Topolins and Red Light Improve the Micropropagation Efficiency of Passion Fruit (*Passiflora edulis* Sims) ‘Tainung No. 1’

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Abstract. Passion fruit is a commercial crop of economic importance worldwide, with recent increases in demand for high-quality plants for commercial production. Plant tissue culture is widely used for the mass propagation of many commercial crops, however its application on passion fruit is challenged by the problem of low reproducibility, leaf chlorosis, and growth retardation resulted from in vitro culture. The aim of this study was to evaluate the effects of cytokinins and light quality on in vitro culture of nodal segments of passion fruit ‘Tainung No. 1’. Three aromatic cytokinins were tested in a modified MS basal medium. The bud proliferation rates of segments initiated on a media containing 1 mg·L⁻¹ *meta*-topolin riboside (*m*TR) or benzyladenine (BA) were not significantly different at the same concentration. Buds cultured on medium supplemented with *m*TR grew and elongated for 4 weeks, while buds on a medium containing BA formed rosettes. After transfer to a medium without plant growth regulators (PGRs), shoots rooted spontaneously within 8 weeks. Furthermore, the effects of continuous propagation under a high proportion of red light affected the subsequent plant growth. Red LED induced an increase in the chlorophyll content (2.71 mg·g⁻¹) compared with other light qualities (1.05–2.63 mg·g⁻¹) and improved plantlet quality. Acclimated plants were grown in the field, and the flower morphology and fruit set were of commercial quality. Findings showed that replacing BA with *m*TR as the main cytokinin and using a high proportion of red light during the tissue culture induction period produced high-quality plantlets in 3 months. This system is economical and will be further developed for the commercial propagation of passion fruit vines in the future.

The genus *Passiflora* L. is a group of about 520 species that grow as vines, shrubs, or small trees in tropical, subtropical, and occasionally temperate areas (Ulmer and MacDougal, 2004). Also known as passion flowers, numerous species in this genus are economically important because of the taste and nutritional value of their fruits (i.e.,

passion fruit), the pharmaceutical properties of their leaves, or the ornamental value of their flowers (Huh et al., 2017; Mikovski et al., 2019).

Passiflora species are propagated through seeds, cuttings, air-layering, or grafts (Faleiro et al., 2019; Ragavendran et al., 2012). In Taiwan, the main cultivar is Tainung No. 1, a hybrid of *Passiflora edulis* and *P. edulis* f. *flavicarpa*. ‘Tainung No.1’ is conventionally propagated by grafting, but this method does not provide enough planting materials to satisfy the ever-increasing demand for international plants export. Therefore, an in vitro culture technique should be established to clone this superior genotype.

Tissue culture of *Passiflora* species could be used to mass produce healthy plantlets for commercial growers and to produce ornamental hybrids through somatic hybridization, to produce medicinal and secondary metabolites, and for in vitro conservation. Among the in vitro culture systems, organogenesis is the primary pathway for regener-

ation of *Passiflora* (Mikovski et al., 2019; Otoni et al., 2013). For some medicinal *Passiflora* spp., there are reported callus culture systems and micropropagation protocols, while in vitro techniques have been discussed for conservation (Ożarowski, 2011; Ożarowski and Thiem, 2013; Pacheco et al., 2016). Nevertheless, micropropagation of *Passiflora* is not yet commercially feasible because adult material does not work well as an initial explant and has a low proliferation rate (Rocha et al., 2020). Furthermore, for each species within the genus, the reported culture conditions vary and each need to be optimized to overcome inherent tissue culture-induced problems, such as leaf chlorosis and growth retardation, and to increase production (Monteiro et al., 2000).

Cytokinins are a class of plant growth regulators widely used in *Passiflora* tissue culture. Benzyladenine (BA) is usually used for adventitious shoot induction and axillary shoot proliferation. The BA concentration should be reduced to enhance further elongation of shoot buds (Ożarowski and Thiem, 2013; Rocha et al., 2015). However, when BA is used in tissue culture for many plants, it is known to cause in vitro abnormalities, including hyperhydricity, shoot-tip necrosis, difficulty in rooting and acclimatization, and histogenic instability, in species as varied as *Aloe polyphylla* (Bairu et al., 2007), *Beta vulgaris* (Kubaláková and Strnad, 1992), *Barleria greenii* (Amoo et al., 2011), *Hordeum vulgare* (Huyluoglu et al., 2008), *Magnolia* spp. (Parris et al., 2012), *Petunia* (Bogaert et al., 2006), and ‘Williams’ banana (Bairu et al., 2008).

