Plant Regeneration through Protocorm-like Bodies Induced from Nodal Explants of *Syngonium podophyllum* ‘White Butterfly’

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Abstract. *Syngonium podophyllum* ‘White Butterfly’, one of the most popular ornamental foliage plants, is propagated almost exclusively through in vitro shoot culture. Ex vitro rooting, however, has been associated with severe Myrothecium leaf spot (*Myrothecium roridum* Tode ex Fr.). The objective of this study was to establish a method for regenerating well-rooted plantlets before ex vitro transplanting. Leaf and petiole explants were cultured on a Murashige and Skoog (MS) basal medium supplemented with N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU), N-phenyl-N'-1,2,3-thiadiazol-5-ylurea (TDZ), 6-benzyladenine (BA), or N-isopentenylaminopurine (2iP) with α -naphthalene acetic acid (NAA) and 2,4-dichlorophenoxyacetic acid (2,4-D), respectively. Calli formed from leaf explants cultured on the basal medium supplemented with CPPU or TDZ with 2,4-D or with NAA as well as from petiole explants cultured on the medium supplemented with BA, CPPU, or TDZ with 2,4-D or NAA. The calli, however, failed to differentiate, and shoot organogenesis did not occur. Culture of nodal explants on the MS basal medium supplemented with 9.84 μ M 2iP, 8.88 μ M BA, 8.07 μ M CPPU, or 9.08 μ M TDZ with 2.26 μ M 2,4-D resulted in the formation of protocorm-like bodies, adventitious shoots, and subsequently well-rooted plantlets. MS basal medium supplemented with 19.68 μ M 2iP and 1.07 μ M NAA resulted in the highest percentage (92.9%) of nodal explants producing protocorm-like bodies and an average of 16.9 well-rooted plantlets per nodal explant. Adventitious shoots were able to root in the initial induction medium, but better root development occurred after shoots with protocorm-like bodies were transferred onto MS basal medium supplemented with 9.84 μ M 2iP and 2.69 μ M NAA. Regenerated plantlets were stable and grew vigorously with 100% survival rates after ex vitro transplanting to a container substrate in a shaded greenhouse.

Syngonium podophyllum Schott, commonly known as arrowhead vine, goosefoot plant, or nephthytis, belongs to the family Araceae and occurs indigenously on humid forest floors of Central and South America (Croat, 1982). As a result of their attractive foliar variegation and tolerance to low-light environments, cultivars from *S. podophyllum* in their juvenile stage have been widely produced as ornamental foliage plants and used as living specimens for interiorscaping (Chen et al., 2005). There are $\approx$ 30 commercial cultivars available in the foliage plant industry with ‘White Butterfly’ being the most popular over the last 30 years (Chen et al., 2002). Traditionally, arrowhead vine is propagated through eye cuttings, one leaf with one or two stem nodes (Chen and Stamps, 2006). Eye cuttings, however, can carry and spread diseases such as *Xanthomonas* blight caused by *Xanthomonas campestris*

pv. *dieffenbachiae* (Chase, 1989; Chase et al., 1988).

Since the late 1980s, arrowhead vine has been micropropagated using shoot tips (Kane, 2000; Miller and Murashige, 1976). Shoot tips are inoculated on Murashige and Skoog (MS) medium containing 14.8 μ M N-isopentenylaminopurine (2iP) and 5.7 μ M IAA for establishment (Stage I) and then cultured on MS medium containing 98.4 μ M 2iP only for multiplication (Stage II) (Kane, 2000; Miller and Murashige, 1976). Stage II shoot clusters are divided into single microcuttings and rooted directly in soilless substrate (Kane, 2000). Chen and Henny (2008) estimated that 19 million plantlets of arrowhead vine are annually micropropagated worldwide, which has greatly reduced the incidence of diseases carried by eye cuttings. Recently, however, Myrothecium leaf spot (*Myrothecium roridum* Tode ex Fr.) has become the most common disease of arrowhead vine. This opportunistic airborne fungal pathogen particularly occurs during the ex vitro rooting of microcuttings after shoot culture because the cutting base is especially susceptible to this pathogen. Sometimes

more than 80% of microcuttings are infected (Norman, personal communication). No commercial cultivars are resistant to this pathogen (Norman et al., 2003).

