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THE EFFECT OF LOW TEMPERATURE ON GROWTH AND DEVELOPMENT OF *PHALAENOPSIS*

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Abstract

Trials were set to assess low temperature effects on growth and development of *Phalaenopsis*. Different temperature treatment groups were set to maintain an average day/night temperature of 29/21, 26/20, 26/18(℃). *Phalaenopsis* Red Swan, Big Chili and Fuller's Sunset were all coordinated by the cold-induced release of reproductive bud dormancy. The morphological development of flower bud differentiation was similar in Phalaenopsis Big Chili and Sogo Yukidian: flower organ differentiation began with the formation of the outer tepal primordia, followed by the inner tetal primordia and finally, by the lip and column (stamen and carpel) primordia. The earlier the flower bud started differentiation, the earlier the flower bloomed. The inflorescence primordium differentiation started under 26/18 and 26/20 and no flower stalks emerged under 29/21 within 50 d. Different temperature treatments did not affect significantly the length of flower stalk. But temperature played a possible important role in leaf growth. High temperature (29/21) favored vegetative growth than 26/20 and 26/18. It was found ABA and GA_3 levels in apical meristems of stalk increased before flower bud differentiation. High level of IAA decreased when the stalk began rapid elongation (longer than 5cm), and ZR levels at apical meristems were higher than at leaves.

Key words: *Phalaenopsis*; Low temperature; Flower bud differentiation; Growth; Development; Hormone.

Abbreviations: ABA – abscisic acid, IAA–indole-3-acetic acid, ZR–zeatin riboside，GA_3–gibberellic acid

Introduction

Phalaenopsis (*Orchidaceae*) is the most popular cultivated orchid in the world ((Lee *et al*., 2018; Hsu *et al*., 2018; Kwon *et al*., 2017; Kim *et al*., 2015). *Phalaenopsis* production phase can be divided into two phases: a vegetative growth phase and a floral inductive development phase. Quantitative expression of plant growth can accurately represent physiological maturity during different growth phases. Jin (2005) continuously recorded the relative growth rate of the leaves of four cultivars of *Phalaenopsis*. The Plastochron Index (PI) established showed that the leaves grew exponentially (r^2 values between 0.90 and 0.97) and leaves that emerged before the reproductive phase can't be fully assessed by the PI (Jin *et al*., 2005). Thus, there must be some relationship between reproductive growth and vegetative growth.

In China *Phalaenopsis* mainly comes into the market during the Spring Festival. So the timing of flower bud development is crucial in order to produce plants commercially with a controllable anthesis. Temperature is a primary environmental factor affecting growth and development in *Phalaenopsis* (An *et al*., 2013). In commercial cultivation, high temperature is required to inhibit flowering until the *Phalaenopsis* plants can support the flower quality and low temperature promotes flowering (Lee *et al*., 2015). Guo & Lee (2006) found that the day/night temperature of 32/28 and 29/25°C resulted in the highest total net CAM CO_2 fixation in vegetative *Phal.* TS97 plants than higher (33/29°C) and lower temperatures (21/16°C).

It was found that the process of flower bud differentiation in *Phalaenopsis* could be divided into six phases, initial differentiation, inflorescence primordium differentiation, flower primordium differentiation, sepal primordium differentiation, petal primordium differentiation, column and pollinia differentiation (Wei *et al*., 2010). However, the physiological processes taking place in the apical meristems, leaves and roots during the flower bud initiation and development periods have been studied only to a limited extent. Zeng (2008) found that relative high content of endogenous ZR in leaves of *Phalaenopsis* was the key factor of floral bud formation. In straoberry, relatively higher ABA and CTKs levels but relatively lower GAs and IAA levels required during flower bud differentiation were detected (Wan *et al*., 2018). Better understanding of flower development mechanism and physiology is important for florescence regulation.

The objective of the present research was to investigate the influence of cold treatment to growth and development and the changes of endogenous hormone during the flower bud initiation. The results will be very useful in developing management strategies to enhance the flowering of *Phalaenopsis* by controlling temperature or using exogenous application of plant hormones.

