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Changes in Morphological Characteristics, Regeneration Ability, and Polysaccharide Content in Tetraploid *Dendrobium officinale*

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Abstract. Dendrobium officinale **Kimura et Migo is a famous traditional Chinese medicinal plant. It produces various phytochemicals, particularly polysaccharides, which have nutraceutical and pharmaceutical values. To increase its biomass production and polysaccharide content, our breeding program has generated a series of polyploid cultivars through colchicine treatment of protocorm-like bodies (PLBs). The present study compared two tetraploid cultivars, 201-1-T_1 and 201-1-T_2, with their diploid parental cultivar, 201-1, in an established in vitro culture system. Tetraploid '201-1-T_1' and '201-1-T_2' had shorter leaves and shorter and thicker stems and roots, and they produced higher biomass compared with the diploid cultivar. The length and width of stomata significantly increased, but stomatal density decreased in tetraploid cultivars. The PLB induction rates from the stem node explants of the tetraploid cultivars were significantly higher than those of diploid. However, the PLB proliferation of tetraploids was lower than that of the diploid. The mean number of plantlets regenerated from tetraploid PLBs was also lower than that of the diploid after 4 months of culture. Polysaccharide contents in stems, leaves, and roots of 6-month-old tetraploid plantlets were significantly higher than those of diploids. The polysaccharide content in the stem of '201-1-T_1' was 12.70%, which was a 2-fold increase compared with the diploid cultivar. Our results showed that chromosome doubling could be a viable way of improving *D. officinale* in biomass and polysaccharide production.**

Dendrobium Sw. is one of the largest genera in the family Orchidaceae, and it comprises more than 1500 species (Cheng et al., 2019) that are widely distributed in Asia, Australia, and Europe and produced as ornamental flowering plants (Lam et al., 2015). In China, *Dendrobium*, commonly known as "Shi Hu," has been used as traditional Chinese medicine for thousands of years (Cheng et al., 2019). More than 78 species of *Dendrobium* are native to China, and 50 of them have medicinal value (Chen et al., 2012; Xu et al., 2013). They are a source of tonic, astringent, analgesic, antipyretic, and anti-inflammatory substances (Cakova et al., 2017). Regarded as one of the most famous *Dendrobium* in China since the Tang dynasty ≈1300 years ago, *D. officinale* Kimura et Migom has been used to eliminate heat evil, remediate stomach problems, moisten the lung, relieve cough, nourish the body, and prolong lifespan (Alonso-Sande et al., 2009; Wang et al., 2018; Yuan et al., 2014). More than 190 compounds have been isolated from *D. officinale* (Tang et al., 2017), of which polysaccharide is one of the major active constituents, exhibiting immunomodulatory and cardioprotective activities (Dou et al., 2016; He et al., 2016). Stems usually contain a high concentration of polysaccharide (He et al., 2017). Due to its health benefits and long growth cycle, wild *D. officinale* has been exploited to near extinction. *D. officinale* has been considered a threatened species and is listed as critically endangered on the International Union for Conservation of Nature (IUCN) Red List (Critically Endangered A4c ver. 3.1) (Jin et al., 2016; Qian et al., 2014). Despite previous attempts to revive *D. officinale*, an urgent need for conserving wild *D. officinale* still exists (Li et al., 2018a; Liu et al., 2015; Teixeira da Silva et al., 2014b). To meet the demand for commercial production, new cultivars should be developed and methods for efficient propagation should be established (Ding et al., 2008).

Current efforts regarding *D. officinale* breeding include germplasm collection, identification, evaluation, and conventional hybridization. Breeding objectives are largely focused on improving morphological characteristics and biomass production (Teixeira da Silva et al., 2016). Only a limited number of cultivars have been released thus far (Chen et al., 2018). Chromosome manipulation is an effective way of orchid improvement (Hossain et al., 2013). Because orchid seeds produce protocorm during germination, and because in vitro regeneration induces protocorm-like bodies (PLBs) (Morel, 1960; Teixeira da Silva et al., 2015), protocorms or PLBs can be easily treated with colchicine for manipulation of chromosomes (Yang et al., 2013; Zhan and Cheng, 2011). PLBs are composed of many meristematic centers that differentiate into shoots and roots (Cui et al., 2008). An advantage of propagation through PLBs is that a large number of plantlets can be regenerated, thus enhancing propagation efficiency (Chen and Wei, 2018). Through colchicine treatment, autopolyploids have been induced from several *Dendrobium* species, including *D. chrysotoxum* (Atichart, 2013), *D. devonianum* (Li et al., 2005), *D. nobile* (Vichiato et al., 2014), *D. ochreatum* (Wang et al., 2017), and *D. officinale* (Yang et al., 2013; Zhan and Cheng, 2011). Autopolyploidy results in more floral pieces with larger petals and/or sepals, prolonged flower lifespan, and increased fertility (Chaicharoen, 1995; Chaicharoen and Saejew, 1981; Ketsa et al., 2001; McConnell and Kamemoto, 1993; Vichiato et al., 2014). Furthermore, autopolyploidy enhances plant overall size, including increased leaf width and thickness and biomass production (Chaicharoen and Saejew, 1981; del Pozo and Ramirez-Parra, 2014; Dudits et al., 2016; Jiang et al., 2014; Vichiato et al., 2007).