Meta-topolin (*m*T), or 6-(3-hydroxybenzylamino)-purine, first isolated from poplar leaves (*Populus × canadensis* Moench., cv. Robusta), was shown to have a stronger cytokinin activity and may be useful as an alternative to BA (Mok et al., 2005; Mutui et al., 2012; Ornellas et al., 2017; Strnad et al., 1997). Exogenously applied *m*T delayed chlorophyll degradation in excised radish cotyledons (Palavan-Ünsal et al., 2002a) and wheat leaves (Palavan-Ünsal et al., 2002b; Palavan-Ünsal et al., 2004), increased the in vitro-shoot multiplication rate of *Aloe ferox* (Bairu et al., 2009) and *Pelargonium sidoides* (Moyo et al., 2012), reduced shoot-tip necrosis in micropropagated *Harpagophytum procumbens* (Bairu et al., 2011) and *Ulmus glabra* (Mirabbasi and Hosseinpour, 2014), and reduced senescence in in vitro-propagated rose (Bogaert et al., 2006) and *Pelargonium × hortorum* cuttings (Mutui et al., 2012). The application of *meta*-topolin riboside (*m*TR) induced a higher chlorophyll a/b ratio in banana shoot-tip culture (Aremu et al., 2012b). Also, *m*T-treated regenerants showed better in vitro rooting rates and acclimatization (Aremu et al., 2012a; Gentile et al., 2014). To the best of our knowledge, there have been no reports evaluating *m*T and/or *m*TR in the currently used *Passiflora* in vitro culture protocols or on how they affect the micropropagation efficiency.

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The influence of light in regulating plant growth and development has been well documented. Using light-emitting diodes (LEDs) as the light source has been found to have positive effects on in vitro shoot and root development of various species, resulting in improved adaptability and growth after transfer to soil (Gupta and Agarwal, 2017). Over the decades, the use of monochromatic red and blue LEDs alone or in combination has been reported during plant morphogenesis both in vivo and in vitro (Agarwal and Dutta Gupta, 2016; Batista et al., 2018; Bula et al., 1991; Dutta Gupta and Jatothu, 2013; Gupta and Agarwal, 2017; Lee et al., 2011; Massa et al., 2008; Ramirez-Mosqueda et al., 2016). However, the effects of different and combined LED light qualities during in vitro culture of *Passiflora* has not been reported.

In this study, we determined the effects of aromatic cytokinins (BA, *mT*, and *mTR*) and LED light qualities [blue (B), green (G), red (R), and infrared (IR), singly and in combination] on direct bud multiplication, elongation, and rooting of the Tainung No. 1 cultivar of passion fruit. The optimized propagation system was efficient and amenable to large-scale vegetative production.

Materials and Methods

Plant material, disinfection, initial culture, and culture conditions. The *Passiflora* hybrid cultivar Tainung No. 1 was cultured in the greenhouse (Horticultural Experiment Station, National Chung Hsing University). During the cultivation period, the average annual temperature was 25.3 °C with an average relative humidity of 66.9%, and the daily average maximum and minimum temperatures were 32.9 and 17.6 °C, respectively. Nodal segments containing axillary buds were cut off from mature vines as explants for initial in vitro culture. Explants were surface-disinfected using 2% sodium hypochlorite (NaOCl) and 1–2 drops of Tween 20 for 20 min, and then washed in distilled water three times in a laminar flow hood. Nodal segment with a single bud was placed vertically into a test tube (20 × 150 mm; PYREX Glass Test Tube No. 9820) containing 9 mL of a shoot induction medium (SI medium), which consisted of MS basal salts and vitamins (Murashige and Skoog, 1962), 100 mg·L⁻¹ *myo*-inositol (Sigma-Aldrich Co.), 170 mg·L⁻¹ NaH₂PO₄ (Hayashi Pure Chemical Ind., Co., Ltd., Japan), and 30 g·L⁻¹ sucrose (Taiwan Sugar Corporation, Taiwan) and was solidified using 0.86% agar (Trade Mark, Taiwan). An initial experiment was conducted using various concentrations of benzyladenine (BA; 0, 0.1, 0.2, and 1 mg·L⁻¹, Sigma-Aldrich Co.) and α -naphthalene acetic acid (NAA; 0 and 0.1 mg·L⁻¹, Sigma-Aldrich Co.). We added BA and NAA to the medium before the pH was adjusted to 5.7 with 1 N HCl or NaOH before autoclaving (1.2 kg/cm² of pressure, 121 °C, 20 min). Explants were maintained at 25 ± 1 °C under a 12-h photoperiod at a light intensity of 55.6 μ mol·m⁻²·s⁻¹ (daylight fluorescent tubes FL-

30D/29, 40 w; China Electric Co, Taipei, Taiwan). Each treatment included 10 test tubes, which considered individual replications. The experiments were repeated three times.

