Regeneration of *Chlorophytum amaniense* ‘Fire Flash’ Through Indirect Shoot Organogenesis

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Abstract. *Chlorophytum amaniense* Engl. ‘Fire Flash’ is a popular exotic ornamental foliage plant as a result of its unique coral-colored midribs and petioles and tolerance to interior low light levels. Currently, demand for propagative materials exceeds the availability of seeds. This study was intended to develop an in vitro culture method for rapid propagation of this cultivar. Leaf and sprouted seed explants were cultured on a Murashige and Skoog basal medium supplemented with different cytokinins with 1.1 μM α -naphthalene acetic acid (NAA) or 2.3 μM 2,4-dichlorophenoxyacetic acid (2,4-D). Leaf explants showed poor responses in callus production and no adventitious shoots were obtained. Callus formation frequencies from sprouted seeds were 71% and 85% when induced by 9.8 μM N₆-(2-isopentyl) adenine (2iP) with 1.1 μM NAA and 9.1 μM N-phenyl-N'-1,2,3-thiadiazol-5-ylurea (TDZ) with 1.1 μM NAA, respectively. Adventitious shoots occurred after the induced calluses were subcultured on the same concentrations of TDZ or 2iP with NAA. Shoot formation frequencies from calluses cultured on TDZ with NAA and 2iP with NAA were 92% and 85%, and the corresponding mean shoot numbers were 37 and 31 per piece of callus (1 cm³), respectively. Adventitious shoots rooted at 100% after transferring to the basal medium containing 4.4 μM 6-benzylaminopurine (BA) with 2.7 μM NAA. Plantlets, after transplanting to a soilless substrate were easily acclimatized in a shaded greenhouse under a photosynthetic photon flux (PPF) density of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Regenerated plants grew vigorously without undesirable basal branching or distorted leaves. This newly established regeneration method can provide the foliage plant industry with a means for rapidly propagating ‘Fire Flash’ liners in a year-round fashion.

Chlorophytum amaniense Engl. is native to the rain forests of east Africa (Dress, 1961). There is only one cultivar in this species, commonly known as ‘Fire Flash’; other names include Fire Glory, Mandarin Plant, Sierra Leone Lily, and Tangerine. *C. amaniense* ‘Fire Flash’ was recently introduced commercially as an exotic ornamental foliage plant (Chen et al., 2005) and enjoys considerable market success.

‘Fire Flash’ has a self-heading and upright growing style, broad lanceolate leaves, and bright coral-colored midribs and petioles (Fig. 1A). The unique coral-colored petioles and midribs contrasting with dark green leaves make it a sought after cultivated foliage plant specimen. Another important characteristic of ‘Fire Flash’ is its ability to tolerate interior conditions under a low light level of 8 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for 8 months or longer (Chen et al., 2005).

As a result of its unique color and adaptation to low light levels, ‘Fire Flash’ production has been dramatically increased since its introduction. Unlike *C. comosum* (Thunb.) Jacques (Spider Plant), ‘Fire Flash’ does not produce stolons, and no small plantlets are produced from its apex and node. Propagation through seed and division has not met the growing demand for starting materials. Commercial tissue culture laboratories have begun producing liners for the ornamental plant industry; however, single liner plugs frequently develop multiple shoots after transplanting, resulting in unsalable plants with small distorted leaves (Fig. 1B). Thus, a new and reliable protocol for regenerating high-quality ‘Fire Flash’ liners is needed.

In addition to ornamental value, the rhizomes of ‘Fire Flash’ form nearly oval tubers, which may contain antitumor steroidal saponins as do other species of *Chlorophytum* Ker Gawl. Steroidal saponins have been isolated from *C. arundinaceum* Baker (Tandon and Shukla, 1997), *C. borivilianum* Sant. & Fernan. (Seth et al., 1991), *C. comosum* (Mimaki et al., 1996), and *C. malayense* Ridl. (Qiu et al., 2000) and have shown cytotoxicity in vitro against several human cancer cell lines (Mimaki et al., 1996).

