



Article

Flowering Time and Physiological Reaction of *Dendrobium nobile* Lindl in Response to TDZ Application

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Abstract: The objective of this work was to analyze the effect of Thidiazuron (TDZ) treatment on floral initiation, flowering time, ornamental characteristics and physiological metabolism of potted *Dendrobium nobile*. Three TDZ concentrations (200, 500 and 1000 mg L⁻¹) were applied as solution to water the root zone of the plants. Control plants (plants watered with water) showed a good vegetative development but no floral branches. TDZ greatly influenced the flowering process. For all the tested TDZ concentrations, the first flower bud occurred at 55–60 days after the last irrigation (DAI), the highest TDZ concentration showing the major delay in its occurrence. The initial flowering (30% of flowered plants) began 47 days after the first flower bud initiation with no statistical differences among the treatments. Plants treated with TDZ 500–1000 mg L⁻¹ showed the longest period of flowering (about 32 days) and the single flowers delayed the withering of about 2–3 days compared to the lowest TDZ treatment (200 mg L⁻¹). The number of flowers, floral branches and flowering percentage were distinctly influenced by the TDZ concentration. The highest percentage of flowering (40%) was scored when plants were watered with a TDZ solution at 500 mg L⁻¹ and this was a performant treatment providing the best morphological flower features for the ornamental value of this plant. Among the physiological factors affecting the flowering, this study showed that TDZ increased the relative membrane permeability which facilitated the transport of macromolecular flower-forming substances into and out of the membrane. Therefore, the membrane permeability change could be an indicator of shifts in physiologically active substances during the flowering transition process in *Dendrobium nobile* plants.

Keywords: *Dendrobium nobile*; TDZ; flower bud formation; early flowering; physiological metabolism



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1. Introduction

The regulation of flowering time is an important issue to guarantee the year-round production of ornamental crops and to enable the flower synchronization of cross-parents for successful fertilization in a crossbreeding protocol. In addition, flower initiation is one of the critical life-history phases for ornamental plants. Generally, the environmental response and endogenous pathways stimulate flower bud formation to ensure successful reproduction [1,2].

Endogenous pathways, including the genes of flowering initiation, hormones, carbohydrate levels, miRNA and floral competence, form an integrated network to regulate the flowering time [3–7]. Plant growth regulators (PGR) have been proven to act as exogenous hormones and therefore they are widely used to interfere with floral initiation in ornamental crops [8]. Thidiazuron (TDZ), which is one of the phenylurea derivatives considered as the most active cytokinin-like substance, acts as a multifunctional PGR in regulating floral induction, explant regeneration and dormancy breaking, as well as the leaf postharvest senescence process [9–11].

TDZ successfully induced the in vitro florigens of soybean, *Pennisetum glaucum*, and contributed to early flowering by shortening the required time at the vegetative growth stage [10,12]. Normally, TDZ addition to Murashige and Skoog Medium (MS media) can stimulate explant in vitro regeneration, resulting in adventitious shoots, callus production and protocorm-like body (PLB) proliferation [9,13–15].

As a preservative ingredient, 100 μM TDZ applied as a foliar spray at the preharvest stage or added in post-harvest solution significantly prolonged the vase life of *Dianthus caryophyllus* [16], *Gladiolus grandiflora* [11] and *Calendula officinalis* [17] by enhancing solution uptake, superoxide dismutase (SOD) activity and membrane stability index (MSI). Additionally, TDZ could inhibit carotenoid degradation, flower abscission and leaf senescence [18,19].

Genus *Dendrobium*, consisting of approximately 1000 species around the world, is mainly distributed in tropical and subtropical areas. China has more than 100 wild species of *Dendrobium*, with the southwest region being particularly rich; many species have high ornamental value [20]. *D. nobile* is one of the appreciated Chinese medicinal plants but it is used in breeding programs to obtain hybrids of *D. nobile* 'Spring Dream'.