Chromosome manipulation of *D. officinale* has been primarily centered on morphological changes, and limited attention has been given to the regeneration ability during in vitro culture and active phytochemicals such as polysaccharide. We believe that in addition to morphological changes, polyploidized *D. officinale*, such as tetraploids, should have increased contents of polysaccharide and exhibit differences in plant regeneration during in vitro culture. Our breeding program has produced a series of *D. officinale* cultivars with different ploidy

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levels. The present study was performed to test our hypothesis by comparing two tetraploid cultivars, 201-1-T_1 and 201-1-T_2, with their parental diploid cultivar, 201-1, and to determine if morphological characteristics, regeneration ability, and polysaccharide content were changed due to the induced autopolyploidization.

Materials and Methods

Plant materials. Diploid *D. officinale* hybrid 201-1 ($2n = 38$) and tetraploid cultivars 201-1-T_1 and 201-1-T_2 ($2n = 76$) were used in this study. Their appearance, root tip cell chromosome numbers, and relative DNA contents that were analyzed using flow cytometry (Cui et al., 2009) are presented in Fig. 1. The two tetraploids were selected from a regenerated population of '201-1' after the PLBs were treated with colchicine. Hybrid 201-1 has a rapid growth rate and a desirable growth form, and it has been used as a new cultivar. The two tetraploids have been stable during in vitro culture and in greenhouse production.

Analysis of morphological characteristics. Young shoots (≈1 cm) derived from PLBs were grown in glass culture vessels (height, 100 mm; diameter, 65 mm; and volume, 330 mL) containing MS medium (Murashige and Skoog, 1962) supplemented with 2.2 µM 6-BA (6-benzylaminopurine), 0.5 µM NAA (naphthaleneacetic acid), 30 $g \cdot L^{-1}$ sucrose, 0.3 $g \cdot L^{-1}$ active carbon (AC), and 7 $g \cdot L^{-1}$ agar with a pH of 5.8. There were five shoots per culture vessel and nine vessels per cultivar. The experiment was arranged as a randomized complete block design (RCBD). Each culture vessel was considered an experimental unit, each block had three vessels per cultivar, and there were three blocks. Young shoots were maintained in a culture room under fluorescent tube with a light intensity of 20 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Six months after culture, plantlet height, stem diameter, leaf number, leaf width and length, root number, root length, and diameter were recorded using an electronic digital caliper. The fresh weight of entire plantlets, after roots were washed to remove agar and blotted on paper towels, was weighed using an electronic balance.

Determination of stomatal dimension and density. Another experiment that was identical to the aforementioned morphological analysis was conducted, but it was used to analyze stomatal dimension and density based on the method described by Stoddard (1965). Briefly, a drop of clear nail polish was applied to the abaxial surface of the third leaf from the meristem per plantlet. After drying, a piece of Scotch 3M transparent tape (Scotch