After 4 weeks of culturing, elongated shoots were transferred to the plant growth regulator (PGR)-free PA2L medium, which contained MS salts and vitamins, 100 mg·L⁻¹ *myo*-inositol, 170 mg·L⁻¹ NaH₂PO₄, 20 g·L⁻¹ sucrose, 1 g·L⁻¹ peptone, 1 g·L⁻¹ activated charcoal, 150 mL·L⁻¹ coconut water (KOH; BABI Corp International, Thailand), 6 g·L⁻¹ potato powder (PhytoTechnology Laboratories), was solidified using 0.86% agar, and a pH adjusted to 5.2 before autoclaving. The other culture conditions were the same as above.

Effects of BA, meta-topolin (*mT*), and meta-topolin riboside (*mTR*) on proliferation and elongation of nodal segments. To test if the shoot quality could be improved by substituting another aromatic cytokinin for the BA, *mT* (OlChemIm Ltd., Czech Republic) and *mTR* (OlChemIm) were considered as targets. In the initial experiment, the best bud growth was seen with 1 mg·L⁻¹ BA and 0.1 mg·L⁻¹ NAA, and this was thus used as the control to compare with substitution of 1 mg·L⁻¹ *mT* or *mTR*. While the BA and NAA were added to the SI medium before autoclaving, the *mT* and *mTR* were sterilized through a 0.22- μ m MF-Millipore Membrane Filter (Merck, Ireland) before one was added to the autoclaved SI medium supplemented with NAA. Plantlets derived from initial culture on the PA2L medium were cut into nodal segments as explants to clarify the effects of the three cytokinins on bud multiplication and elongation.

Light quality affects plantlet growth and chlorophyll content of explants during in vitro culture. A custom system (Fig. 1A) designed for tissue-cultured plantlets and equipped with eight sets of LEDs (Nano Bio Light Technology Co., Ltd., Taiwan) as light sources (Chen et al., 2016; Fang et al., 2011; Lai et al., 2018) was used to test the influence of light quality on the growth of nodal segments. Eight sets of LED chips were mounted on the lips of the tissue culture vessels in a combination of blue (B), green (G), red (R), and infrared (IR) LEDs. Each vessel had the same photosynthetic photon flux density, 42 μ mol·m⁻²·s⁻¹. The LED peaks for B, G, R, and IR were 450 ± 2 nm, 525 ± 3 nm, 660 ± 5 nm, and 730 ± 5 nm, respectively. Two different B/G/R/IR ratios were used to produce either cool white light (26:26:26:2) or warm white light (10:45:51:4) with a spectrum like daylight. Four LED sets had chip ratios of 3B:3R:3IR, 1B:1G:7R, 1B:7R:1IR, and 1B:8R. The other two sets had only one band each, namely 9R and 9B (Lai et al., 2018). The light spectrum of each set is shown in Fig. 1B. Five nodal segments were vertically cultured in one vessel containing SI medium with 1 mg·L⁻¹ *mTR* and 0.1 mg·L⁻¹ NAA (based on the results of the second experiment, above), with three vessels for each of the eight light quality combinations. After 4 weeks of culturing, the vessels were removed

from the LED system. In the laminar flow hood, the nodal segments were examined and the bud numbers, leaf numbers, tendrils numbers, and callus formation rate were recorded. These explants were then subcultured to the PA2L medium and moved to the culture room with the same conditions under fluorescent lights as described above. After 8 weeks of additional culture under fluorescent lights, the height, bud, and root numbers, and chlorophyll content of the plantlets were measured. Plant height was measured by a ruler after the taking out of the flask. The average numbers of bud and roots were counted for six plantlets. For the analysis of chlorophyll concentration, three leaf discs (8 mm in diameter) from each plant were collected and extracted with 5 mL N, N-Dimethylformamide (DMF) for 24 h at 4 °C in darkness, following the protocol of Moran and Porath (1980). The absorbance was measured with a spectrophotometer (U2000; Hitachi, Japan) at 664.5 and 647.0 nm, respectively. The concentrations of chlorophyll a and chl b were calculated using the formula described by Inskeep and Bloom (1985).