Replacing ex vitro rooting with ex vitro transplanting should offer a solution to controlling Myrothecium leaf spot in arrowhead vine. Microcuttings could be rooted in vitro and then transplanted ex vitro. Such a procedure, however, could be extremely labor-intensive and commercially difficult. Another solution could be the regeneration through either somatic embryos or protocorm-like bodies in which well-rooted plantlets could be produced for ex vitro transplanting. Regeneration through somatic embryos or protocorm-like bodies is also the desired system for genetic transformation. Somatic embryogenesis has been reported with *S. podophyllum* ‘Variegatum’ in which healthy plantlets were regenerated using petiole explants (Zhang et al., 2006). There is no reported protocorm-like body formation in the more commercially valuable *S. podophyllum* ‘White Butterfly’. Thus, the objective of this study was to develop an efficient method for regeneration of disease-free, well-rooted plantlets of *S. podophyllum* ‘White Butterfly’ for ex vitro transplanting.

Materials and Methods

Plant materials. Shoot tips ($\approx$ 8 to 10 cm long), including the youngest leaf and stem, were excised from 1-year-old *S. podophyllum* ‘White Butterfly’ stock plants grown in a shaded greenhouse under a maximum photosynthetically photon flux density of 300 μ mol·m⁻²·s⁻¹ at the University of Florida’s Mid-Florida Research and Education Center in Apopka, FL. Leaves and petioles were excised from stem sections and surface-sterilized by immersing in 70% ethanol for 45 s and then soaking in a 20% Clorox (Clorox Co., Oakland, CA) (1.2% NaOCl) solution for 25 min. Defoliated stems with nodal buds were surface-sterilized by immersing in 70% ethanol for 1 min and soaking in 20% Clorox for 40 min. After pouring off the Clorox solution, leaves, petioles, and stems were rinsed three times with sterile water.

Medium and cultural conditions. Murashige and Skoog mineral salts and vitamins (Murashige and Skoog, 1962) with 2.5% (w/v) sucrose and 0.6% (w/v) agar (Sigma, St. Louis, MO) were used as a basal medium. The pH of the medium was adjusted to 5.8 with 1 M KOH before autoclaving at 121 °C for 25 min. Plant growth regulator solutions were filter-sterilized and added to autoclaved basal medium when the temperature dropped to 50 °C. Three experiments were conducted for plant regeneration. The first experiment was the culture of leaf and petiole explants on MS basal medium in the dark at 25 °C for 8 weeks and then placed under a 16-h photoperiod provided by cool-white fluorescent tubes at a photon flux density of 8 μ mol·m⁻²·s⁻¹ for 8 weeks or directly under the photon flux density of 8 μ mol·m⁻²·s⁻¹ for 16 weeks. Growth regulators in the culture

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medium were 8.07 μM N-(2-chloro-4-pyridyl)-N'-phenylurea (CPPU) or 9.08 μM N-phenyl-N'-1,2,3-thiadiazol-5-ylurea (TDZ) with 1.07 μM α -naphthalene acetic acid (NAA) or with 2.26 μM 2,4-dichlorophenoxyacetic acid (2,4-D), which were based on the formula of Zhang et al. (2006). Additionally, the test also included 8.88 μM 6-benzyladenine (BA) or 9.84 μM 2iP with 2.26 μM 2,4-D or with 1.07 μM NAA. The second experiment was a preliminary test with nodal explants under the aforementioned light conditions using the basal medium containing 9.84 μM 2iP, 8.88 μM BA, 8.07 μM CPPU, or 9.08 μM TDZ with 2.26 μM 2,4-D. The third experiment was intended to identify the optimal combination and concentration of growth regulators for regenerating plantlets from nodal explants under the light conditions. Nodal explants were cultured on the basal medium supplemented with 9.84, 19.68, or 29.52 μM 2iP with 1.07 μM NAA or with 2.26 μM 2,4-D as well as 9.08 and 13.62 μM TDZ alone or with 1.07 μM NAA or 2.26 μM 2,4-D.

Sterilized leaves were cut into 1.5- to 2.0-cm squares, and petioles and nodes were cut into 1.0-cm long segments in 100 $\times$ 15-mm sterile petri dishes (Fisher Scientific, Inc., Pittsburgh, PA). Leaf, nodal, and petiole explants were transferred onto petri dishes containing 20 mL of basal medium supplemented with the aforementioned different growth regulators. Leaf explants were placed with the ad-axial surface up; nodal and stem explants were placed horizontally. Petri dishes were sealed with parafilm M (Fisher Scientific, Inc.). There were six explants per petri dish in Expts. 1 and 2, but four nodal explants per dish in Expt. 3. Expt. 3 was repeated three times.