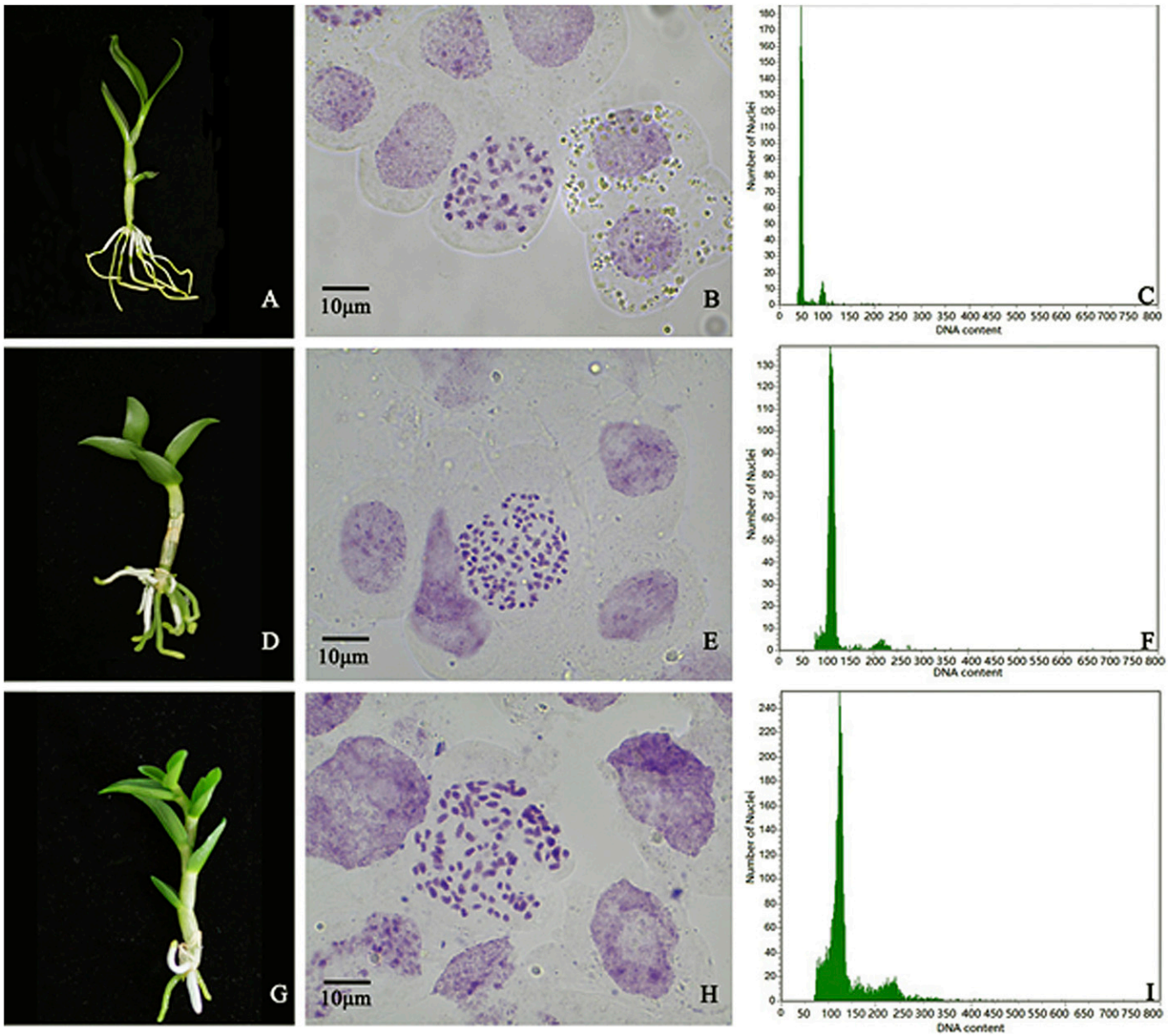

Fig. 1. The plantlet appearance, chromosome number in root tip cell, and relative DNA content in leaves of the diploid ($2n = 38$) parental cultivar 201-1 (**A**–**C**) and two tetraploid ($2n = 76$) cultivars 201-1-T_1 (**D**–**F**) and 201-1-T_2 (**G**–**I**) of *D. officinale*.

3M, St. Paul, MN) was placed on top of dried nail polish. The polish with an imprint affixed to the tape was peeled off and mounted on the microscope slides for observation. Stomata were viewed using a compound biological microscope, and the images were displayed on a computer via the microscope's built-in camera (Moticam2306; 3.0 M Pixel USB 2.0) with Motic Images Advanced 3.2 software (Motic Group, Hong Kong, China). Digital images of the leaf and stomata imprints were viewed at 400× and 1000× magnification and saved as a digital tagged image file (.tif) with 1024 × 768-pixel resolution for the later analysis. The images of the leaf imprint were analyzed, and the stomatal lengths were measured using Image J2 software. Fifteen images per cultivar were observed.

Evaluation of PLB induction, proliferation, and plant regeneration. Stems ≈4 to 5 cm in length were cut from '201-1', '201-1-T_1', and '201-1-T_2' plantlets. After removal of leaves, the stems were decontaminated with 70% ethanol for ≈1 min. Then, they were thoroughly rinsed with sterilized distilled water for 30 s. Finally, they were cut into segments ≈0.4 to 0.5 cm in length and inoculated on half-strength MS medium supplemented with 0.44 µM 6-BA, 20% coconut water (CW), 30 g·L^{-1} sucrose, 7 g·L^{-1} agar, and 0.1 g·L^{-1} AC with a pH of 5.8. The experiment was also set as an RCBD. Each block had three vessels per cultivar, nine stem explants per culture vessel, and a total of three blocks. Stem explants were cultured in the dark for 1 month. The number of stem explants with PLBs were counted, and the percentage of PLB induction was calculated based on the number of stem segments with PLBs in each vessel divided by the total number of stem segments in the same vessel.

To assess the growth of PLBs, PLBs of each cultivar were cultured on half-strength MS medium supplemented with 0.88 µM 6-BA, 0.5 µM NAA, 20% CW, 30 g·L^{-1} sucrose, and 7 g·L^{-1} agar, respectively, with a pH of 5.8. Again, the experiment was an RCBD. There were 9 PLBs with a total fresh weight of 1 g per culture vessel, 10 vessels per block, and a total of 3 blocks. They were initially cultured in the dark for 1 month. Then, the weight of PLBs in each vessel was measured by the electronic balance. The growth or proliferation coefficient of PLBs was calculated based on the weight of PLBs after 1 month of culture divided by the initial weight of PLBs.