Previous studies indicated that *Dendrobium* flowering could be influenced by TDZ directly through in vivo application or indirectly by influencing the flowering of the ex vitro plantlets when applied during the in vitro culture. Wen et al. (2013) proved that TDZ could promote the flowering of *D. nobile* by affecting the transcriptions of some transcription factors and signal genes [21]. Supplementing TDZ to the MS medium successfully induced the early flowering in ex vitro plantlets of *D. capra* and *D. nobile* [22,23]. The tissue-cultured plantlets of *D. officinale* produced a high percentage of inflorescences (83.2%) and normal flowers (73.6%) provided that the in vitro culture was carried out for 9 weeks on MS medium supplemented with TDZ 0.1 mg L^{-1} [24]. Some studies indicated that TDZ can further increase the frequency of floral bud formation when used in combination with other PGRs [10,23]. It was reported that TDZ combined with auxins such as 1-naphthylacetic acid (NAA) and Paclobutrazol (PP₃₃₃) efficiently improved floral buds compared to its single application [22]. An optimal in vitro protocol to induce a high rate of flower buds and blossomed flowers in tissue-cultured plants of *D. officinale* indicated the use of a combination of TDZ with other PGRs (0.3 mg L^{-1} PP₃₃₃ + 0.5 mg L^{-1} 6-Benzylaminopurine (6-BA) + 0.5 mg L^{-1} NAA + 0.06 mg L^{-1} TDZ) [25]. Foliar spraying and localized irrigation at root level are appropriate methods for the in vivo application. TDZ (30 mg L^{-1}) when applied as foliar spray and through root zone watering was beneficial in inducing floral bud formation but decreased the flower size and shortened the flowering duration in potted plants of *Dendrobium* 'Sunya Sunshine' [26]. Generally, the beneficial effect of TDZ on *Dendrobium* flowering is related to several factors such as the species, application method, the concentration of this growth regulator and if it is used alone or in combination with other PGRs.

The flowering switch in higher plants is profoundly affected by various pathways. Usually, the plant hormone status is a traditional way to check the effects of the growth regulator. Additionally, endogenous carbohydrate levels and some autonomous pathways can modulate the process of floral initiation. There is little information available for TDZ effects on floral induction and the physiological metabolism. Current evidence suggests that carbohydrate and chlorophyll metabolisms involve the molecular regulatory mechanism of floral initiation [24,27,28]. Generally, the transport of macromolecular flower-forming substances in and out of the membrane could lead to the membrane permeability change.

The objective of this research was to analyze the effect of three concentrations of TDZ applied via root irrigation on the floral morphogenesis and flowering time of *D. nobile*. In particular, the flowering features and the physiological metabolism in response to TDZ rates were studied.

2. Materials and Methods

2.1. Plant Material and Experimental Conditions

The work was carried out in the greenhouses of the Southwest Forestry University Department of Horticulture and Landscape Architecture, Kunming, Yunnan, China.

The experimental plant material was set with 2–3-year-old seedlings of *D. nobile* obtained through in vivo division. Attention was paid to select homogeneous seedlings (3–4 branches, 22–25 cm height) which were transplanted into pots (diameter: 18 cm) filled with a commercial potting mix, composed of 50% coconut fiber and 50% tree bark (NATURAL COCONUT FIBER, China). From May to September 2020, seedlings were cultivated in the greenhouse according to standard cultivation practices (T/HCHMA 0002-2021 Technical regulation of *Dendrobium nobile*) and maintained under a 65% polywoven shading net. Then, the plants were moved to the incubator at 25 °C/12 °C (day/night) temperature with a daily light intensity of 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and 80% relative air humidity (HR). After three months under these conditions, the experiment started.

The plant growth regulator TDZ (Shanghai EKEAR Biotechnology Limited, Shanghai, China) was applied at concentrations of 200, 500 and 1000 mg L^{-1} ; plants were watered with 220 mL solution, volume which was considered to saturate the water content of the culture substrate. Plants watered with 220 mL water were used as the control. All samples were subjected to initial watering on 27 December 2020 when the soil water potential dropped at a threshold value equal to 40–50% of saturated water content. Plants were watered every 15 days and were completely irrigated three times during the whole process.

The experimental unit consisted of 8 uniform plants and each treatment had three replicates.

2.2. Flowering and Morphological Characterization of Flowers

Plants were monitored from December 2020 to April 2021 and the morphological observations on floral differentiation and formation were carried out after the last watering. The number of branches and the flowering characteristics were observed in each experimental unit and recorded as number of flower spikes, number of inflorescences per branch, number of flowers per inflorescence and total number of flowers. Early flower bud initiation was recorded and the flowering process was followed from the first flowering opening to the fading flowering.

When plants reached the full bloom, the flower morphology was assessed through the length of flower inflorescence, the length and width of the petal, middle sepal and lateral sepal and the lip height.

2.3. Physiological Characteristics Measurements

Three physiological parameters were examined at 13, 23, 33 and 102 days after the last irrigation (DAI), corresponding to the different stages of transition from vegetative growth to reproductive growth. More precisely, 13, 23, 33 and 102 DAI represented the time related to vegetative bud stage (stage I), transitional stage (stage II), inflorescence development stage (stage III) and bloom stage (stage IV), respectively.