Histological observation. Samples collected from different culture periods of nodal explants were taken weekly and fixed in FAA (formalin:glacial acetic acid:70% ethanol at 5:5:90 by volume). After dehydration through an alcohol-xylol series, the samples were embedded in Paraplast with a 56 to 58 $^{\circ}\text{C}$ melting point. The sections 7 to 8 μm thick were stained with either Safranin-Fast green or Heidenhain's iron-alumhematoxylin and mounted on Permount[®] (Fisher Scientific, Inc.). All the sections were observed under a Nikon OPTIPHOT microscope (Nikon Co., Tokyo, Japan) and photographed using a Canon S3 IS digital camera (Canon Inc., Tokyo, Japan).

Data collection and analysis. A completely random design was used for the three experiments. Each petri dish was considered an experimental unit, and each treatment had eight replications. Explants that responded to the induction were recorded per petri dish from 4 to 16 weeks after culture, and frequencies of the response were calculated. Means and SES for the frequencies of explants that produced calli or protocorm-like bodies as well as the number of adventitious shoots were calculated. Thus, data included the percentage of explants with callus or protocorm-like body formation and the number of shoots produced per explants. After checking normal distribution, data were analyzed by analysis of variance (SAS GLM; SAS Insti-

tute, Cary, NC), and means separations were determined using Fisher's protected least significant differences at the 5% levels.

Root development and ex vitro plantlet establishment. The basal medium supplemented with 9.84 μM 2iP and 2.69 μM NAA was used for root development. Regenerated plantlets were separated, washed free of agar using tap water, and transplanted into a sphagnum peat-based medium (Vergro Container Mix A; Verlite Co., Tampa, FL) consisting of Canadian peat, vermiculite, and perlite in a 3:1:1 ratio based on volume. Potted plants were grown in a shaded greenhouse under a maximum photosynthetically photon flux density of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, temperature range of 20 to 28 $^{\circ}\text{C}$, and relative humidity of 70% to 100%. Survival rates of plantlets in the shaded greenhouse were recorded 2 months after transplanting.

Results

Expt. 1: Leaf and petiole explants. Leaf explants cultured in the dark slightly expanded and became yellow or light brown in 5 weeks. White calli occurred at the cut edges 8 weeks later. All treatments were able to induce callus formation with frequencies varying from 10.4% to 37.5% (Table 1). Leaf explants cultured under the 16-h photoperiod turned brown in 5 weeks. Whitish green calli appeared at the cut edges in 12 weeks after culture on MS medium supplemented 8.07 μM CPPU or 9.08 μM TDZ with 2.26 μM 2,4-D or with 1.07 μM NAA (Fig. 1A). Frequencies of leaf explants with callus formation varied from 9.3% to 20.4% (Table 1). Calli, however, did not occur in medium supplemented with the other growth regulators. Regardless of culture in darkness or light, calli derived from leaf explants grew slowly and did not differentiate. There was no adventitious shoot formation from leaf explants.

Petiole explants cultured in darkness expanded and became yellow in 4 weeks. White calli formed at the cut edges 10 to 12 weeks later. Frequencies of petiole explants with

calli ranged from 16.7% to 31.7% (Table 2). Petiole explants cultured under the 16-h photoperiod turned brown or dark in 4 weeks. After 10 weeks, yellowish white calli appeared at the cut edges of petiole explants cultured on MS basal medium supplemented with 8.88 μM BA, 8.07 μM CPPU, or 9.08 μM TDZ with 2.26 μM 2,4-D or with 1.07 μM NAA (Fig. 1B), but did not occur on the medium containing 2iP. Frequencies of petiole explants cultured under light with callus formation varied from 10.0% to 18.8% (Table 2). Similar to the results of leaf explants, calli derived from petiole explants showed slow growth and did not differentiate on the same medium. Adventitious shoots did not occur.