Acclimatization and transplantation. After subculture in the PA2L medium for 8 weeks, plantlets with well-developing roots and shoots (measuring $\approx$ 5 cm from the base of the shoot to the shoot tip) were planted in 6-inch plastic pots containing peatmoss (Klasmann-Deilmann GmbH, Germany) and covered by plastic wrap with several holes for about 2 weeks in a temperature gradient incubator (TG3; FIRSTEK, Taiwan) at 25 °C. The plantlets were then moved to a mist bed for another 2 weeks. Surviving plantlets were individually transferred to black plastic pots and cultured in the greenhouse.

Statistical analysis. All experiments were arranged in a completely randomized design (CRD). Statistical analysis was conducted using the SAS 9.4 software (Institute Inc., NC). The data, expressed as percentages, were transformed using arc sine before an analysis of variance (ANOVA) and converted back to original scale (Compton, 1994). Data were subjected to ANOVA, and differences between mean values among treatments were tested using least significant difference multiple range tests at a 5% level of significance ($P \leq 0.05$).

Results and Discussion

Effects of BA, meta-topolin (*mT*), and meta-topolin riboside (*mTR*) on nodal segment induction in tissue culture. BA is the most used cytokinin for the micropropagation of *Passiflora*, although some side effects, such as leaf chlorosis and retarded growth, have been reported (Monteiro et al., 2000). To determine if a topolin-type cytokinin could be substituted for BA, we first compared a proliferation media containing a range of BA concentrations. In medium without PGRs, only 8.1% of the buds elongated; while in medium with only 0.1 mg·L⁻¹ NAA, only 11.4% elongated. Additionally, the browning rates were higher than on media

Fig. 1. The custom LED system (Mini E Light) equipped with eight sets of LEDs as light sources for nodal segment culture. (A) The system has two layers and 12 vessels per layer. Each layer has an independent timer to control day/night light cycle and light quantity (bar = 10 cm). (B) Spectral distribution of LEDs and fluorescent lamps. The spectral irradiance was recorded with a spectroradiometer and software system (FieldSpec HandHeld, ASD Inc. CO). B = blue light (450 ± 2 nm); G = green light (525 ± 3 nm); R = red light (660 ± 5 nm); IR = infrared (730 ± 5 nm); CW = cool white light [26:26:26:2 (B:G:R:IR)]; WW = warm white light [10:45:51:4 (B:G:R:IR)]. CK = fluorescent lamps in a tissue culture room.

Table 1. Effects of BA and NAA on the culture of nodal segments of passion fruit cultivar Tainung No. 1.

BA ($\text{mg}\cdot\text{L}^{-1}$) ^a	NAA ($\text{mg}\cdot\text{L}^{-1}$)	Bud sprouting (%) ^b	Bud multiplication (%)	Callus formation (%)	Bud browning (%)
0.0	0.0	8.1 ± 4.2 c	0.0 ± 0.0 b	16.2 ± 8.5 a	75.7 ± 12.7 a
0.1	0.0	33.8 ± 12.5 abc	9.5 ± 9.5 ab	11.4 ± 5.9 a	45.2 ± 8.6 abc
0.2	0.0	40.5 ± 13.3 a	9.5 ± 4.8 ab	29.0 ± 0.5 a	21.0 ± 12.4 c
1.0	0.0	18.1 ± 11.7 abc	33.3 ± 17.2 a	19.1 ± 12.6 a	29.5 ± 17.3 bc
0.0	0.1	11.4 ± 5.9 bc	8.1 ± 4.2 ab	19.5 ± 5.2 a	61.0 ± 13.3 ab
0.1	0.1	36.7 ± 14.0 ab	16.2 ± 8.5 ab	12.9 ± 8.4 a	34.3 ± 13.5 bc
0.2	0.1	11.4 ± 5.9 bc	36.7 ± 18.2 a	16.2 ± 1.9 a	35.7 ± 10.9 bc
1.0	0.1	14.3 ± 14.3 bc	31.9 ± 13.7 a	20.0 ± 20.0 a	33.8 ± 20.7 bc

^aBasal medium contained MS salts, $100 \text{ mg}\cdot\text{L}^{-1}$ myo-inositol, $30 \text{ g}\cdot\text{L}^{-1}$ sucrose, $170 \text{ mg}\cdot\text{L}^{-1}$ NaH_2PO_4 , and $8.6 \text{ g}\cdot\text{L}^{-1}$ agar; the pH was adjusted to 5.7 before autoclaving.