The relative membrane permeability was assessed indirectly by detecting electrolyte leakage or conductivity of the leaf tissues according to the method described by Sairam, 1994 [27]. The leaf tissue (500 mg) was picked and placed in 20 mL double-distilled water (DDW) in two separate tubes. The first was incubated at 25 °C for 30 min and its conductivity was recorded as C1. The second tube was incubated at 100 °C for 20 min and its conductivity was designated as C2. The conductivity value was measured using an Elico DDS-307 Conductivity meter (INESA Scientific instrument Co., Ltd., Shanghai, China). Membrane stability index (MSI) was determined by a ratio of C1 to C2, expressed in percent.

The relative chlorophyll content (%) was determined using a chlorophyll meter (SPAD-502, Konica Minolta, Japan) by detecting the optical concentration difference at two wavelengths between 650 nm and 940 nm. SPAD value is widely used to stand for the relative amount of chlorophyll in vivo. The fully expanded leaf from the upper one-third of the

canopy was considered for the measurement. Three samples were randomly chosen for measurements and 10 leaves were tested for each treatment. The chlorophyll relative content was measured with SPAD (SPAD-502, Konica Minolta, Japan) meter from 11.00 a.m. to 13.00 p.m. at the three above said times (13, 23, 33 DAI) and the additional time (102 DAI) corresponding to the reproductive bloom stage. The measurement of each clean leaf was repeated three times.

The soluble sugars were estimated by the anthrone method with glucose as the standard [28]. The leaf was sampled from the upper branch. Approximately, 10 g of tested and fresh leaves was milled to a fine powder and dried. Each sample was obtained in three portions, and put into graduated Pyrex tubes (150 mm × 25 mm); 5 mL distilled water was added and sealed with sealing film. Then, it was extracted in boiling water for 30 min (twice); the extract was filtered into a 25 mL flask and the tubes and residue were rinsed repeatedly to fix the volume on the scale. The anthrone reagent (5 mL) was pipetted into Pyrex tubes and frozen in ice water. A 1 mL solution was layered on the acid, cooled for 5 min and then thoroughly mixed. Afterwards, it was incubated in boiling water and then cooled in water for 5 min. Absorbance was measured at 620 nm with a UD751GD spectrophotometer (INESA Scientific instrument Co., Ltd., Shanghai, China) using blank as a reference, and the standard curve was plotted with standard solution.

2.4. Data Collection and Statistical Analysis

The data collection started after the last irrigation. An axillary bud was considered to be present when the bud reached 0.20 cm diameter. The time to reach initial flowering and full bloom was assumed to occur when the percentage of blooming plants reached 30% and 60%, respectively. Fading flowering was considered when 80% of the flowers withered. The bloom duration referred to the days from the initial flowering to the fading stage. The duration of a single flower was assessed by the average flowering period of 10 flowers from each treatment.

The influence of TDZ was assessed by evaluating 24 plants used as the population for the observation on the number of flowers/plant, number of flowers/inflorescence, number of inflorescences/branch, number of floral branches/plant and number of branches/plant. Flowering percentage for each treatment was calculated according to the formula: (Total number of floral branches/Total number of branches) × 100. A floral bud of *D. nobile* usually produces an inflorescence. The number of flowers per inflorescence was determined by the number of flowers emerging from each flower bud.

Measurements on flower morphological characteristics were performed on 10 flowers randomly selected from each treatment. The length of flower inflorescence, the length and width of the petal, middle sepal and lateral sepal and the height of the lip were examined with a vernier caliper.

The leaves for the physiological test were obtained from the upper branch [29]. Three representative plants were randomly chosen for measurements in each treatment and measurements for each plant were repeated three times repetitively from December 2020 to April 2021.

The significance of TDZ rates on each growth index and physiological parameter was assessed by one-way analysis of variance (ANOVA) using EXCEL (Microsoft Company, Albuquerque, NM, USA). In the case of significance tests of differences, data were analyzed using an ANOVA and Fisher's LSD test at a significance level of 5% ($p = 0.05$).

3. Results

3.1. The Influence of TDZ on Flowering

Plants treated with TDZ showed a consistent flowering increase (Table 1). When plants were watered only with water (control), no flowering was observed although plants showed a vigorous vegetative growth. TDZ increased the number of stems bearing flowers, the lower TDZ concentrations (200 and 500 mg L⁻¹) being more effective.