Previous research on regeneration of *Chlorophytum* has primarily focused on *C. borivilianum* and *C. arundinaceum*, traditional medicinal

plants. In vitro propagation methods were initially established using shoot explants (Dave et al., 2003; Kemat et al., 2010; Purohit et al., 1994; Suri et al., 1999) based on Murashige and Skoog (MS) medium (Murashige and Skoog, 1962) fortified with BA. Later, direct organogenesis methods were developed from immature floral buds and inflorescence axis (Samantaray et al., 2009; Sharma and Mohan, 2006) using MS medium containing BA or kinetin (KN) with 2,4-D, indole-3-acetic acid, NAA, or indole-3-butyric acid. Direct organogenesis methods were also developed from shoot crown explants (Lattoo et al., 2006) and from immature inflorescences of *C. arundinaceum* (Samantaray et al., 2009). However, there has been no documentation regarding methods of regeneration for *C. amaniense*.

This study was intended to evaluate the responses of leaf and sprouted seed explants to different combinations of growth regulators and to develop a new and reliable method for regeneration of *C. amaniense* liners with high quality for commercial ornamental and pharmaceutical production.

Materials and Methods

Plant materials. Young leaves were excised from ‘Fire Flash’ stock plants grown in a shaded greenhouse under a maximum PPF density of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ at the University of Florida’s Mid-Florida Research and Education Center, Apopka, FL. Seeds were harvested from greenhouse-grown stock plants and soaked in distilled water for 1 h; only those precipitated in the bottom of a beaker were used. The leaves and seeds were surface-sterilized by immersing in 70% ethanol for ≈ 1 min and soaking in 20% Clorox (1.2% NaOCl; Clorox Co., Oakland, CA) solution for 25 min, respectively. After pouring off the Clorox solution, leaves and seeds were rinsed three times with sterile distilled water. Leaf explants were produced by cutting the sterilized leaves into $\approx 4\text{-cm}^2$ pieces (2 cm $\times$ 2 cm) in sterile petri dishes. Leaf and seed explants were then ready for inoculation.

Medium preparation. The basal medium was comprised of MS mineral salts and vitamins, 3.0% (w/v) sucrose, and 0.65% (w/v) agar (Sigma, St. Louis, MO). Medium pH was adjusted to 5.8 with 1 M KOH before autoclaving at 121 $^{\circ}\text{C}$ for 25 min. When the medium temperature dropped to ≈ 50 $^{\circ}\text{C}$, filter-sterilized stock solutions of BA, N-(2-Chloro-4-pyridyl)-N'-phenylurea (CPPU), KN, 2iP, TDZ, 2,4-D, or NAA were added into the autoclaved basal medium based on the combinations and concentrations listed in Tables 1, 2, and 3. The medium was aliquoted to petri dishes (Fisher Scientific Inc., Pittsburgh, PA) at 20 mL each for callus initiation or to Magenta GA-7 vessels (PhytoTechnology Laboratories, Shawnee Mission, KS) at 25 mL each for shoot induction.

Callus induction. Leaf explants were cultured on the MS basal medium supplemented with 2.3 μM 2,4-D or 1.1 μM NAA with 9.1 μM TDZ, 8.1 μM CPPU, 9.3 μM KN, or 9.8 μM 2iP (Table 1). Leaf explants were placed

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Fig. 1. *Chlorophytum amaniense* 'Fire Flash' is a newly introduced exotic ornamental foliage plant. (A) 'Fire Flash' exhibits bright coral-colored midribs and petioles. (B) 'Fire Flash' plants produced in 15-cm pots from in vitro cultured liners provided by commercial tissue culture laboratories showing multiple shoots per pot and distorted leaves. (C) 'Fire Flash' produced in 15-cm pots from liners regenerated using the indirect shoot organogenesis method established in this study, in which each pot has only one shoot and no distorted leaves.

Table 1. Frequency of callus formation from leaf explants of *Chlorophytum amaniense* 'Fire Flash' after 8-week culture on a Murashige and Skoog basal medium^z containing different concentrations of growth regulators.