^bPercentage data were arc sine transformed before analysis of variance; means $\pm$ ses within a column followed by different letters indicate significant differences according to least significant difference multiple range test at $P < 0.05$. Data are mean of three independent experiments.

containing BA (Table 1). In media containing both BA and NAA, the effect of BA on bud multiplication increased with concentration, with $1 \text{ mg}\cdot\text{L}^{-1}$ BA showing the best multiplication rates. By comparison, lower concentrations of BA (0.1 and $0.2 \text{ mg}\cdot\text{L}^{-1}$) caused significant bud elongation but not bud multiplication (Table 1). Considering the various effects on culture initiation, the combination of $1.0 \text{ mg}\cdot\text{L}^{-1}$ BA and $0.1 \text{ mg}\cdot\text{L}^{-1}$ NAA was chosen for further comparison with media containing *mT* or *mTR* as the cytokinin.

Cytokinins show different levels of biological activities: *mTR* = zeatin > benzyladenine riboside (BAR) > *ortho*-topolin riboside (*oTR*) = *para*-topolin riboside (*pTR*) (Kamínek et al., 1987). *Meta*-topolin (*mT*) was slightly more active than *mTR* in wheat senescence and tobacco callus bioassays (Werbrouck et al., 1996). According to previous studies, *mT* is considered the most active of the natural aromatic cytokinins; when used instead of the widely used BA, it can eliminate high callus formation rates, necrosis, and

negative effects at the rooting stage (Bandaralage et al., 2015). In this study, we compared BA, *mT*, and *mTR* during in vitro bud elongation of passion fruit nodal segments. The rates of bud multiplication, callus formation, and bud browning was not significantly different among the three aromatic cytokinins at the same concentration ($1 \text{ mg}\cdot\text{L}^{-1}$; Table 2), but there was a big difference in appearance of the explants after 6 weeks (Fig. 2). Most explants cultured on the BA medium formed a rosette, even after transferring to PGR-free medium for 2–3 intervals of subculturing. Buds cultured on medium supplemented with *mT* or *mTR* grew sustainably and elongated for 4 weeks. Explants grown on the *mTR* medium had larger leaves and an increased number of nodes on the stem (Fig. 2C). After transfer to the PA2L medium, shoots had fully expanded leaves and vigorous roots after 8 weeks. The average plant height was 8.4 ± 2.6 cm, and plants had an average of 11.6 ± 2.6 nodes per plantlet. The average in vitro rooting rate was $88.1 \pm$

0.1% . The average number of in vitro roots per plantlet was 5.4 ± 3.1 , and the length of the longest root averaged 5.6 ± 2.4 cm. These plantlets could be further cut to produce one node that could be brought back into culture to repeat the process again. During 4–5 years of in vitro culture, no somaclonal variation was observed.

Plants derived from one round of multiplication (4 weeks) (Fig. 3A) and one subculture in PA2L medium for 8 weeks (Fig. 3B) were then transplanted to the greenhouse for another 8 weeks for acclimatization (Fig. 3C) before transfer to the field (Fig. 3D). Plants had flower buds 4 months after planting in the field, and the flower morphology were normal (Fig. 3E and F). Flowers were open-pollinated by bees in the field and showed a normal fruit set (Fig. 3G). Mature fruits naturally dropped on the net and had a purple-red peel and oval shape (Fig. 3H). In the present study, a simple and reliable protocol for establishing nodal segment explants of the passion fruit cultivar Tainung No. 1

Table 2. Effects of BA, NAA, and topolins on the culture of nodal segments in 'Tainung No. 1' passion fruit.