For shoot induction, 9 PLBs ≈0.3 to 0.4 cm in diameter per cultivar were cultured on MS medium supplemented with 0.88 µM 6-BA, 0.5 µM NAA, 30 g·L^{-1} sucrose, 0.1 g·L^{-1} AC, and 7 g·L^{-1} agar with a pH of 5.8. The experiment was an RCBD with 10 vessels per block and a total of 3 blocks. They were maintained under a 12-h photoperiod with light intensity of 20 µmol·m^{-2}·s^{-1} for 2 months. The initial times of shoot formation

Table 1. Morphological and stomatal characteristics of diploid and tetraploid cultivars of *D. officinale* grown under in vitro culture conditions.[z]

Parameter	Diploid 201-1	Tetraploid 201-1-T_1	Tetraploid 201-1-T_2
Stem length (cm)	5.43 ± 0.36 a[y]	4.20 ± 0.17 b	3.03 ± 0.24 c
Stem diameter (cm)	0.28 ± 0.02 b	0.42 ± 0.02 a	0.44 ± 0.03 a
Leaf no.	5.70 ± 0.49 a	5.40 ± 0.47 a	4.80 ± 0.35 a
Leaf length (cm)	2.14 ± 0.11 a	1.55 ± 0.09 b	1.29 ± 0.37 c
Leaf width (cm)	0.78 ± 0.04 a	0.77 ± 0.03 a	0.73 ± 0.05 a
Root no.	6.30 ± 0.63 a	7.00 ± 0.95 a	7.20 ± 0.35 a
Root length (cm)	5.74 ± 0.38 a	2.24 ± 0.13 b	2.17 ± 0.14 b
Root diameter (cm)	0.13 ± 0.006 b	0.19 ± 0.007 a	0.21 ± 0.007 a
Fresh weight (g)	0.92 ± 0.07 b	2.14 ± 0.17 a	1.95 ± 0.57 a
Stomata no. (1000 µm^2)	54.40 ± 0.83 a	30.30 ± 1.76 b	30.50 ± 0.76 b
Stomata cell length (µm)	37.36 ± 0.67 c	45.99 ± 0.74 a	42.13 ± 0.64 b
Stomata cell width (µm)	19.10 ± 0.26 c	25.53 ± 1.28 a	22.26 ± 0.74 b
Stomata area (µm^2)	1535.31 ± 37.33 b	2392.45 ± 123.78 a	2172.37 ± 90.16 a

[z]All data represent means ± SE (n = 3).
[y]Different letters within the same row indicate a significant difference at $P \leq 0.05$ based on Duncan's multiple range test.