Growth regulator concentrations (μM) in basal medium						Frequency of explants with callus ^y	
TDZ	CPPU	KN	2iP	NAA	2,4-D	Light culture ^x	Dark culture
9.1					2.3	17 ± 4 a ^w	48 ± 6 a
9.1				1.1		24 ± 3 a	52 ± 5 a
	8.1				2.3	0	19 ± 4 c
	8.1			1.1		0	17 ± 4 c
		9.3			2.3	0	19 ± 2 c
		9.3				0	20 ± 2 c
			9.8	1.1	2.3	9 ± 3 b	39 ± 5 b
			9.8	1.1		19 ± 4 a	41 ± 6 ab

^zBasal medium comprised of Murashige and Skoog mineral salts and vitamins, 2.5% (w/v) sucrose, and 0.6% (w/v) agar, of which TDZ = N-phenyl-N'-1,2,3-thiadiazol-5-ylurea, CPPU = N-(2-Chloro-4-pyridyl)-N'-phenylurea, KN = kinetin, 2iP = N₆-(2-isopentyl) adenine, NAA = α-naphthalene acetic acid, and 2,4-D = 2,4-dichlorophenoxyacetic acid.

^ySix explants per petri dish and 12 dishes per treatment.

^xExplants were cultured under a 16-h photoperiod provided by cool-white fluorescent tubes with a photon flux density of 50 μmol·m⁻²·s⁻¹.

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

Table 2. Frequency of callus formation from sprouted seed explants of *Chlorophytum amaniense* 'Fire Flash' after 8-week culture on a Murashige and Skoog basal medium^z containing different concentrations of growth regulators under a photon flux density of 50 μmol·m⁻²·s⁻¹.

Growth regulator concentrations (μM) in basal medium							Frequency of sprouted seed explants with callus ^y
TDZ	CPPU	KN	2iP	BA	NAA	2,4-D	
9.1						2.3	44 ± 8 c ^x
9.1					1.1		85 ± 4 a
	8.1					2.3	0
	8.1				1.1		21 ± 3 d
		9.3				2.3	25 ± 4 d
		9.3			1.1		23 ± 3 d
			9.8			2.3	37 ± 5 c
			9.8		1.1		71 ± 7 b
				8.9		2.3	19 ± 4 d
				8.9	1.1		25 ± 3 d

^zBasal medium comprised of Murashige and Skoog mineral salts and vitamins, 2.5% (w/v) sucrose, and 0.6% (w/v) agar, of which TDZ = N-phenyl-N'-1,2,3-thiadiazol-5-ylurea, CPPU = N-(2-Chloro-4-pyridyl)-N'-phenylurea, KN = kinetin, 2iP = N₆-(2-isopentyl) adenine, BA = 6-benzylaminopurine, NAA = α-naphthalene acetic acid, and 2,4-D = 2,4-dichlorophenoxyacetic acid.

^ySix sprouted-seed explants per petri dish and 12 dishes per treatment.

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

with the adaxial surface up, six explants per dish and 12 dishes per treatment. The explants were cultured both in the dark and under a 16-h photoperiod provided by cool-white fluorescent tubes with a photon flux density of 50 μmol·m⁻²·s⁻¹.

Seed explants were initially cultured on the basal medium without plant growth regulators

in the dark for 10 d. There were six seeds per dish and a total of 140 dishes was used for seed germination. Sprouted seeds were then cultured on the basal medium supplemented with 2.3 μM 2,4-D or 1.1 μM NAA with 9.1 μM TDZ, 8.1 μM CPPU, 9.3 μM KN, 9.8 μM 2iP, or 8.9 μM BA (Table 2) with six sprouted seeds per dish and 12 dishes per treatment. The

sprouted seed explants were cultured under the aforementioned light conditions only.

Shoot induction. Callus clumps induced from sprouted seeds by TDZ and NAA, TDZ and 2,4-D, 2iP and NAA, or 2iP and 2,4-D were cut into ≈1-cm³ pieces (≈150 mg in fresh weight) and cultured on the basal medium supplemented with the same combination of growth regulators as they were initially cultured, but varying in concentrations, i.e., 2.3 μM 2,4-D or 1.1 μM NAA with 4.5, 9.1, and 13.6 μM TDZ as well as 2.3 μM 2,4-D or 1.1 μM NAA with 4.9, 9.8, and 14.8 μM 2iP (Table 3). There were four callus pieces per Magenta GA-7 vessel and eight vessels per treatment, which were cultured under the mentioned light conditions.

