morphological characteristics between diploid–tetraploid chimeric plants and diploid plants (Figure 2). Balloon vine has biternate leaves. Leaflets of diploids do not overlap one another (Figure 2A). The leaflet size and shape of the chimeric plants were separated into two types. One was very similar to diploids, and leaflets didn't overlap (Figure 2B). The other had larger and thicker leaflets that overlapped one another (Figure 2C). The

chimeras with larger and thicker leaflets invariably produced larger fruits than diploids. Organ size varied among plants and shoots, even though all chimeras had tetraploid cells. In general, the shoot meristem of plants has three cell layers (L1, L2, and L3), which are maintained after differentiation into organs. In leaves, the epidermis consists mainly of L1 cells, the palisade layer mainly of L2 cells, and the spongy parenchyma mainly of L3 cells (Sussex, 1989). According to Dermen (1960), cytochimeras with an L1–L2–L3 ploidy of 2*x*–4*x*-2*x* are much more likely to be tetraploids than those with either 2*x*-2*x*-4*x* or 4*x*-2*x*-2*x*. Adaniya and Tamaki (1991) reported that cytochimeric *Allium wakegi* with an L1-L2-L3 ploidy of 2*x*-4*x*-4*x* showed similar growth characteristics to tetraploids, but 4*x*-2*x*-2*x* plants were similar to diploids. In our study, the ploidy of each cell layer was not measured, but the ploidy of L2 may be important for determining organ size in the balloon vine, too.

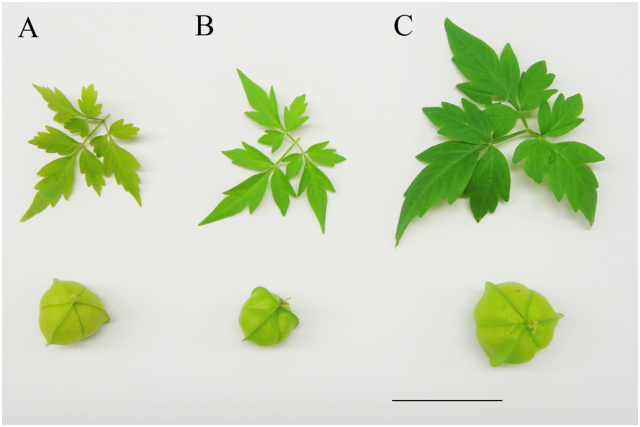

Figure 2. Leaves and fruits of balloon vines with different ploidy levels: (A) diploid, (B, C) diploid–tetraploid chimeras. Scale bar = 5 cm.

Tetraploid seeds obtained from diploid–tetraploid chimeras and their progenies were used for octoploid induction by treatment with 10 mM colchicine for 24, 48, or 72 h (Table 2). Five plants survived 24 h treatment, but none survived 48 or 72 h. The survival ratio in 24 h treatment (2.8%) was much lower than that of diploid seeds under the same condition (42.4%). The tetraploid balloon vines grew more slowly than the diploids, so their growth characteristics should be different. We suspect that the colchicine concentrations that trigger growth inhibition were also different. Treatment at a lower concentration or for a shorter duration might be optimal for octoploid induction. Untreated tetraploids had a peak relative fluorescence intensity of about 200 (Figure 1C). One survivor of treatment with 10 mM for 24 h showed two peaks of about 200 and 450 with nearly the same cell counts (Figure 1D), and thus appears to have been a tetraploid-octoploid chimera. The other four survivors had a peak at about 200 and are likely to have been tetraploids. The tetraploid–octoploid chimera had thicker, crumpled leaves and grew more slowly than both diploids and tetraploids.

Table 2. Effects of colchicine treatment on induction of polyploids in tetraploid balloon vine.

Colchicine conc.		**10 mM**	
Duration (h)	24	48	72
No. of seeds treated	177	47	22
No. of survivals	5	0	0
No. of tetraploids	4	0	0
No. of octoploids	1	0	0
Survival ratio (%)	2.8	0.0	0.0
Polyploidization ratio (%)	0.6	0.0	0.0

Polyploidization ratio (%) = No. of tetraploids / No. of seeds examined × 100.

Literature cited

Adaniya, S., and Tamaki, S. (1991). Colchicine-induced cytochimeras of *Allium wakegi* Araki and their growth characteristics. J. Jpn. Soc. Hortic. Sci. *60* (*1*), 105–112 http://dx.doi.org/10.2503/jjshs.60.105.

Dermen, H. (1960). Nature of plant sports. Amer. Hort. Mag. *39*, 123–173.

Ferrucci, M.S. (2000). Cytotaxonomy of Sapindaceae with special reference to the tribe *Paullinieae.* Genet. Mol. Biol. *23* (*4*), 941–946 http://dx.doi.org/10.1590/S1415-47572000000400039.

Galbraith, D.W., Harkins, K.R., Maddox, J.M., Ayres, N.M., Sharma, D.P., and Firoozabady, E. (1983). Rapid flow cytometric analysis of the cell cycle in intact plant tissues. Science *220* (*4601*), 1049–1051 http://dx.doi.org/10.1126/science.220.4601.1049. PubMed

Sugiura, T. (1931). A list of chromosome numbers in angiospermous plants. Bot. Mag. *45* (*535*), 353–355 http://dx.doi.org/10.15281/jplantres1887.45.353.

Sussex, I.M. (1989). Developmental programming of the shoot meristem. Cell *56* (*2*), 225–229 http://dx.doi.org/10.1016/0092-8674(89)90895-7. PubMed

Taylor, E.W. (1965). The mechanism of colchicine inhibition of mitosis; I. Kinetics of inhibition and the binding of H3-colchicine. J. Cell Biol. *25* (*1*), 145–160. PubMed

Urdampilleta, J.D., Coulleri, J.P., Ferrucci, M.S., and Forni-Martins, E.R. (2013). Karyotype evolution and phylogenetic analyses in the genus *Cardiospermum* L. (Paullinieae, Sapindaceae). Plant Biol (Stuttg) *15* (*5*), 868–881 http://dx.doi.org/10.1111/j.1438-8677.2012.00679.x. PubMed