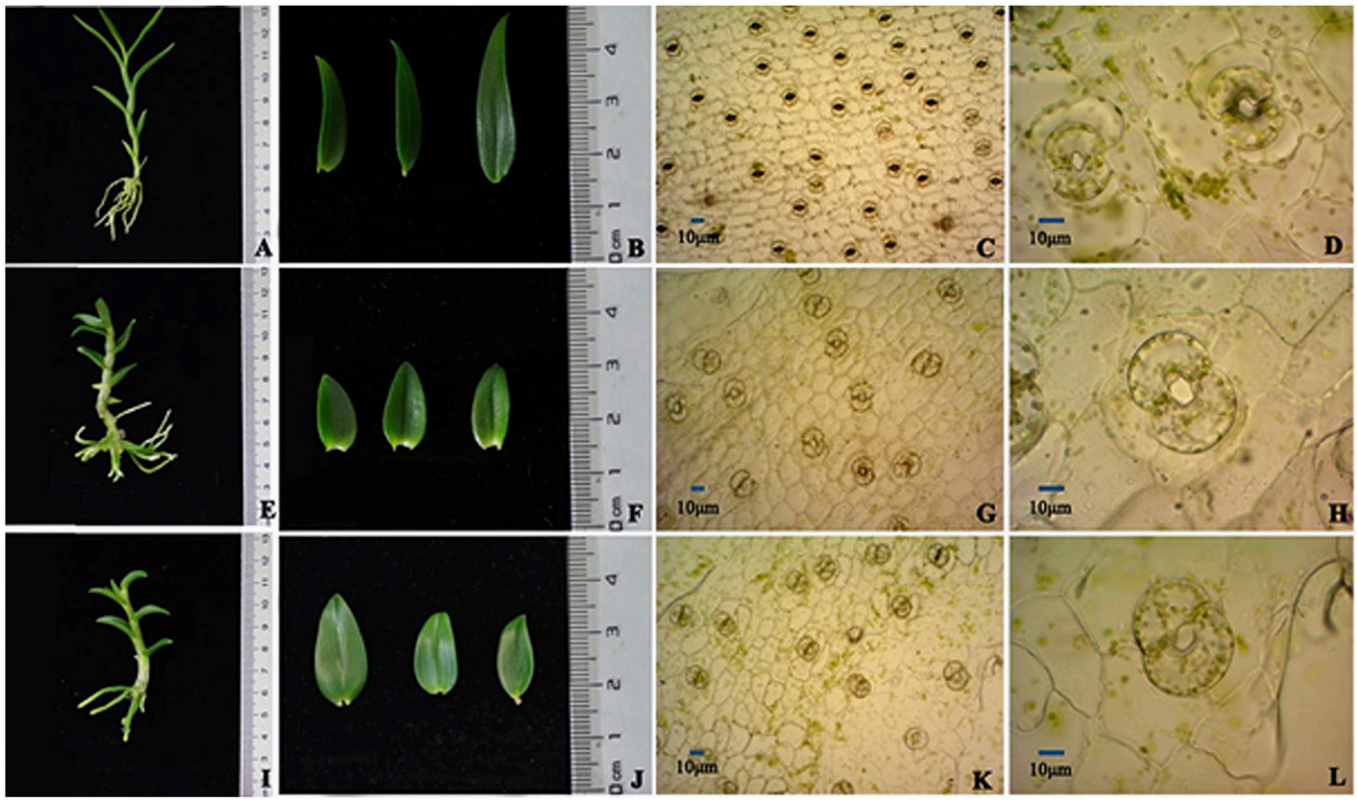

Fig. 2. Six-month-old plantlets, their leaves, stomatal density (400× magnification), and stomatal size (1000× magnification) of the diploid cultivar 201-1 (**A**–**D**) and tetraploid cultivars 201-1-T_1 (**E**–**H**) and 201-1-T_2 (**I**–**L**) of *D. officinale*.

and root formation were recorded. The numbers of shoots and roots from each PLB were recorded after 2 months of culture.

Analysis of polysaccharides content. To determine the polysaccharide content, an experiment that was the same as the one used for the aforementioned morphological analysis was conducted. After 6 months of growth, polysaccharide contents in leaves, stems, and roots were measured using the standard protocol published by Chinese Pharmacopoeia (Committee for the Pharmacopoeia of P R China, 2015). Briefly, 50 g of the stems, leaves, and roots of plantlets were placed in a brown envelope and dried in an electrical oven at 60 to 65 °C to a constant weight. After being ground into powder, a 0.3-g sample was mixed in 200 mL of deionized water and refluxed in a syncore parallel reactor (Büchi, Switzerland) for 2 h at 100 °C; after cooling, the solution was diluted to 250 mL in volumetric flask. The solution was filtered using a vacuum filter; 2 mL of filter solution was transferred to a centrifuge tube after adding 7 mL of absolute ethanol. Then, the tube was kept in refrigeration at 4 °C for 1 h, followed by centrifuging at 6000 rpm for 20 min using a centrifuge (Allegra X-15R; Beckman Coulter, Fullerton, CA). After being washed with 8 mL of 80% ethanol twice, the precipitate was dissolved in heated water and diluted to 25 mL. Then, 1 mL of the solution, 1 mL of 5% phenol, and 5 mL of pure sulfuric acid solution were transferred to a 10-mL glass tube and well-mixed. Next, the tube was boiled in boiling water for 20 min. After being cooled

Table 2. Induction of PLBs from stem explants of diploid and tetraploid cultivars of *D. officinale*.[z]

Cultivar	Day PLB initiation	PLB induction rate (%)
201-1	13.4 ± 1.20 b[y]	40.18 ± 2.95 b
201-1-T_1	16.6 ± 0.40 a	56.03 ± 7.01 a
201-1-T_2	17.4 ± 0.74 a	55.75 ± 4.42 a

[z]All data represent means ±SE (n = 3).
[y]Different letters within the same column indicate a significant difference at $P \leq 0.05$ based on Duncan's multiple range test.