Table 1. Influence of TDZ treatments on vegetative growth and flowering of *Dendrobium nobile* plants watered at root level with TDZ solution. The percentage of flowering was calculated according to the formula: (Floral branches per plant/Branches per plant) \times 100. Data were collected after the last irrigation. For each variable, values followed by the same small letter are not significantly different at $p = 0.05$.

TDZ Level (mg L ⁻¹)	Number					Percentage of Flowering (%)
	Flowers/Plant	Flowers/ Inflorescence	Inflorescences/ Branch	Floral Branches/Plant	Branches/Plant	
0	-	-	-	-	92 \pm 0.81 a	0.00 d
200	68 \pm 0.77 b	2.60 \pm 0.44 a	1.60 \pm 0.38 a	24 \pm 0.52 b	90 \pm 0.81 a	26.84 \pm 6.21 b
500	97 \pm 1.01 a	1.50 \pm 0.33 a	2.70 \pm 0.48 a	38 \pm 0.62 a	95 \pm 1.05 a	40.38 \pm 7.41 a
1000	52 \pm 0.74 c	1.60 \pm 0.34 a	1.70 \pm 0.37 a	17 \pm 0.40 c	87 \pm 0.87 a	19.66 \pm 4.89 c
LSD _{0.05}	6.58 *	1.34	1.60	2.60 *	7.45	3.78 *

LSD_{0.05} means the least squared difference at the $p \leq 0.05$. * means within the same column followed by different lowercase letters are significantly different at 0.05 level of single-factor ANOVA.

Nevertheless, the number of flowers per inflorescence and the number of inflorescences per branch showed no statistical significance for all the tested TDZ concentrations (Table 1).

Generally speaking, 55–60 days were necessary from the last irrigation with TDZ (DAI = days after the last irrigation) to notice the first appearance of the flower buds with significant differences among the TDZ treatments (Table 2). The time span from flower bud appearance to the onset of flowering was the same for all TDZ concentrations tested (47 d) as well as the time required to reach the full bloom was similar for all the TDZ treatments (52–54 d). The highest TDZ concentrations (500 mg L⁻¹ and 1000 mg L⁻¹) significantly retarded the flower withering and prolonged the flowering period, compared to the low TDZ concentration (200 mg L⁻¹) (Table 2). ANOVA showed that TDZ treatment had a significantly positive impact on blooming period of single flowers too.

Table 2. Influence on flowering of *Dendrobium nobile* plants watered at root zone with TDZ solutions. Initial flowering and full bloom were considered when 30% and 60% of flowered plants were observed, respectively. Fading flowering was when 80% of the flowers withered.

TDZ Level (mg L ⁻¹)	Days after the Last Irrigation (DAI)				Flowering Period (No. Days)	Blooming Period of Single Flower (No. Days)
	First Flower Bud Initiation	Initial Flowering	Full Bloom	Fading Flowering		
0	-	-	-	-	-	-
200	55 \pm 0.32 c	102 \pm 0.58 a	109 \pm 0.60 a	128 \pm 0.66 b	27 \pm 0.32 b	20.30 \pm 0.40 c
500	58 \pm 0.39 b	105 \pm 0.75 a	112 \pm 0.66 a	136 \pm 0.78 a	32 \pm 0.40 a	21.70 \pm 0.31 b
1000	60 \pm 0.45 a	107 \pm 0.79 a	112 \pm 0.63 a	138 \pm 0.82 a	32 \pm 0.38 a	23.20 \pm 0.34 a
LSD _{0.05}	1.46 *	4.77	3.64	5.29 *	1.27 *	1.15 *

LSD_{0.05} means the least squared difference at the $p \leq 0.05$. * means within the same column followed by different lowercase letters are significantly different at 0.05 level of single-factor ANOVA.

3.2. The Influence of TDZ on Flower Morphology

Flower morphology of *D. nobile* was greatly influenced by TDZ treatments. Furthermore, the ANOVA analyses revealed that there were significant differences among the three TDZ tested concentrations. Treatment with 500 mg L⁻¹ TDZ had a good inductive effect on the largest size of floral organs, including the length and height of the petal, sepal and lip. Specifically, petal length, middle sepal length and lateral sepal length reached 5.16 cm, 5.12 cm and 4.87 cm, respectively (Figure 1). This TDZ treatment was proven to be the best in enhancing the flower characteristics compared to the other TDZ concentrations (200 and 1000 mg L⁻¹).