Data collection and analysis. A completely randomized design was used for the induction of callus and adventitious shoots. Each petri dish or Magenta GA-7 vessel was considered an experimental unit. Explants or calluses that responded to the induction were recorded weekly after culture initiation. The frequencies of explants with callus formation and calluses with shoots were calculated from data collected 8 weeks after initial inoculation. Mean numbers of shoots per callus piece were counted 12 weeks after callus culture. After checking normal distribution, data were analyzed by analysis of variance (SAS GLM; SAS Institute, Cary, NC), and mean separations were determined using Fisher's protected least significant differences at the 5% levels.

Rooting and ex vitro plantlet establishment. Shoots (≈3 to 4 cm) were excised and transferred to Magenta GA-7 vessels containing ≈25 mL of the basal medium supplemented with 4.4 μM BA with 2.7 μM NAA for rooting and continuous growth. Four weeks after placing into rooting medium, well-rooted plantlets were washed free of agar using tap water and transplanted individually into 10-cm pots containing a soilless substrate with Canadian peat, vermiculite, and perlite in a 3:1:1 ratio. The potted plants were grown in a shaded greenhouse under a PPF density of 200 μmol·m⁻²·s⁻¹, a temperature range of 20 to 28 °C, and relative humidity of 70% to 100%. Six weeks later, the plants were transplanted into 15-cm pots for finished plant production in the same greenhouse conditions. Survival rate, shoot numbers per pot, and any growth disorders were visually assessed 2 months later.

Results

Callus induction. Leaf explants cultured under light conditions turned brown and then darker within 2 weeks after inoculation. Five weeks later, a few light brownish calluses appeared at the cut edges of some leaf explants cultured on the basal medium supplemented with 9.1 μM TDZ or 9.8 μM 2iP with either 2.3 μM 2,4-D or 1.1 μM NAA. Callus formation did not occur in basal medium containing the other growth regulators. The callus formation frequencies ranged from 9% to 24% (Table 1). Leaf explants cultured in the dark expanded slightly and became yellow and then brown in 4 weeks. Light brownish callus occurred in the

Table 3. Frequency of adventitious shoot formation from sprouted-seed explants derived callus piece (1 cm³) and shoot number of *Chlorophytum amaniense* 'Fire Flash' after culture on a Murashige and Skoog basal medium^z containing different concentrations of growth regulators.

Growth regulator concentrations (μM) in basal medium				Frequency of shoot formation from callus pieces ^y	Shoot no. per callus piece ^x
TDZ	2iP	NAA	2,4-D		
4.5		1.1		71 ± 6 b ^w	25 ± 2 c
4.5			2.3	25 ± 3 e	8 ± 1 e
9.1		1.1		85 ± 5 ab	31 ± 3 b
9.1			2.3	23 ± 4 e	10 ± 1 de
13.6		1.1		37 ± 4 d	14 ± 2 d
13.6			2.3	10 ± 3 f	7 ± 1 e
	4.9	1.1		60 ± 8 c	19 ± 2 d
	4.9		2.3	21 ± 3 e	14 ± 2 d
	9.8	1.1		92 ± 4 a	37 ± 2 a
	9.8		2.3	21 ± 4 e	13 ± 2 de
	14.8	1.1		42 ± 4 d	15 ± 3 d
	14.8		2.3	8 ± 3 f	4 ± 1 e

^zBasal medium comprised of Murashige and Skoog mineral salts and vitamins, 2.5% (w/v) sucrose, and 0.6% (w/v) agar, of which TDZ = N-phenyl-N'-1,2,3-thiadiazol-5-ylurea, 2iP = N₆-(2-isopentyl) adenine, NAA = α-naphthalene acetic acid, and 2,4-D = 2,4-dichlorophenoxyacetic acid.

^yFour callus piece (1 cm³) per Magenta GA-7 vessel and eight vessels per treatment. The frequencies were calculated based on data collected 8 weeks after culture.

^xShoot numbers were calculated based on data collected 12 weeks after culture.