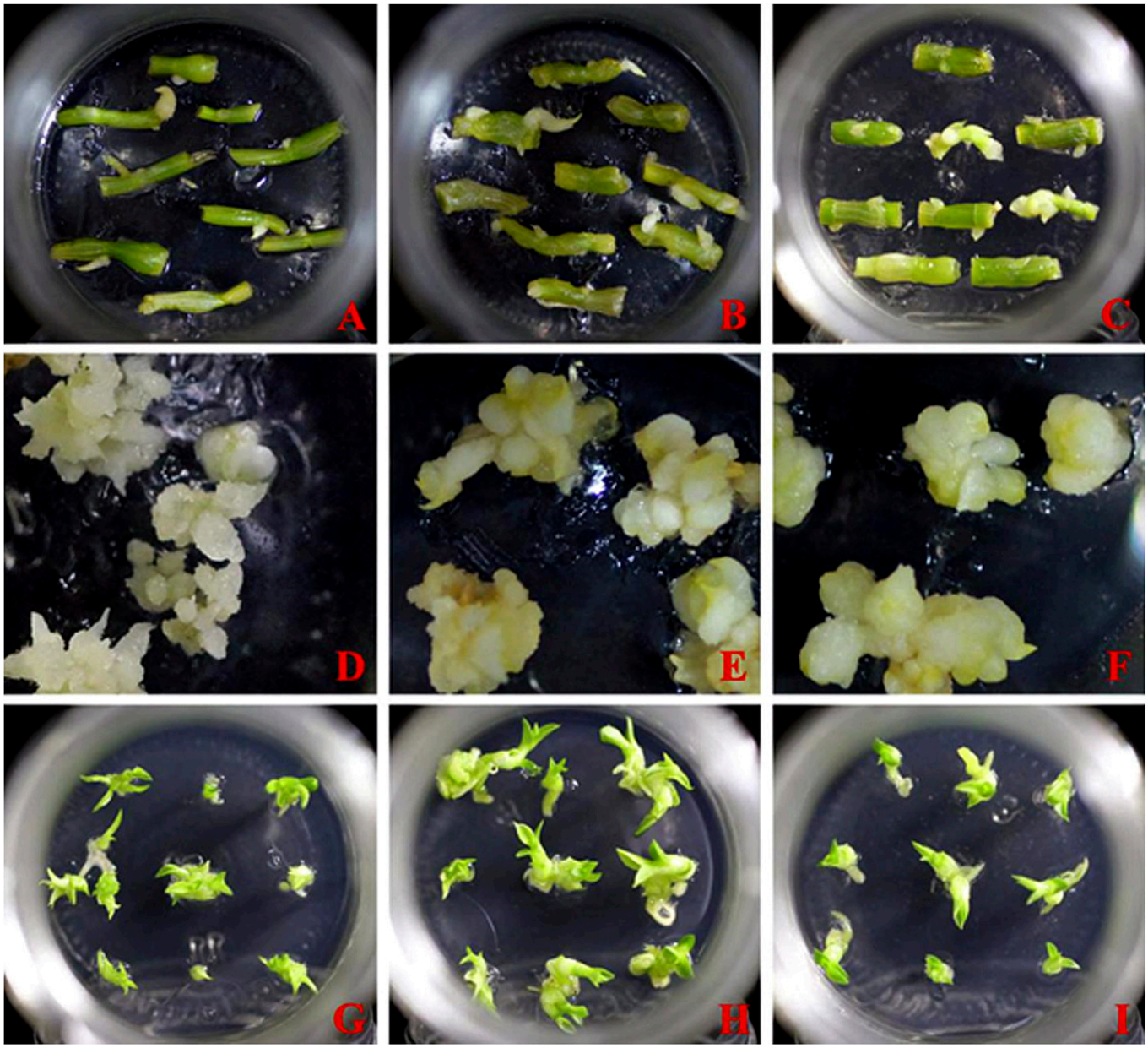

Fig. 3 The induction (**A**–**C**) and proliferation (**D**–**F**) of protocorm-like bodies (PLBs) and plant regeneration (**G**–**I**) from PLBs of the diploid cultivar 201-1 (**A**, **D**, **G**) and tetraploid cultivars 201-1-T_1 (**B**, **E**, **H**) and 201-1-T_2 (**C**, **F**, **I**) of *D. officinale.*

in ice water for 5 min, the solution was transferred to the cuvette to measure the absorbance with an ultraviolet spectrophotometer. The content of polysaccharides was calculated as glucose in the solution. This experiment was repeated three times.

Statistical analysis. Collected data were subjected to an analysis of variance (ANOVA) using SPSS 13.0 for Windows (SPSS, Chicago, IL). When significant differences occurred, means were separated using Duncan's multiple range test with $P < 0.05$ considered significant.

Results

Morphological changes. Two tetraploid '201-1-T_1' and '201-1-T_2' exhibited marked differences in several morphological characteristics compared to diploid '201-1' (Table 1). Leaf, stem, and root lengths of the tetraploid cultivars were significantly shorter than that of the diploid cultivar (Fig. 2). The stem and root diameters were much greater than those of the diploid. However, leaf number, leaf width, and root number, were similar among the three cultivars. As a result, fresh weights of the tetraploids were 2-fold higher than those of the diploid (Table 1). The length and width of stomata cells as well as the stomatal area of tetraploid plants were much larger than those of the diploid cultivar, whereas the stomatal density significantly decreased in tetraploid plants (Table 1, Fig. 2).

Differences in PLB induction, proliferation, and regeneration. Significant differences in the induction of PLBs were observed between diploid and tetraploid cultivars. The time required to induce PLBs in the diploid was shorter than that of the tetraploids. The PLB induction rates for tetraploids were 56.03% and 55.75% compared with 40.18% for the diploid (Table 2, Fig. 3A–C). However, there were no significant differences between the two tetraploid lines regarding the PLB induction time and rate.

The proliferation of PLBs among the three cultivars varied significantly. The mass of PLBs produced by the diploid was less compared with that of the two tetraploid cultivars (Fig. 3D–F). The proliferation coefficients of the tetraploid PLBs were significantly higher than that of the diploid: up to 3.69 and 3.03 for the tetraploids compared with 2.31 for the diploid (Fig. 4). Furthermore, tetraploid '201-1-T_1' exhibited a higher PLB proliferation rate than tetraploid '201-1-T_2'.

Shoot regeneration from PLBs were also different between the diploid and tetraploids (Table 3, Fig. 3G–I). Shoot initiation took ≈14 d in the diploid but ≈16 d in the tetraploids (Table 3). The mean number of shoots regenerated from diploid PLBs was significantly higher than that regenerated from tetraploid PLBs, and the time of root initiation in the diploid occurred much earlier than that in the tetraploids.