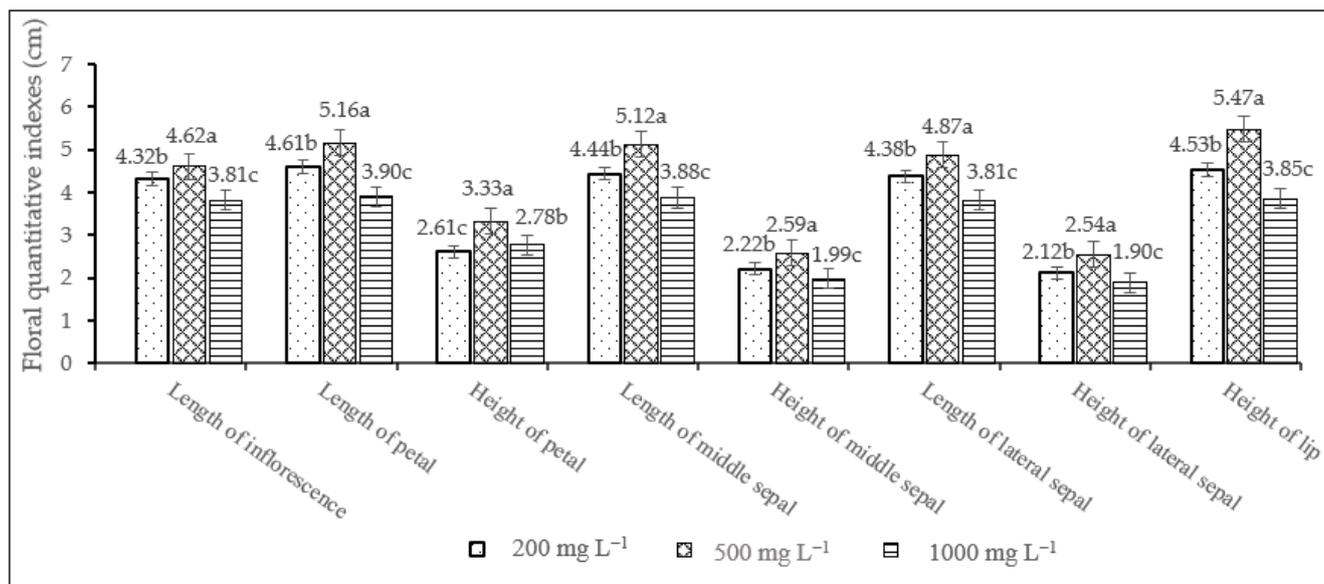


Figure 1. The effect of different TDZ concentrations on eight floral quantitative characteristics of *D. nobile*. $LSD_{0.05}$ means the least squared difference at the $p \leq 0.05$. For each characteristic, different lowercase letters are significantly different at 0.05 level of single-factor ANOVA.

3.3. Effect of TDZ on Soluble Sugar Content during the Flowering Development

According to Zhang et al. (2019), *Dendrobium nobile* plants sprayed with TDZ (30 mg L⁻¹) were able to switch to the flowering phase after one month of the treatment [26]. In our experiment, compared to the control (plants watered only with water), TDZ significantly affects the soluble sugar content of *Dendrobium* at all four developmental stages (Figure 2A). Generally, when TDZ was applied, the soluble sugar content was significantly raised from the vegetative bud stage to the inflorescence development stage. Plants treated with TDZ 200 mg L⁻¹ showed an increase in sugar content over the three developmental stages according to a linear correlation ($y = 1.093x - 6.5557$ $R^2 = 1$); the highest sugar content value (56.18 ± 0.48 mg g⁻¹) was reached at the inflorescence development stage, and afterwards, a significant decrease was observed (25.11 ± 0.31 mg g⁻¹). When 500 mg L⁻¹ TDZ was used, a slight increase in sugar content was scored between the inflorescence development stage (25.10 ± 0.52 mg g⁻¹) and the bloom stage (29.09 ± 0.97 mg g⁻¹). Plants treated with 1000 mg L⁻¹ TDZ showed a significant decrease in sugar content from the inflorescence development stage (40.58 ± 0.67 mg g⁻¹) to the bloom stage (25.33 ± 0.58 mg g⁻¹).

At bloom stage, the sugar content was almost similar in the control plants and in plants treated with the three TDZ concentrations (Figure 2A).

These results suggest that the TDZ treatment on *D. nobile* affected the soluble sugar content already at the lowest TDZ concentration tested (200 mg L⁻¹). A moderate rate (500 mg L⁻¹ TDZ) did not significantly upgrade the soluble sugar at the transitional stage. Taking into consideration the floral characteristics scored when plants were treated with 500 mg L⁻¹ TDZ, it could be argued that at this concentration, no important fluctuation of the soluble sugar content was observed in the critical period from vegetative bud until the inflorescence development stage.