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

Fig. 2. Morphogenesis of *Chlorophytum amaniense* 'Fire Flash'. (A) Light brownish colored callus produced from leaf explants cultured on a Murashige and Skoog basal medium containing 9.1 μM N-phenyl-N'-1,2,3-thiadiazol-5-ylurea (TDZ) and 1.1 μM α-naphthalene acetic acid (NAA). (B) Sprouted seeds after 10 d dark culture on the basal medium devoid of growth regulators. (C) Light green calluses produced from sprouted seed explants cultured on the basal medium supplemented with 9.1 μM TDZ and 1.1 μM NAA. (D) Two bud primordia and (E) multiple primordia occurred after the induced callus was subcultured on the same basal medium containing the same concentrations of TDZ and NAA. (F-G) Multiple shoots appeared when cultured in Magenta GA-7 vessels. (H) Individual shoots rooted in Magenta GA-7 vessels. (I) Regenerated plantlets grew in a 10-cm pot filled with a soilless substrate in a shaded greenhouse. Bars = 1 mm.

cut edges of explants 2 weeks later (Fig. 2A). All the growth regulator treatments induced callus formation, but induction frequencies varied significantly (Table 1). The highest frequencies were 48% to 52% induced by TDZ with either 2,4-D or NAA followed by 39% to 41% induced by 2iP with either 2,4-D or NAA. Other growth regulator combinations

resulted in callus formation frequencies 20% or less. Calluses became brown and dark and died within 10 weeks under lighted conditions. No shoot organogenesis occurred in leaf explants irrespective of growth regulator combinations.

Sprouted seed explants. Seeds germinated on basal medium devoid of growth regulators

after 10 d in the dark (Fig. 2B). When the sprouted seeds were transferred to callus induction medium and cultured under the 16-h photoperiod light conditions, they grew into seedlings with reduced root growth. Approximately 3 to 4 weeks later, white callus appeared from the base of seedling stems, resulting in the shrinkage of shoots. The shoots were removed ≈2 weeks later, and the callus rapidly proliferated into large, greenish clumps (Fig. 2C). Of the growth regulators evaluated, 9.1 μM TDZ with 1.1 μM NAA was able to induce 85% of sprouted seed explants to produce callus followed by 9.8 μM 2iP with 1.1 μM NAA that resulted in 71% of explants producing callus (Table 2). Some calluses induced by 9.1 μM TDZ with 1.1 μM NAA or 9.8 μM 2iP with 1.1 μM NAA produced adventitious shoots in the initial culture medium. Growth regulators in other combinations or concentrations only induced 44% or less of sprouted seed explants to form callus.

Shoot induction. When callus pieces initially induced by TDZ or 2iP with NAA or with 2,4-D were cultured on the basal medium containing the same growth regulator combinations (TDZ with NAA or 2,4-D, 2iP with NAA or 2,4-D) (Table 3), callus proliferated and produced compact callus clumps in 4 weeks. Ten days later, bud primordia appeared (Fig. 2D) followed by adventitious shoots (Fig. 2E-G). In general, shoot formation frequencies and shoot numbers per callus piece were much higher from callus pieces induced by either TDZ or 2iP with NAA than by their respective combinations with 2,4-D (Table 3). Among them, 9.8 μM 2iP with 1.1 μM NAA induced 92% of callus pieces to produce shoots, and shoot numbers averaged 37 per callus piece. Similarly, 9.1 μM TDZ with 1.1 μM NAA resulted in 85% of callus pieces producing shoots, and shoot numbers averaged 31 per callus piece. The next most successful combination was 4.5 μM TDZ with 1.1 μM NAA, which stimulated 71% of callus pieces to form shoots, and shoot numbers averaged 25 per callus piece. The other combinations or concentrations of growth regulators showed lower shoot formation frequencies (60% or less) and lower shoot numbers (19 or less) than the aforementioned three combinations.

Rooting, acclimatization, and greenhouse production. Few adventitious shoots produced roots in the shoot induction medium. However, when shoots were transferred to the basal medium containing 4.4 μM BA with 2.7 μM NAA, all shoots produced multiple roots and some petioles or stems exhibited a light coral color at their early stage (Fig. 2H). After transplanting into 10-cm pots, the regenerated plants grew vigorously in the soilless substrate (Fig. 2I). All plantlets survived in the shaded greenhouse. Visual examination of finished plant production indicated that all plants maintained a single shoot per pot and exhibited no distorted growth (Fig. 1C).