Materials and Methods

Uniform *Phalaenopsis* Red Swan, Big Chili Fuller's Sunset and Sogo Yukidian plants with six mature leaves were transplanted into 11.7-cm-diameter plastic pots filled with sphagnum moss as a growing medium. Plants were placed in the greenhouse in Yantai Agricultural Science and Technology Institute, Shandong province, China (37°29′2"N, 121°16′26"E, 6 meters above sea level) under an average day/night temperature of 30/25°C and natural photoperiod. Three treatment groups were set in different rooms of the greenhouse: control check (CK),

treatment group 1 (T1), treatment group 2 (T2). The cooling and shading systems were programmed to maintain an average day/night temperature of 29/21°C, 26/20°C, 26/18°C respectively from 20th Aug. to 20th Sep., 2013. 100 samples in every group of the 3 cultivars were chosen, and the length of every newly emerged leaf and stalk was recorded every day.

Phalaenopsis amabilis Big Chili and Sogo Yukidian were collected every 2d from the time of stalk sprouting (30th Sep.) to the time of flowering. The apical meristems were observed under a stereomicroscope (Olympus E4500, Japan) and recorded using a camera (Olympus E4500) mounted on the stereomicroscope to determine floral stage development on five fresh materials.

Samples of leaves, roots and apical meristems were obtained at different growth and development stages of the three cultivars in T2: normal temperature treatment (NTT), low temperature treatment (LTT), inflorescence primordium differentiation (IPD), 5cm stalk (5S), 10 cm stalk (10S), 25 cm stalk (25S), 45 cm stalk (45S), flower bud (FB), early anthesis (EA) and middle anthesis (MA). Five plants were sampled to analyze the distribution of the endogenous hormones ABA, IAA, zeatin riboside (ZR) and GA_3 on flower bud differentiation and development. After freezing in liquid nitrogen for 30 min, the sample was stored in the refrigerator (-20°C). After adding 6 ml extracting solution (80% methanol, containing 1mmol/L 2-tert-Butyl-4-methylphenol), 1g plant sample was ground and stored at 4°C for 4 h to extract the hormones. Resultant extraction was centrifuged (8 min, 3500 revolution per minute). The supernatant was taken out, and 1 ml extracting solution was added into the precipitate. After extracting for 1h at 4°C, the supernatant was centrifuged again and the supernatants were merged together. The supernatant was separated by C-18 solid phase extraction column, transferred into a 5 mL centrifuge tube, dried by nitrogen, eliminated methanol from extractions and diluted into a constant volume by 2 ml sample diluents, in which there was 1ml/L tween-20, 1g/L gelatin and PBS (Phosphate Buffer solution: 8g/L NaCl, 0.2 g/L KH_2PH_4, 2.96 g/L $Na_2HPO_4.12H_2O$, pH=7.5). The levels of ABA, IAA, ZR and GA_3 were determined by Enzyme-linked Immunosorbent Assays (ELISA) (He *et al*., 2009; Zhang *et al*., 2011).The ELISA kit was supported by China Agricultural University. The optical density was measured at 490nm on the ELISA analytical instrument (SPECTRAmaxPLUS384), the standard curves were draw and the levels of ABA, IAA, ZR and GA3 were calculated respectively. The experiment was repeated three times and the average value was taken for statistical analysis.

Results and Discussion

Morphological development of flower: The morphological development of flower bud differentiation in *Phalaenopsis* cultivars was non-synchronized but the sequence was generally similar. In the UD phase (Fig. 1E), the apical meristem was surrounded by bract primordia. There was no dormant bud under the first bract and the flower bud initiation started under the 6th or 7th bract (Fig. 3), and the buds in the middle of the first bract and the flower bud were all dormant buds. The flower bud initiation started at different time in different cultivars, for example, the stalk length of Big Chili was 5cm while Sogo Yukidian was 7.5cm. After the differentiation, the dormant flower bud, which took the form of a broad, low dome, was changed into the flower meristem, marking the onset of reproductive growth (Fig. 1A, F). Floral organogenesis began with the initiation of 5 petals which (Fig. 1) would develop into out tepals and inner tepals (Fig. 2A, B, F, G). PD phase started when the length of stalk was 7.5cm (Big Chili) and 15cm (Sogo Yukidian) (Fig. 1B,G). Primordia of the column (Fig. 2D, I) became visible (Fig. 1C, H) when the stalk was 15cm in length. The morphological development of flower bud differentiation in *Phalaenopsis* cultivars was non-synchronized but the sequence was generally similar: flower organ differentiation began with the formation of the outer tepal primordia, followed by the inner tepal primordia and finally, by the lip and column (stamen and carpel) primordia.