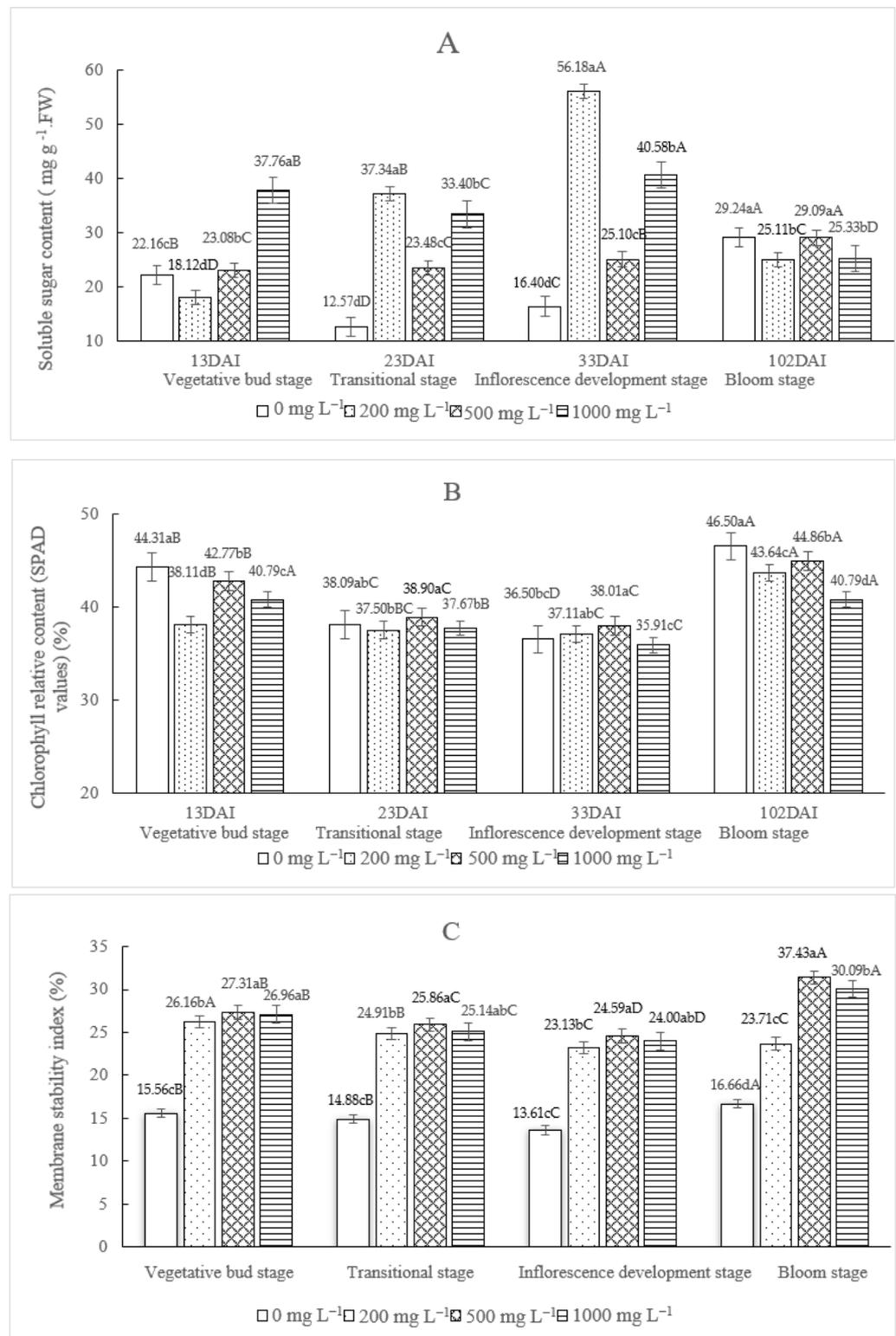


Figure 2. The effect of different concentrations of TDZ on the soluble sugar content (A), chlorophyll content (B) and relative membrane permeability (C) in *Dendrobium nobile*. Plants were kept under a controlled temperature in the incubator (temp: 25 °C/12 °C (day/night), light: 90 $\mu\text{mol m}^{-2} \text{s}^{-1}$, relative air humidity (HR): 80%). For each stage, different lowercase letters indicated significantly different means (one-way ANOVA, $p \leq 0.05$). For each TDZ concentration, different capital letters indicated significantly different means (one-way ANOVA, $p \leq 0.05$).