Differences in polysaccharide content. The polysaccharide content in stems was much greater than that in leaves, and the content in leaves was higher than that in roots, regardless of ploidy levels. Polysaccharide contents in stems, leaves, and roots of tetraploid cultivars were significantly higher than those in the diploid (Table 4). The contents of polysaccharide in stems, leaves, and roots of '201-1-T_1' were 2.04-fold, 2.81-fold, and 2.22-fold higher than those in the diploid cultivar. Similarly, polysaccharide contents in stems, leaves, and roots of '201-1-T_2' were 1.61-fold, 2.74-fold, and 3.49-fold higher than those in the diploid line. Additionally, the polysaccharide contents of the two tetraploids were different. In roots, the polysaccharide content of '201-1-T_2' (2.41%) was significantly higher than that of '201-1-T_1' (1.53%); however, in stems, the polysaccharide content of '201-1-T_2' (10.03%) was lower than that of '201-1-T_1' (12.70%).

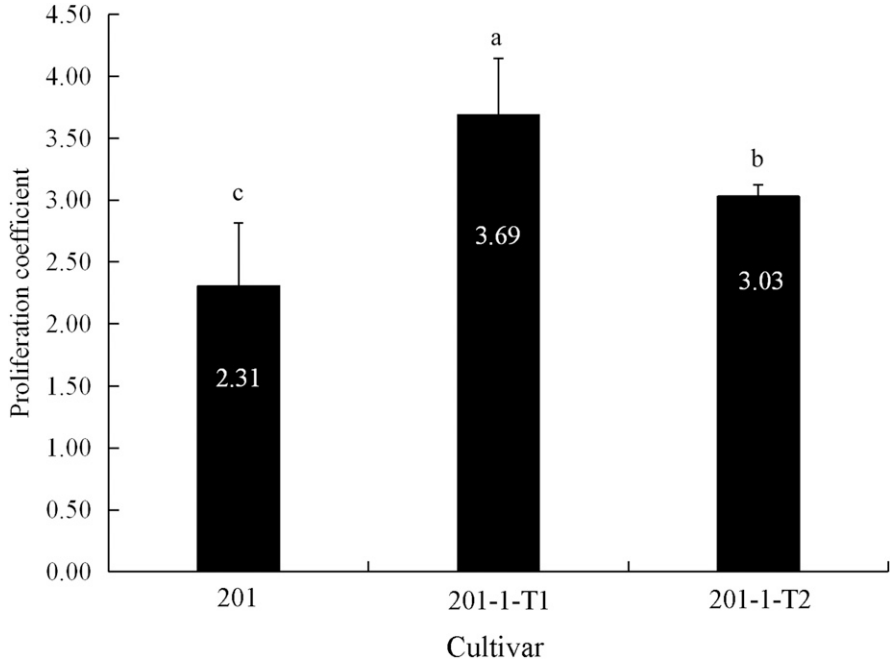

Fig. 4. The proliferation coefficient of the diploid cultivar 201-1 and tetraploid cultivars 201-1-T_1 and 201-1-T_2 of *D. officinale*. Bars represent SE, and letters above the bars indicate significant difference at $P \leq 0.05$ based on Duncan's multiple range test.

Discussion

Dendrobium officinale is an important ornamental flowering plant worldwide. It is also a famous traditional Chinese medicinal plant. With the increasing recognition of its nutraceutical and pharmaceutical values, production of *D. officinale* in China increased rapidly from 3900 ha in 2014 to 7066 ha in 2016 (Cheng et al., 2019). The increased production acreage was also accompanied by high demand for new cultivars that can produce higher biomass with increased polysaccharide contents. Our research of *D. officinale* developed as a series of cultivars with different ploidy levels. The present study tested our hypothesis that gene abundance due to chromosome doubling could increase biomass production and polysaccharide contents. The results showed that tetraploid cultivars produced significantly higher fresh weights compared with the diploid cultivar. Polyploidization also changed the regeneration ability of tetraploid cultivars. The polysaccharide contents in stems, leaves, and roots of 6-month-old tetraploid plantlets were either nearly doubled or more than doubled in comparison with that of the diploid parental cultivar. Our study showed that chromosomal doubling is a viable way of

Table 3. Shoot regeneration and root formation from protocorm-like bodies (PLBs) of diploid and tetraploid cultivars of *D. officinale*.[z]

Cultivar	Day of initial shoot appearance	Mean number of shoots per PLB	Day of initial root appearance	Mean no. of roots per plantlet
201-1	13.90 ± 0.46 a[y]	2.90 ± 0.43 a	28.90 ± 0.67 b	0.90 ± 0.23 a
201-1-T_1	16.50 ± 0.34 b	1.70 ± 0.15 b	34.30 ± 0.58 a	0.70 ± 0.21 a
201-1-T_2	15.80 ± 0.33 b	1.20 ± 0.13 b	33.50 ± 0.34 a	0.65 ± 0.13 a

[z]All data represent means ± SE (n = 3).
[y]Different letters within the same column indicate significant difference at $P \leq 0.05$ level based on Duncan's multiple range test.

Table 4. Polysaccharide contents in diploid and tetraploid plantlets of *D. officinale*.[z]

Cultivar	Stems (%)	Leaves (%)	Roots (%)
201-1	6.22 ± 0.05 c[y]	1.59 ± 0.12 b	0.69 ± 0.08 c
201-1-T_1	12.70 ± 0.19 a	4.47 ± 0.14 a	1.53 ± 0.07 b
201-1-T_2	10.03 ± 0.14 b	4.37 ± 0.15 a	2.41 ± 0.12 a

[z]All data represent means ± SE (n = 3).
[y]Different letters within the same column indicate a significant difference at $P \leq 0.05$ based on Duncan's multiple range test.

improving biomass and polysaccharide production in *D. officinale*.