Expt. 2: Nodal explants. Small, nodule-like structures appeared directly from nodal explants and quickly enlarged when cultured on MS basal medium containing 9.84 μM 2iP, 8.88 μM BA, 8.07 μM CPPU, or 9.08 μM TDZ with 2.26 μM 2,4-D 5 weeks after culture (Fig. 1C). The nodule-like structures resembling protocorm-like bodies as described by Morel (1960) became green and differentiated to form round protuberances and subsequently globular structures (Fig. 1D). The globular structures formed shoot primordia (Fig. 1E-F). Shoots and then roots occurred (Fig. 1G). The mean frequencies of protocorm-like body formation ranged from 28.6% in medium containing 8.07 μM CPPU and 2.26 μM 2,4-D to 71.9% in medium containing 9.08 μM TDZ and 2.26 μM 2,4-D. Adventitious shoot numbers averaged from 5.4 to 11.5 per nodal explant (Table 3).

Histological observation. Histological analysis showed that protocorm-like bodies emerged directly from nodal explants and there was a direct vascular connection between explant and protocorm-like body (Fig. 2A). A differentiated protocorm-like body (Fig. 2B) showed the young leaf and the main shoot meristem and lateral shoot meristem. Later, the shoot elongated with shoot meristem and axillary buds (Fig. 2C).

Expt. 3: Optimization test. The test of different concentrations of 2iP with 1.07 μM

Table 1. Frequency of callus formation from leaf explants of *Syngonium podophyllum* 'White Butterfly' cultured on a Murashige and Skoog basal medium supplemented with different growth regulators^a.

Basal medium ^b	Frequency of explants with callus formed (%)	
	Dark culture	Cultured under light ^c
9.84 μM 2iP + 2.26 μM 2,4-D	18.4 $\pm$ 3.6 bc ^w	0
9.84 μM 2iP + 1.07 μM NAA	10.4 $\pm$ 3.1 c	0
8.88 μM BA + 2.26 μM 2,4-D	16.7 $\pm$ 3.1 bc	0
8.88 μM BA + 1.07 μM NAA	16.8 $\pm$ 4.4 bc	0
8.07 μM CPPU + 2.26 μM 2,4-D	37.5 $\pm$ 4.1 a	20.4 $\pm$ 3.7 a
8.07 μM CPPU + 1.07 μM NAA	25.9 $\pm$ 2.9 b	15.0 $\pm$ 3.0 b
9.08 μM TDZ + 2.26 μM 2,4-D	24.1 $\pm$ 2.9 b	14.6 $\pm$ 3.8 b
9.08 μM TDZ + 1.07 μM NAA	20.4 $\pm$ 2.4 b	9.3 $\pm$ 2.9 bc

^aSix leaf explants per petri dish and eight petri dishes per treatment.

^bBasal medium comprises Murashige and Skoog mineral salts and vitamins, 2.5% (w/v) sucrose, and 0.6% (w/v) agar.

^cCultured under a 16-h photoperiod provided by cool-white fluorescent tubes at a photo flux density of 8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

^wDifferent letters within a column represent significant difference at $P \leq 0.5$ by Fisher's protected least significant difference test.

2iP = N-isopentenylaminopurine; 2,4-D = 2,4-dichlorophenoxyacetic acid; NAA = α -naphthalene acetic acid; BA = 6-benzyladenine; CPPU = N-(2-chloro-4-pyridyl)-N'-phenylurea; TDZ = N-phenyl-N'-1,2,3-thiadiazol-5-ylurea.

Fig. 1. Morphogenesis of *Syngonium podophyllum* 'White Butterfly'. (A) Whitish green calli appeared at the cut edges of a leaf explant. (B) Yellowish white calli appeared at the cut edges of petiole explants. (C) Small, nodule-like structures appeared directly from nodal explants and quickly enlarged. (D) The nodule-like structures resembling protocorm-like bodies became green and differentiated to form round protuberance and subsequently globular structures. (E–F) The globular structures formed shoot primordia. (G) Shoots and then roots occurred (arrows). (H) Better root development occurred after shoots with protocorms were transferred onto a Murashige and Skoog basal medium supplemented with $9.84 \mu\text{M}$ 2iP and $2.69 \mu\text{M}$ NAA. (I) Regenerated plants grew vigorously in a shaded greenhouse. Bars = 1 mm.

Table 2. Frequency of callus formation from petiole explants of *Syngonium podophyllum* 'White Butterfly' cultured on a Murashige and Skoog basal medium supplemented with different growth regulators^a.