3.4. Effect of TDZ on Chlorophyll Content during the Flower Development

The chlorophyll relative content (SPAD value) in the control plants showed a significant decrease until the inflorescence development stage; at the bloom stage, a significant increase was observed in both the control and TDZ treatments (Figure 2B). Generally speaking, and also in the TDZ-treated plants, a decrease in the SPAD values was scored during the three developmental stages followed by a significant increase at the bloom stage (Figure 2B). However, in the control plants, a more pronounced decrease could be observed until the inflorescence developmental stage (17.63% decrease in SPAD value in the control compared to 2.62%, 11.13% and 11.96% decrease in SPAD value when 200, 500 and 1000 mg L⁻¹ TDZ was applied). At blooming stage, the leaf chlorophyll relative content in the control and the treated plants was not significantly different (Figure 2B).

3.5. The Change in Relative Membrane Permeability

Relative membrane permeability increased significantly under the three TDZ concentrations and it was significantly higher than the control at all the developmental stages (Figure 2C). Generally, the MSI% slightly decreased over the three stages until the bloom stage; at the bloom stage, a significant increase in MSI% was scored both for the control and the three TDZ applications; in the case of TDZ 200 mg L⁻¹, the MSI% was stable between the inflorescence development stage and the bloom stage. Looking at the bloom stage, it is interesting to highlight that the MSI% significantly increased from the control until the 500 mg L⁻¹ TDZ treatment. At this TDZ concentration, the highest values were scored and a consequent significant decrease was then observed at 1000 mg L⁻¹ TDZ.

4. Discussion

Flowering is a critical event which implies the transition from the vegetative to the reproductive phase. This process is influenced by several environmental and endogenous signals [30]. Among them, the effects of phytohormones on *orchid* flowering have been studied [31]. TDZ applied as the only growth regulator during the in vitro propagation of *D. capra* and *D. nobile* caused a low rate of flower bud formation in the ex vitro plantlets [10,22]. Some researchers considered that a combination of various hormones was more effective to induce the flower transition in *Dendrobium*. An inflorescence induction of 100% was observed when microshoots of *D. wangliangii* were cultured on 1/2 MS medium with 2 mg L⁻¹ TDZ and 0.5 mg L⁻¹ NAA [32]. Besides NAA, paclobutrazol (PP₃₃₃) was found to be beneficial for a high frequency of floral induction. Wang et al. (2014) proved that a combination of 0.05–0.1 mg L⁻¹ TDZ and 1.0 mg L⁻¹ NAA could produce more than 50% floral bud induction and 84% normal flowers in *D. officinale* [23]. Similarly, they could successfully induce the early flowering if the plantlets of *D. capra* were in vitro cultured in MS medium containing TDZ and NAA over an 11-month period [22]. High TDZ rates with a warm temperature (exceeding 25 °C) usually led to flower deformation.

When the in vivo irrigation technique was applied, the TDZ effect could be influenced mostly by the watering method and cultivation environment, such as substrates, air temperature and humidity. Sphagna moss and coconut shell could achieve a relatively better water holding capacity than pine bark. *D. 'Sunya Sunshine'* treated with 30–120 mg L⁻¹ TDZ in a combination of foliar spaying and root irrigation exhibited no significant difference in floral induction rate, which was more than 80% in all treatments. Compared with the control group, the flower number per inflorescence and flower size decreased and the flowering duration was shortened [26]. Nevertheless, the floral bud formation percentage in nodes of *D. wardianum* was 84.3% after in vivo spraying twice with 200 mg L⁻¹ Paclobutrazol (PP₃₃₃) and 200 mg L⁻¹ TDZ mixed solution in vivo [33]. It was presumed that the species greatly determined the TDZ concentration response. Undoubtedly, an appropriate rate of TDZ would enhance the *Dendrobium* flower traits and the early flowering. Our research found that a moderate rate of TDZ (500 mg L⁻¹) could induce a high percentage of flower formation, retard the flower withering and prolong the flowering period. Moreover, TDZ

significantly improved the number of flowers, number of floral branches and the size of floral organs.

Our study showed that TDZ greatly improve the blooming rate of *D. nobile* but, compared to previous results (Zhang et al., 2019), the percentage of flowering (about 20–40% depending on TDZ concentration) was poor [26]. Several reasons could account for that. Firstly, the substrate greatly affected the TDZ effect because the pine bark reduced TDZ adsorption. Secondly, foliar spraying combined with root irrigation, as used in the previous studies, could better enhance the TDZ uptake and utilization with an effect on flowering percentage. The results suggested that an integrated network constructed by different ways of application and the use of several growth regulators could effectively regulate the flowering transition of *Dendrobium*.