Cytokinin ^z	Expanded leaves (%) ^y	Bud multiplication (%)	Callus formation (%)	Bud browning (%)
0	16.7 ± 8.5 a	1.7 ± 1.7 b	0.0 ± 0.0 a	81.7 ± 7.5 a
1 mg·L ⁻¹ BA	15.1 ± 8.3 a	47.5 ± 13.1 a	4.8 ± 4.8 a	32.6 ± 5.4 b
1 mg·L ⁻¹ <i>mT</i>	16.1 ± 9.6 a	39.6 ± 23.0 a	0.0 ± 0.0 a	44.3 ± 13.9 b
1 mg·L ⁻¹ <i>mTR</i>	17.4 ± 3.2 a	51.0 ± 7.3 a	0.0 ± 0.0 a	31.6 ± 5.3 b

^zBasal medium contains MS salts, 100 mg·L⁻¹ *myo*-inositol, 30 g·L⁻¹ sucrose, 170 mg·L⁻¹ NaH₂PO₄, 0.1 mg·L⁻¹ NAA, 8.6 g·L⁻¹ agar; the pH was adjusted to 5.7 before autoclaving. BA = benzyladenine; *mT* = *meta*-topolin; *mTR* = *meta*-topolin riboside.

^yPercentage data were arc sine transformed before analysis of variance; means ± ses within a column followed by different letters indicate significant differences according to least significant difference multiple range test at *P* < 0.05. Data are mean of three independent experiments.

Fig. 2. Nodal segments were cultured on medium supplemented with one of three cytokinins for 6 weeks. (A) 1 mg·L⁻¹ BA; (B) 1 mg·L⁻¹ *meta*-topolin; (C) 1 mg·L⁻¹ *meta*-topolin riboside. Scale bars = 0.7 cm (A), 1 cm (B), and 1 cm (C).

Fig. 3. Nodal segments of 'Tainung No. 1' passion fruit pictured during *in vitro* culture, acclimation, flowering, and fruit set in the field. (A) Elongated shoot from nodal segment cultured on a *mTR* medium (bar = 8.6 mm). (B) Healthy plantlets with fully expanded leaves and vigorous roots (bar = 1.6 cm). (C) Plantlets acclimated in a greenhouse (bar = 5.4 cm). (D) Plant after transfer from greenhouse to the field (bar = 8.8 cm). (E) Plants with flower buds 4 months after planting in soil (bar = 52.2 cm). (F) Open flower with normal shape and structures (bar = 1.1 cm). (G) Flowers were pollinated by bees and showed normal fruit set (bar = 41.4 cm). (H) Mature fruit had normal color and shape (bar = 3.2 cm).

was established using the *mTR* medium for node-bud elongation and the PGR-free medium for spontaneous rooting.

Numerous studies have discussed the promising of using *mT* in many tissue culture systems (Aremu et al., 2014; Bairu et al.,

2006; Benmahioul et al., 2012; Bogaert et al., 2006; Lata et al., 2016; Werbrouck et al., 1996). Using *mTR* improved the survival rate of potato cultures (Baroja-Fernández et al., 2002), proved to be better than BA in terms of multiplication rate and quality of shoots in

Baleria greenii (Amoo et al., 2011), and promoted the shoot multiplication rate over an equimolar concentration of BA in banana culture (Bairu et al., 2008). The superiority of *mT* and its derivatives over BA has been attributed to its faster translocation in plant

Table 3. Growth and development of nodal segments after 30 d of growth under different light qualities (LEDs) on shoot initiation (SI) medium.

Treatment ^z	Bud no. ^y	Leaf no.	Tendrils no.	Callus formation (%) ^x
CW	1.3 ab	2.7 a	0.1 a	22.5 ab
9B	1.3 a	2.2 a	0.0 b	15.0 b
3R3B3IR	1.1 ab	2.4 a	0.0 b	15.0 b
7R1G1B	1.2 ab	2.6 a	0.0 b	22.5 ab
WW	1.1 ab	2.4 a	0.0 b	30.0 ab
9R	1.1 ab	2.3 a	0.0 b	40.0 a
8R1B	1.0 b	2.4 a	0.0 b	25.0 ab
7R1B1IR	1.2 ab	2.5 a	0.0 b	22.5 ab

^zLight was provided by a combination of blue (B), green (G), red (R), and infrared (IR) LEDs. Cool white light (CW) and warm white light (WW) were of a spectrum like that of daylight.

^yFive nodal segments in each vessel supplemented with SI medium, 1 mg·L⁻¹ *meta*-topolin riboside (*mTR*), and 0.1 mg·L⁻¹ NAA. Explants were maintained at 25 ± 1 °C under a 12-h photoperiod at a photosynthetic photon flux density of 42 μmol·m⁻²·s⁻¹.