Basal medium ^y	Frequency of explants with callus formed (%)	
	Dark culture	Cultured under light ^x
$9.84 \mu\text{M}$ 2iP + $2.26 \mu\text{M}$ 2,4-D	18.5 ± 3.3 bc ^w	0
$9.84 \mu\text{M}$ 2iP + $1.07 \mu\text{M}$ NAA	16.7 ± 3.5 c	0
$8.88 \mu\text{M}$ BA + $2.26 \mu\text{M}$ 2,4-D	24.1 ± 4.0 b	14.8 ± 3.4 ab
$8.88 \mu\text{M}$ BA + $1.07 \mu\text{M}$ NAA	20.4 ± 4.6 b	10.0 ± 2.7 c
$8.07 \mu\text{M}$ CPPU + $2.26 \mu\text{M}$ 2,4-D	31.7 ± 4.6 a	16.7 ± 3.9 a
$8.07 \mu\text{M}$ CPPU + $1.07 \mu\text{M}$ NAA	24.1 ± 4.0 b	11.7 ± 2.6 c
$9.08 \mu\text{M}$ TDZ + $2.26 \mu\text{M}$ 2,4-D	31.5 ± 4.3 a	18.8 ± 2.1 a
$9.08 \mu\text{M}$ TDZ + $1.07 \mu\text{M}$ NA	22.9 ± 3.0 b	16.7 ± 3.1 a

^aSix petiole explants per petri dish, eight petri dishes per treatment.

^yBasal medium comprises Murashige and Skoog mineral salts and vitamins, 2.5% (w/v) sucrose, and 0.6% (w/v) agar.

^xCultured under a 16-h photoperiod provided by cool-white fluorescent tubes at a photo flux density of $8 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

^wDifferent letters within a column represent significant difference at $P \leq 0.5$ by Fisher's protected least significant difference test.

2iP = *N*-isopentenylaminopurine; 2,4-D = 2,4-dichlorophenoxyacetic acid; NAA = α -naphthalene acetic acid; BA = 6-benzyladenine; CPPU = *N*-(2-chloro-4-pyridyl)-*N'*-phenylurea; TDZ = *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea.

NAA and $2.26 \mu\text{M}$ 2,4-D, respectively, showed that the basal medium containing $19.68 \mu\text{M}$ 2iP and $1.07 \mu\text{M}$ NAA resulted in 92.9% of the nodal explants producing protocorm-like bodies. Adventitious shoots

appeared from protocorm-like bodies. On average, 16.9 shoots were regenerated from each explant (Table 4). Frequencies of protocorm-like body formation in medium supplemented with 9.84, 19.68, or

$25.52 \mu\text{M}$ 2iP with $2.26 \mu\text{M}$ 2,4-D were similar averaging from 66.7% to 70.8%; and mean shoot numbers varied from 10.2 to 12.9.

The use of TDZ only at 9.08 and $13.62 \mu\text{M}$ induced 87.5% and 67.9% of nodal explants to form protocorm-like bodies corresponding to 10.5 and 9.4 adventitious shoots, respectively. TDZ at $9.08 \mu\text{M}$ with NAA at $1.07 \mu\text{M}$ also resulted in a frequency (85.7%) similar to TDZ at $9.08 \mu\text{M}$ only. The protocorm-like body frequencies induced by $9.08 \mu\text{M}$ TDZ with $2.26 \mu\text{M}$ 2,4-D and $13.62 \mu\text{M}$ TDZ with $1.07 \mu\text{M}$ NAA or with $2.26 \mu\text{M}$ 2,4-D were lower ranging from 42.9% to 71.4%. Adventitious shoots per nodal explant averaged from 8.7 to 12.0.

Adventitious shoots were able to produce roots in the initial induction medium (Fig. 1G), but better root development occurred after shoots with protocorms were transferred onto the basal medium supplemented with $9.84 \mu\text{M}$ 2iP and $2.69 \mu\text{M}$ NAA (Fig. 1H). Up to 17 well-rooted plantlets were produced from a single nodal explant.

Ex vitro transplanting. After washing off the agar, plantlets were separated and directly transplanted into 4-cm diameter plastic pots containing the peat-based substrate. More

Table 3. Frequency of protocorm-like body (PLB) formation and mean number of adventitious shoots per nodal explant of *Syngonium podophyllum* 'White Butterfly' cultured on a Murashige and Skoog basal medium supplemented with different growth regulators^a.