The flower stalks in ‘Red Swan’ emerged first, then in ‘Fuller’s sunset’, and in ‘Big Chili’ emerged last. The earlier the flower bud started differentiation, the earlier the flower bloomed. No flower stalks emerged within 50d under 21°C (CK) at night. All the treatments (T1 and T2) of the three cultivars displayed the stalks grew as the time went on. The flower stalk in ‘Red Swan’ was the longest, while in ‘Fuller’s Sunset’ was the shortest. No significant differences existed between T1 and T2 in the same cultivar (Fig. 4). This indicated that different temperature treatments did not affect the length of flower stalk.

All the treatments displayed shorter length of leaves than the control (CK) (Fig. 5). The control was significantly higher than the treatments, but there were no significant differences among the treatments themselves, indicating that the temperature played a possible important role in leaf growth. The temperature in CK (29/21°C, day/night) was more beneficial to vegetative growth than in T1 (26/20°C, day/night) and T2 (26/18°C, day/night).

Plant morphogenesis proceeds through a multitude of events which occur according to a spatial and temporal sequence (Tran, 1999). Temperature is the main factor controlling the morphogenesis of *Phalaenopsis.* Chen (2008) found that the formation of reproductive stem of *Phalaenopsis aphrodite* was noticeably inhibited by a constant warm temperature, but induced by a fluctuating warm day and cool night condition. Moreover, different night temperature greatly affected both the stomatal conductance and the contribution of ambient and respiratory CO_2 to the nocturnal accumulation of malate. But the amounts of nocturnal accumulated malate and daily deposited starch appeared to have no significant difference between the two groups (28°C day/28°C night and 28°C day/20°C night). In our present investigation, it shows that all the treatments (26/20°C, 26/18°C) displayed shorter length of leaves than the control (29/21°C). Therefore, the amounts of nocturnal accumulated malate and daily deposited starch in plants under different temperature conditions were more or less the same, but the use of the energy in plants was totally different. Reproductive growth used a number of energy which should have been used to promote vegetative growth.