However, some studies revealed the adverse effect of TDZ application on abnormal bud appearance [34,35]. No abnormal flowers of *D. nobile* were observed under our experimental conditions. Endogenous regulation involves many crucial chemical signals to modulate the repression and activation of florigen gene expression, such as *FLC* (FLOWERING LOCUS C), *FLD* (FLOWERING LOCUS D) and *FT* (FLOWERING LOCUS T) [36–38]. The previous studies revealed that TDZ upregulated flowering genes' expression [39,40]. In orchids, a low temperature (10 °C) was beneficial for the vernalization of *D. nobile* by upregulating the expression of *DnFT* and, *DnVRN1* expression but decreasing *DnMFT* (*Dendrobium nobile* FLOWERING LOCUS T) and *DnVRN1* (*Dendrobium nobile* VERNALIZATION 1) expression [40,41].

Zheng et al. 2017 reported that *D. spring* could not finish the floral differentiation at 26/21 °C (day/night). A relative low night temperature (≤ 17 °C) was conducive to its floral initiation [42]. Tian et al. 2007 found that its floral differentiation rate could reach 64.74% at 10 °C for 30 d [43]. A continuous temperature of 12 °C at night could enhance the flowering induction and early flowering of *D. nobile* in our experiment, although it produced a relatively low flower formation rate. Further research should be carried out to discuss whether a lower temperature could improve a high rate of floral differentiation in *Dendrobium*.

Carbohydrates are believed to play a crucial role in the regulation of flowering. *TPS1* (TREHALOSE-6-PHOSPHATE SYNTHASE 1) could inhibit its floral initiation regardless of favorably inductive environmental conditions [31]. Seo et al. (2011) proved that the INDETERMINATE DOMAIN transcription factor *AtIDD8* regulated photoperiodic flowering by modulating the sugar transport and metabolism of *Arabidopsis thaliana* [44].

In our experiment, the soluble sugar content (SSC) was correlated with the occurrence of flower buds, whereas there was no distinct fluctuation at the transition stage. The 500 mg L⁻¹ TDZ treatment sustained a stable level of soluble sugar content. We could speculate that during the reproductive development, a sugar signal regulation occurred mainly at the inflorescence differentiation and blooming phase under this average TDZ concentration. An intensive study should be performed to obtain a sequential and dynamic examination of its content based on the precise scheduling of the whole transition phase.

A previous study indicated that chloroplasts worked as essential sensors to cause nuclear transcriptional changes at the developmental transition stage. In *Arabidopsis*, *PTM*, a PHD transcription factor modulating chloroplast retrograde signaling, could mediate the transcriptional repression of *FLC* through the recruitment of *FVE* (a component of the histone deacetylase complex) [45]. In our research, TDZ irrigation decreased in the chlorophyll content in *D. nobile* from the vegetative phase to the inflorescence development stage (13 DAI, 23 DAI and 33 DAI). However, a more pronounced decrease was shown in the control plants. These results seemed to be irrelevant to the flower transition. We speculated that the chloroplast regulatory network might be involved in multiple responses including carbohydrate synthesis and flowering gene expression. Further research is required regarding the chloroplast signaling regulatory mechanism to determine the tradeoff between vegetative growth and reproductive growth.

TDZ used as a preservative solution could improve the membrane integrity of cut flowers [11,17]. In contrast to the control group, TDZ increased the cell membrane permeability and had a positive rate effect. TDZ facilitated the transport of macromolecular flower-forming substances into and out of the membrane. Therefore, the membrane permeability change could be an indicator of shifts in physiologically active substances during the flowering transition process.

5. Conclusions

Investigations addressed to promote flowering in *Dendrobium* are well appreciated due to the long vegetative period scored in these plants. In our experiments, we found that the TDZ applied to water the root zone of *D. nobile* plants was beneficial in affecting the flowering. In fact, the untreated plants were able to show a good vegetative growth but no flowering. The TDZ-treated plants flowered and the highest percentage of flowering (40%) was recorded when this growth regulator was applied at 500 mg L⁻¹. No significant differences were scored in the time frame necessary to reach the initial and full bloom stage when different TDZ concentrations were tested. On the contrary, when 500 mg L⁻¹ TDZ was applied, the important flower features able to enhance the ornamental value, flower number and flowering period were improved. Among the different physiological parameters studied to elucidate the positive effect of TDZ on *D. nobile* flowering, the relative membrane permeability seems to account for a more efficient transport of nutrients and macromolecules during the flowering process and this could explain the best performing flowering.

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