Chromosome doubling can lead to various morphological and anatomical changes (Corneillie et al., 2018; Sattler et al., 2016; Tantasawat et al., 2012; Teixeira da Silva et al., 2014a). In the present study, tetraploid plantlets had shorter leaf, stem, and root lengths and thicker stem and root diameters as well as increased fresh weights compared with the diploid, representing a phenomenon called the Gigas effect (Knight and Beaulieu, 2008); in other words, chromosome doubling generally causes increased organ size (Osborn et al., 2003). Therefore, tetraploid plants had significantly higher biomass production (Corrêa et al., 2016; Hannweg et al., 2016). Additionally, stomatal size was significantly increased but stomatal density was decreased in tetraploid plants. It has been reported that the number of guard cells per unit area decreased in accordance with the increased level of polyploidy (De Oliveira et al., 2004). Some early studies considered that the lower frequency of stomata in tetraploids was probably due to the larger epidermal and guard cells (Gantait et al., 2011; Mishra. 1997) as well as reduced stomata differentiation (Tu et al., 2018). This phenomenon has also been documented for *Salvia miltiorrhiza* (Gao et al., 1996), *Agastache foeniculum* (Talebi et al., 2017), and *Anoectochilus formosanus* (Chung et al., 2017).

The current study also showed that chromosome doubling changed the regeneration ability through PLBs. The time for PLB initiation from tetraploid stem nodes was longer than that for the diploid. However, the induction rate was higher compared with that of the diploid. Polyploidization can lead to an increased number of internodes (Vichiato et al., 2014). PLBs are generally induced from the nodes of stems. We believe that the increased PLB induction rate could be due to the increased stem nodes in tetraploid plantlets. As a result, the proliferation coefficients of tetraploid PLBs were higher compared with that of the diploid, which was similar to the results of Xie et al. (2017), who indicated that the proliferation rates of tetraploid PLBs were higher than those of triploids and diploids in *Cymbidium hybridum*. This is likely related to the increased cell size of tetraploid plantlets. The time required for shoot appearance from PLBs increased, but the number of shoots produced per PLB piece was reduced in tetraploid cultivars. These results concurred with the results of Sun et al. (2011), who indicated that the polyploid in *Pyrus communis* was accompanied by reduced in vitro shoot and root organogenesis. The failure of completing cytokinesis (Øvrebø and Edgar, 2018) or difficulty progressing through normal mitosis (Comai, 2005) can result in decreased in vitro organogenesis polyploids. Additionally, chromosome doubling causes chromosomal rearrangements, inversions, translocations, and even loss or retention of the duplicated genes (Blanc and Wolfe, 2004). The rearrangement might directly or indirectly affect regeneration ability (Colijn-Hooymans et al., 1994; Kubalakova et al., 1996; Lin et al., 2001).

In vitro polyploidization has a significant role in boosting vigor and metabolite contents in medical plants (Salma et al., 2017), such as overaccumulated shihunidine content in tetraploid PLBs of *Dendrobium* hybrid (Grosso et al., 2018), higher polysaccharide and phenolic compounds in tetraploid pseudobulbs of *Bletilla striata* (Li et al., 2018b), richer stevioside in mixoploids leaves of *Stevia rebaudiana* (Hegde et al., 2015), and superior baicalin content in tetraploid roots of *Scutellaria baicalensis* (Gao et al., 2002). Polysaccharide is the major active component in *D. officinale* (Song et al., 2016), and it has an important role in healing some diseases (Luo et al., 2017; Xie et al., 2016). In this study, tetraploid plantlets produced significantly higher polysaccharide contents in leaves, stems, and roots than diploid plantlets. The results suggested that young plantlets produce abundant polysaccharide, as documented by Yuan et al. (2017), and that the polysaccharide contents in tetraploid plantlets were much higher than those in diploid parental plantlets. Similar results were also reported by Jiang et al. (2014). The increased polysaccharide may be partly attributable to the increased cell size and, thus, thicker leaves and roots of tetraploid plantlets, as mentioned by Hegde et al. (2015). The overexpression of genes following chromosome doubling can also enhance secondary metabolites (Comai, 2005; Majdi et al., 2014; Yun-Soo et al., 2004). Recently, Corneillie et al. (2018) reported that polyploidization in *Arabidopsis thaliana* was associated with a proportional reduction in secondary cell wall deposition, such as lignin and cellulose, and concomitantly increased other cell wall polysaccharides. Whether the cell composition changes have contributed to the increased polysaccharides in the tetraploids '201-1-T_1' and '201-1-T_2' requires further investigation. Nevertheless, our study demonstrated that chromosome doubling represents a workable approach to increasing biomass and polysaccharide production in *D. officinale*, and its application could potentially increase the supply of *D. officinale* materials for commercial use.

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