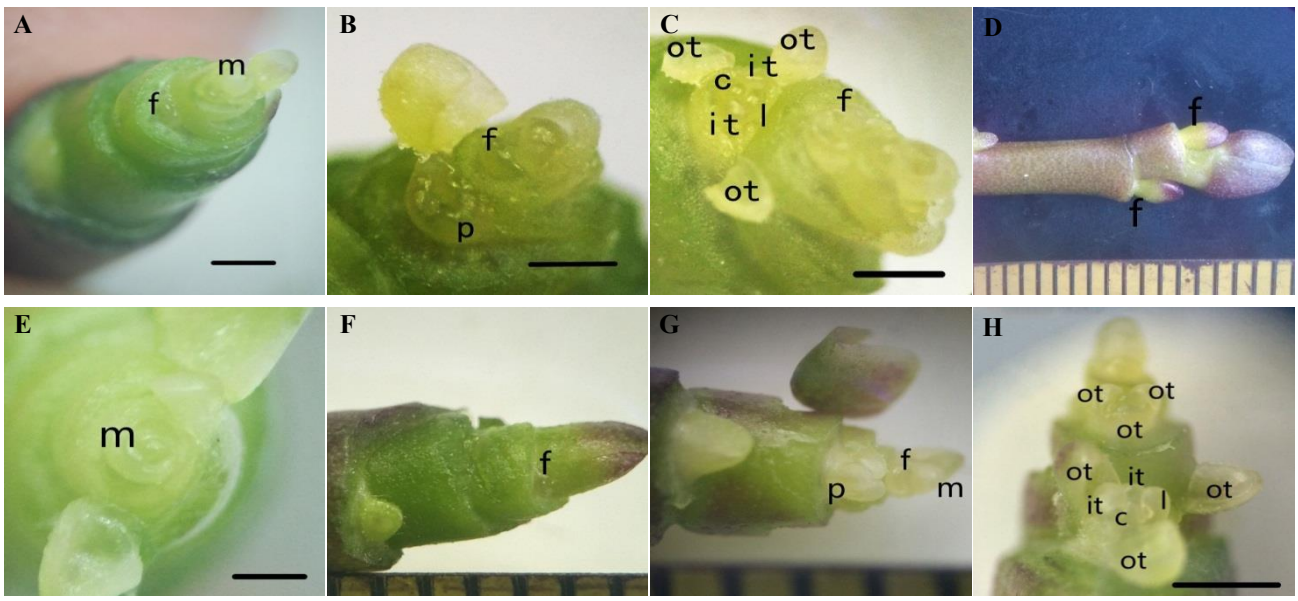

Fig. 1. Apical meristems of *Phalaenopsis* Big Chili and Sogo Yukidian, showing developmental changes from flower bud initiation to differentiation: (A) Flower bud differentiation (FBD) at 5cm stalk of 'Big Chili'; (B) Petal differentiation (PD) at 7.5cm stalk of 'Big Chili'; (C) Column (stamen and carpel) differentiation (CD) at 15cm stalk of 'Big Chili';(D) Flower bud (FB) at 20cm stalk of 'Big Chili'; (E) Undifferentiation (UD) at 5cm stalk of 'Sogo Yukidian'; (F) Flower bud differentiation (FBD) at 7.5cm stalk of 'Sogo Yukidian'; (G) Petal differentiation (PD) at 15cm stalk of 'Sogo Yukidian'; (H) Column (stamen and carpel) differentiation (CD) at 20cm stalk of 'Sogo Yukidian';. Abreviations: m – meristem, f – flower primordia, p –petal primordia, ot- outer tepal primordia, it-inner tepal primordial, l – lip primordia, c-column (stamen and carpel) primordial. Bar: 1mm

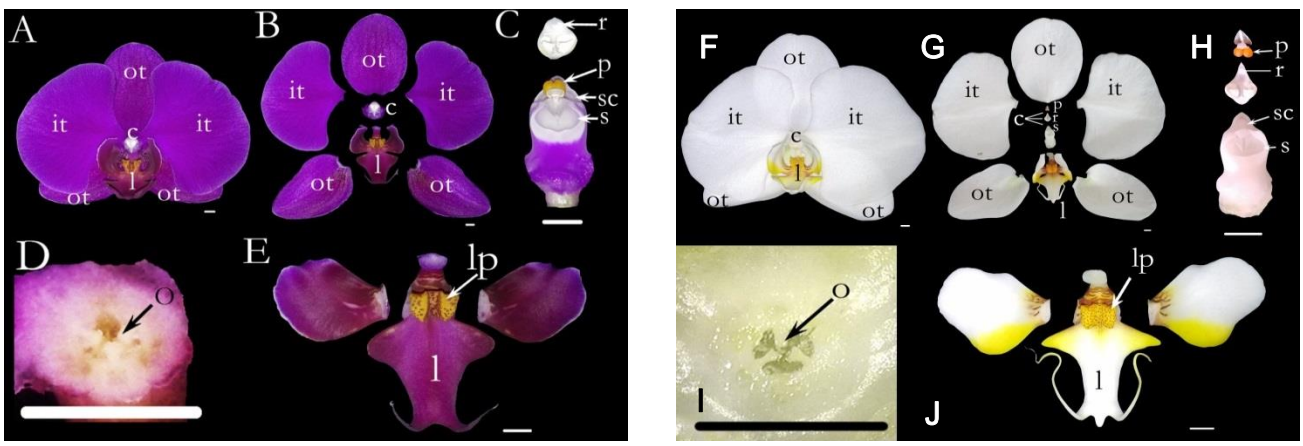

Fig. 2. Flower dissection of *Phalaenopsis* Big Chili (A-E) and Sogo Yukidian (F-J). (A, F) Intact flower; (B, G) Flower dissected into 4 parts; (C, H) column dissection; (D, I) ovule dissection; (E, J) lip dissection.
Abreviations: ot-outer tepal, it-inner tepal, c-column (stamen and carpel), l-lip, lp-lip projection, p-pollinium, r-rostellum, o-ovule, s-stigma, sc-stigmatic cavity. Bar: 5mm

Fig. 3. Flower bud places at different length of stalks, marked in black triangle.

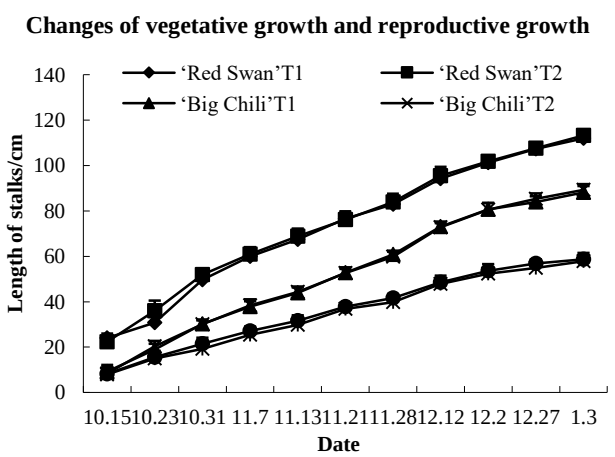

Fig. 4. Length changes of flower stalks after low temperature treatments of *Phalaenopsis*.