^xPercentage data were arc sine transformed before analysis of variance; means ± ses within a column followed by different letters indicate significant differences according to least significant difference multiple range test at *P* < 0.05. Data are mean of three independent experiments.

Table 4. Various LED light qualities had long-lasting effects on plant growth and leaf chlorophyll content in nodal segments after induction to subculture at 8 weeks in 'Tainung No. 1' passion fruit.

Treatment ^z	Plant ht (cm) ^y	Node no.	Leaf no.	Root no.	Max. root length (cm)	Lateral bud no.	chl a (mg·g ⁻¹)	chl b (mg·g ⁻¹)	Total chl (mg·g ⁻¹)
CK	5.4 bc	9.2 a	7.1 a	4.3 a	3.3 b	0.0 a	0.86 b	0.28 d	1.14 d
CW	3.1 d	8.0 a	6.8 a	1.3 bc	0.1 c	0.3 a	1.54 ab	0.57 bc	2.11 abc
9B	5.5 bc	8.8 a	6.7 a	3.7 ab	3.3 b	0.7 a	1.24 ab	0.41cd	1.64 cde
3R3B3IR	7.2 a	8.8 a	6.8 a	4.8 a	2.6 b	0.0 a	0.82 b	0.24 d	1.05 d
WW	4.1 cd	7.3 a	4.3 b	0.3 c	0.0 c	0.2 a	1.30 ab	0.55 bc	1.84 bcd
9R	7.2 a	8.5 a	7.5 a	5.5 a	5.3 a	0.0 a	1.86 a	0.86 a	2.71 a
8R1B	7.2 a	8.0 a	6.3 ab	4.0 ab	3.0 b	0.3 a	1.83 a	0.81 ab	2.63 ab
7R1B1IR	5.9 ab	8.3 a	7.0 a	3.3 ab	2.4 b	0.5 a	1.46 a	0.51 cd	1.97 abc

^zLight was provided by LED chips, except for the CK treatment. A combination of blue (B), green (G), red (R), and infrared (IR) LEDs was used as light source in each treatment. Cool white light (CW) and warm white light (WW) were of a spectrum like that of daylight. CK = nodal segments were cultured in a culture room with daylight fluorescent tubes at a light intensity of 55.6 μmol·m⁻²·s⁻¹.

^yThe nodal segments were cultured in the tissue culture vessels with LED chips mounted on their lips, while the CK treatment was cultured in a glass flask in a culture room. After 4 weeks of light treatment, nodal segments were all transferred to PA2L medium for another 8 weeks in a culture room with daylight fluorescent tubes. Mean values in the same column with different letter(s) indicate significant differences according to least significant difference multiple range test at *P* < 0.05. Data are mean of a total of six independent plants collected from two independent experiments.

Fig. 4. The morphology of nodal segments cultured on a medium containing *meta*-topolin riboside in various light qualities for 4 weeks and then subcultured on a growth medium without plant growth regulators for another 8 weeks. (A) CK = explants cultured in a culture room with daylight fluorescent tubes (bar = 5.4 cm). (B) CW = cool white light (bar = 4.4 cm). (C) 9B (bar = 5.2 cm). (D) 3R3B3IR (bar = 4.6 cm). (E) WW = warm white light (bar = 4.8 cm). (F) 9R (bar = 4.7 cm). (G) 8R1B (bar = 4.5 cm). (H) 7R1B1IR (bar = 4.9 cm).

tissue, which prevents localized accumulation, its different affinity for receptors, and the formation and reversible sequestration of its *O*-glucoside metabolites (Amoo et al., 2012; Aremu et al., 2012a; Kaminek et al., 1987; Mok et al., 2005; Strnad 1997; Subbaraj, 2011; Werbrouck et al., 1996).

Light quality affects plantlet growth and the chlorophyll content of explants during in

vitro culture. While establishing the culture procedure for nodal segments of passion fruit using 1 mg·L⁻¹ *mTR* as the cytokinin in the initial medium, the leaves of plantlets were yellowish-green in color (Figs. 2C and 3B), although this did not affect the survival rate, and new leaves became normal in the greenhouse (Fig. 3C). To increase the appearance of the plantlets, we investigated if changing

the supplied light quality, an important environmental factor in tissue culture, could improve the leaf color. Nodal segments cultured in various LED spectra showed no differences in leaf numbers. A significant difference in the number of buds per segment was seen between the 9B and 8R1B treatments, however, not between 9B and the other treatments. Tendrils were only observed

under the CW treatment. Increasing the proportion of red light resulted in fewer buds and more calli at the node base. Monochromatic red LED (9R) significantly enhanced the callus formation compared with the 9B and 3R3B3IR treatments (Table 3).