Basal medium ^b	Frequency of PLB formation (%)	Mean number of shoots per explant ^c
9.84 μM 2iP + 2.26 μM 2,4-D	67.9 $\pm$ 4.6 a ^w	10.6 $\pm$ 4.4 a
8.88 μM BA + 2.26 μM 2,4-D	39.3 $\pm$ 7.4 b	6.2 $\pm$ 2.5 b
8.07 μM CPPU + 2.26 μM 2,4-D	28.6 $\pm$ 3.6 b	5.4 $\pm$ 1.3 c
9.08 μM TDZ + 2.26 μM 2,4-D	71.9 $\pm$ 7.4 a	11.5 $\pm$ 1.6 a

^aSix node explants per petri dish and eight petri dishes per treatment.

^bBasal medium comprises Murashige and Skoog mineral salts and vitamins, 2.5% (w/v) sucrose, and 0.6% (w/v) agar.

^cCultured under a 16-h photoperiod provided by cool-white fluorescent tubes at a photo flux density of 8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$.

^wDifferent letters within a column represent significant difference at $P \leq 0.5$ by Fisher's protected least significant difference test.

2iP = N-isopentenylaminopurine; 2,4-D = 2,4-dichlorophenoxyacetic acid; BA = 6-benzyladenine; CPPU = N-(2-chloro-4-pyridyl)-N'-phenylurea; TDZ = N-phenyl-N'-1,2,3-thiadiazol-5-ylurea.

than 1000 plantlets were transplanted, and they grew vigorously in the shaded greenhouse (Fig. 11). The plantlets had a survival rate of 100% without the occurrence of *Myrothecium* leaf spot and phenotypic variation.

Discussion

This study was intended to establish a method for efficiently regenerating well-rooted plantlets of *S. podophyllum* 'White Butterfly' through either somatic embryos or protocorm-like bodies. As a result, the study initially followed the protocols used for *S. podophyllum* 'Variegatum' regeneration (Zhang et al., 2006) in which the combinations of CPPU or TDZ with NAA or with 2,4-D were used for inducing somatic embryogenesis from leaf and petiole explants. Similar to the leaf explants of *S. podophyllum* 'Variegatum', leaf explants of *S. podophyllum* 'White Butterfly' only produced calli with no adventitious shoot formation. However, somatic embryos formed directly from petiole explants of *S. podophyllum* 'Variegatum' on a MS basal medium supplemented with 9.08 μM TDZ with 1.07 μM NAA or with 2.26 μM 2,4-D, but did not occur in *S. podophyllum* 'White Butterfly'. Cultivar and even explant differences in regeneration have been well documented (Shen et al., 2008; Skirvin et al., 1994; Zhang et al., 2006). An explanation for this difference could be that cells from leaf and petiole

explants were organogenically less competent. Another possibility could be that growth regulator combinations and/or their concentrations screened were inappropriate for induction of leaf and petiole explants.

Based on the responses of leaf and petiole explants, only four combinations of growth regulators (Table 3) were selected for testing nodal explants. Protocorm-like bodies were formed from nodal explants, adventitious shoots occurred followed by root formation, and subsequently plantlets were produced from the protocorm-like bodies. Protocorm-like bodies were first documented by Morel (1960) when the shoot apex of *Cymbidium* was cultured and later in other orchids (Park et al., 2003; Roy et al., 2007). Protocorm-like bodies are composed of many meristematic centers that are able to differentiate into shoots and roots (da Silva et al., 2000). Protocorm-like bodies, however, are distinguished from somatic embryos by the lack of a single embryonic axis (Norstog, 1979). In the present study, no bipolar structures were found. The protocorm-like bodies of *S. podophyllum* 'White Butterfly' had a direct vascular connection with the nodal explant (Fig. 2A). Adventitious shoots were formed from protocorm-like bodies and roots formed thereafter.

TDZ has been widely used for inducing protocorm-like bodies in orchids (Park et al., 2003; Roy et al., 2007). In the present study, TDZ at 9.08 or 13.62 μM alone or with NAA and 2,4-D induced protocorm-like body for-