Discussion

This study developed an in vitro method of regenerating *C. amaniense* 'Fire Flash' through

indirect shoot organogenesis. Briefly, calluses were induced from sprouted seeds cultured on a MS basal medium supplemented with either 9.8 μM 2iP or 9.1 μM TDZ with 1.1 μM NAA. Adventitious shoots were produced from the calluses subcultured on the same medium (either 9.8 μM 2iP or 9.1 μM TDZ with 1.1 μM NAA). The shoots rooted easily in the basal medium containing 4.4 μM BA and 2.7 μM NAA, and plantlets were acclimatized in a shaded greenhouse under a PPF density of 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. This regeneration method is efficient and reliable because callus formation frequencies were 71% and 85% when sprouted seeds were induced by 9.8 μM 2iP with 1.1 μM NAA and 9.1 μM TDZ with 1.1 μM NAA, respectively. The corresponding shoot formation frequencies from the calluses were 92% and 85% and mean shoot numbers were 37 and 31 per callus piece. Plantlets produced through this procedure grew vigorously in a commercial production setting without undesirable multiple basal shoots and growth distortion.

This established method is different from the previously reported protocols for regenerating *C. arundinaceum* and *C. borivilianum* where synthetic cytokinins of BA and KN were used. The present study found that BA and KN as well as CPPU were ineffective in callus induction. High frequencies of callus and shoot induction were achieved by 2iP or TDZ with NAA (Tables 2 and 3). 2iP is a naturally occurring cytokinin and has been widely used for inducing plant shoot organogenesis, particularly for monocots such as *Syngonium* (Cui et al., 2008), *Dieffenbachia* (Shen et al., 2007; Voyiatzi and Voyiatzis, 1989), *Dracaena* (Liu et al., 2010), and *Epipremnum* (Qu et al., 2002). TDZ has both auxin-like and cytokinin-like activities (Singh et al., 2003). The TDZ induced higher frequencies in callus formation than BA, KN, and CPPU (Tables 1 and 2) and subsequently comparably high frequencies in adventitious shoot formation (Table 3) may suggest that TDZ fulfilled more of a cytokinin-like role in 'Fire Flash' regeneration. Additionally, Sharma and Mohan (2006) reported that BA was a cause of vitrification of regenerated *C. borivilianum*. In our regenerated populations, no vitrified plantlets were observed.

In this study, leaf explants failed to regenerate shoots. In search of reported regeneration methods for *Chlorophytum*, we found that only shoots (Dave et al., 2003; Kemat et al., 2010; Purohit et al., 1994; Suri et al., 1999), immature floral buds, and inflorescence axis (Lattoo et al., 2006; Samantaray et al., 2009; Sharma and Mohan, 2006) were successfully used as explants for regeneration of *C. arundinaceum* or *C. borivilianum*. There has been no report of *Chlorophytum* regeneration from leaf explants. The current study showed that leaf explants quickly became brown and then dark; only a few of those induced by TDZ and 2iP exhibited delayed browning and later formed callus when cultured under light conditions. Leaf explants cultured in the dark showed a delay in browning (≈ 10 d); thus, more explants produced callus. Schubert and

Matzke (1985) documented that in the presence of light, H^+ ion extrusion takes place, which results in increased acidity of culture medium. Light-stimulated H^+ extrusion was also reported by Petzold and Dahse (1988) from leaf discs of *Vicia faba* L. The H^+ extrusion may accelerate the browning process. Kaur et al. (2006) believed that under illumination, the cultures turn brown, thus affecting the division efficiency of protoplasts of *Brassica oleracea* L. and *Brassica napus* L. It is possible that the accelerated browning quickly kills cells of leaf explants of 'Fire Flash' and provides little opportunity for cells to respond to the induction. Although more leaf explants produced callus in the dark culture, the induced calluses still died after culture under lighted conditions. Dark treatments were reported to promote adventitious shoots and embryo-like structures in birch (*Betula pendula* Roth) (Welander, 1988) and somatic embryogenesis in wheat (*Triticum aestivum* L.) (Ozias-Akins and Vasil, 1983). It is worth further investigation if a prolonged dark culture could induce adventitious shoots or somatic embryogenesis from the leaf explants of 'Fire Flash'.

This is the first report of in vitro regeneration of *C. amaniense* 'Fire Flash'. The procedure is simple and efficient, and the regenerated plants were morphologically stable without multiple shoots and distorted growth. The use of this established procedure could significantly increase the propagation of the starting materials of 'Fire Flash' to meet the demand for commercial production.

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