The light quality used during culture initiation also manifested in subsequent plant growth after transfer to the PGR-free medium in the tissue culture room with normal daylight fluorescent tubes. Red LED during initiation increased the shoot length, root number, maximum root length, and chlorophyll content (including chl a and chl b) 8 weeks after transferring to the PGR-free medium. The 3R3B3IR LED combination promoted shoot growth and root number but resulted in shorter root length and the lowest chlorophyll content. The CW and WW treatments significantly inhibited root length compared with the other LED treatments. There was no difference in the numbers of nodes between the treatments, which indicated that internode elongation leads to the recorded increase in plant height in the red light condition (Table 4; Fig. 4). LED treatments yielding higher chlorophyll content are generally associated with improved shoot growth (Liu et al., 2006). However, the responses to different spectral qualities provided by LEDs vary according to plant species (Gupta and Agarwal, 2017). In in vitro-cultured *Pfaffia glomerata*, an equal ratio of red and blue light enhanced the biomass and 20-hydroxyecdysone content (Silva et al., 2020). In contrast, in grape and blueberry, a high proportion of red LEDs accelerated the elongation of regenerated shoots, but blue LED treatment increased the chlorophyll content (Hung et al., 2016; Poudel et al., 2008). Other research showed that blue light promoted stem elongation of eggplant and sunflower seedlings and suppressed stem elongation of leaf lettuce and tomato (Hirai et al., 2006; Li et al., 2017; Mochizuki et al., 2019). Conversely, red LED treatment enhanced chlorophyll content in the shoot culture of banana (Vieira et al., 2015) and *Rehmannia glutinosa* (Manivannan et al., 2015). When leafy single-node cuttings of *Vitis vinifera* 'Manicure Finger' were cultured in vitro under red LED light, stem elongation, leaf growth, and chlorophyll reduction, all associated with shade-avoidance syndrome (SAS), were the primary responses (Li et al., 2017). Red light may promote stem growth by regulating the biosynthesis of gibberellic acid in Norway spruce seedlings (Ouyang et al., 2015), or induce the expression of an auxin inhibitor gene to promote stem and root lengthening in grape (Li et al., 2017). Red light also increased the activity of Phenylalanine ammonia lyase (PAL) in *Dianthus carophyllus* (Manivannan et al., 2017) and the total lignin content in *Triticum aestivum* (Dong et al., 2014).

Promotion of in vitro root induction and development by red LED treatments has been reported for various plant species, including *Tripterospermum japonicum* (Moon et al., 2006), grape (Poudel et al., 2008), banana

(Wilken et al., 2014), *R. glutinosa* (Manivannan et al., 2015), and *Cunninghamia lanceolata* (Xu et al., 2019). The interaction between light quality and cytokinin content in media on multiplication and growth was evaluated during in vitro culture of *Myrtus communis* L. The results indicated that the highest number of shoots was obtained under red LEDs with the lowest concentration of cytokinin in the media (Cioć et al., 2018). In this study, red LED illumination produced superior plantlets, marked by improvements in the in vitro shoot length, chlorophyll content, and leaf and root growth that also acclimatized at a high rate ($\approx 100\%$).

Conclusion

Passion fruit is an important fruit crop around the world. The demands on production and trade in passion fruits are gaining importance globally (Altendorf, 2018; Ramaiya et al., 2019). This growing global market and demand for high-quality plants is beyond the current commercial capacity to supply plants through germinating seeds or by grafting. Simultaneously, the production of passion fruit transplants by tissue culture is often limited by the problems of shoot clustering and leaf yellowing (Monteiro et al., 2000). In Taiwan, the main cultivar is Tainung No. 1, and grafted plants are used for commercial production of fruits both for internal markets and for export. In this study, we established a reliable node-bud proliferation system that uses *mTR* as the cytokinin, yielding a high rate of shoot elongation, and illumination with red light to improve plantlet quality. Plantlets rooted spontaneously in growth medium without auxin, thus avoiding an extra rooting step in the protocol. To the best of our knowledge, this is the first report on the use of topolins and various light qualities for in vitro propagation of *Passiflora*.

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