mation (Table 4). Besides this study, TDZ directly inducing protocorm-like body formation alone has been reported in rose (Tian et al., 2008). In addition to TDZ, this study also showed that 2iP in combination with NAA or 2,4-D induced protocorm-like body formation in *Syngonium*. As stated previously, 2iP was used for shoot culture establishment and multiplication in *S. podophyllum* 'White Butterfly' (Kane, 2000; Miller and Murashige, 1976). Additionally, 2iP in combination with NAA was reported to induce protocorm-like body formation and subsequent regeneration of *Colocasia esculenta* Schott, another species of the family Araceae (Abo El-Nil and Zettler, 1976). In the present study, the highest frequency of protocorm-like body formation and the highest number of adventitious shoots occurred in MS basal medium supplemented 19.68 μM 2iP with 1.07 μM NAA. Thus, it is suggested that the procedure for regeneration of *S. podophyllum* 'White Butterfly' is to culture nodal explants on MS basal medium containing 19.68 μM 2iP and 1.07 μM NAA; adventitious shoots with protocorm-like bodies then should be transferred onto MS basal medium containing 9.84 μM 2iP and 2.69 μM NAA for better root development. Plantlets established in vitro can be directly planted in soilless peat-based substrate in a shaded greenhouse under a maximum photosynthetically photon flux density of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, temperature range of 20 to 28 °C, and relative humidity of 70% to 100%.

This is the first documentation of protocorm-like bodies in *Syngonium*. The established protocorm-like body pathway for regeneration of *S. podophyllum* 'White Butterfly' may represent a new approach for micropropagating this important cultivar to reduce incidence of *Myrothecium* leaf spot compared with the conventional shoot culture method. Because roots are well developed and can readily grow in soilless substrate, transplanting well-rooted plantlets provides little opportunity for *Myrothecium roridum* infection. Additionally, this established protocol may be readily used for stable genetic transformation (Chai et al., 2002), mass multiplication using bioreactors or synthetic seed production (Ara et al., 2000; Young et al.,

Fig. 2. Longitudinal sections of protocorm-like body formation and development from node explants of *Syngonium podophyllum* 'White Butterfly'. (A) A protocorm-like body directly formed from a node explant (Ex) with vascular connection (VS) and early shoot meristem (SM). (B) Differentiated protocorm-like body showing the young leaf (L) the main shoot meristem (SM) and lateral shoot meristem (LSM). (C) Shoot elongation with elongate stem axis (Ax), shoot meristem (SM), and lateral buds (LB).

Table 4. Frequency of protocorm-like body (PLB) formation from nodal explants and mean number of adventitious shoots per explant of *Syngonium podophyllum* 'White Butterfly' cultured on a Murashige and Skoog basal medium supplemented with different growth regulators^c.

Basal medium + plant growth regulators ^a (μM)				Frequency of explants with PLB formation (%)	Mean number of shoots per explant
2iP	TDZ	NAA	2,4-D		
9.84		1.07		71.4 ± 6.5 bc ^x	11.9 ± 1.6 b
19.68		1.07		92.9 ± 4.6 a	16.9 ± 1.8 a
29.52		1.07		78.6 ± 6.5 b	12.3 ± 1.2 b
9.84			2.26	66.7 ± 5.3 c	10.2 ± 1.1 b
19.68			2.26	67.9 ± 4.6 c	12.9 ± 1.5 b
29.52			2.26	70.8 ± 10.0 bc	10.5 ± 1.8 b
	9.08			87.5 ± 5.6 ab	10.5 ± 1.6 b
	9.08	1.07		85.7 ± 5.1 ab	12.1 ± 1.6 b
	13.62	1.07		64.3 ± 5.1 c	12.0 ± 1.5 b
	9.08		2.26	71.4 ± 6.5 bc	9.9 ± 1.1 bc
	13.62		2.26	42.9 ± 7.1 d	8.7 ± 1.2 c
	13.62			67.9 ± 7.1 c	9.4 ± 0.8 bc

^aFour nodal explants per petri dish and eight petri dishes per treatment. Data were pooled from three repeated experiments.

^bBasal medium comprises Murashige and Skoog mineral salts and vitamins, 2.5% (w/v) sucrose, and 0.6% (w/v) agar and explants were cultured under a 16-h photoperiod provided by cool-white fluorescent tubes at a photo flux density of 8 μmol·m⁻²·s⁻¹.

^cDifferent letters within a column represent significant difference at $P \leq 0.5$ by Fisher's protected least significant difference test.

2iP = *N*-isopentenylaminopurine; TDZ = *N*-phenyl-*N'*-1,2,3-thiadiazol-5-ylurea; NAA = α -naphthalene acetic acid; 2,4-D = 2,4-dichlorophenoxyacetic acid.

2000), and for cryopreservation as described for orchids (Nikishina et al., 2007).

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