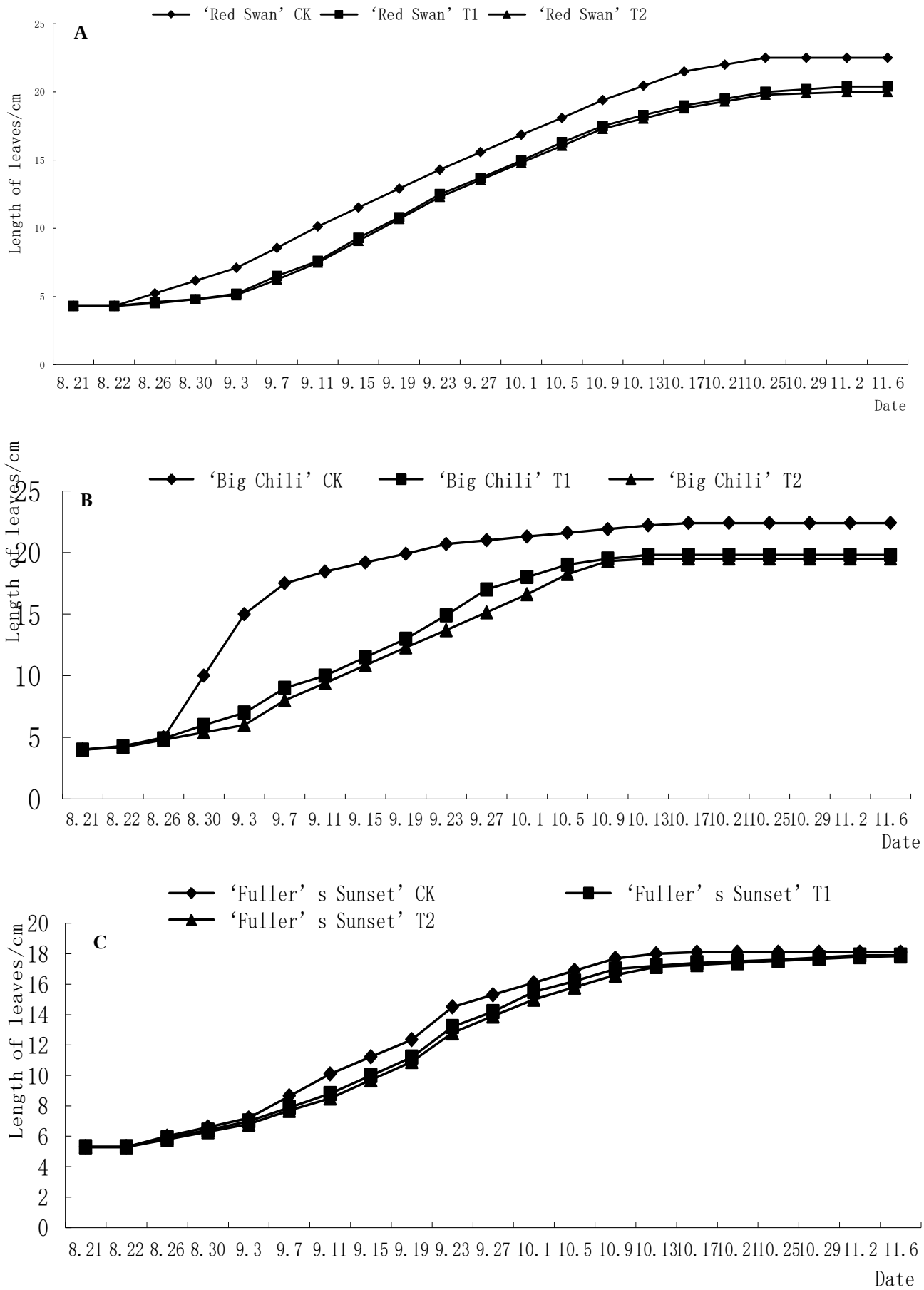

Fig. 5. Length of new leaves in different temperature treatments of three *Phalaenopsis* cultivars.
A. 'Red Swan' B. 'Big Chili' C. 'Fuller's Sunset'

Endogenous hormones changes

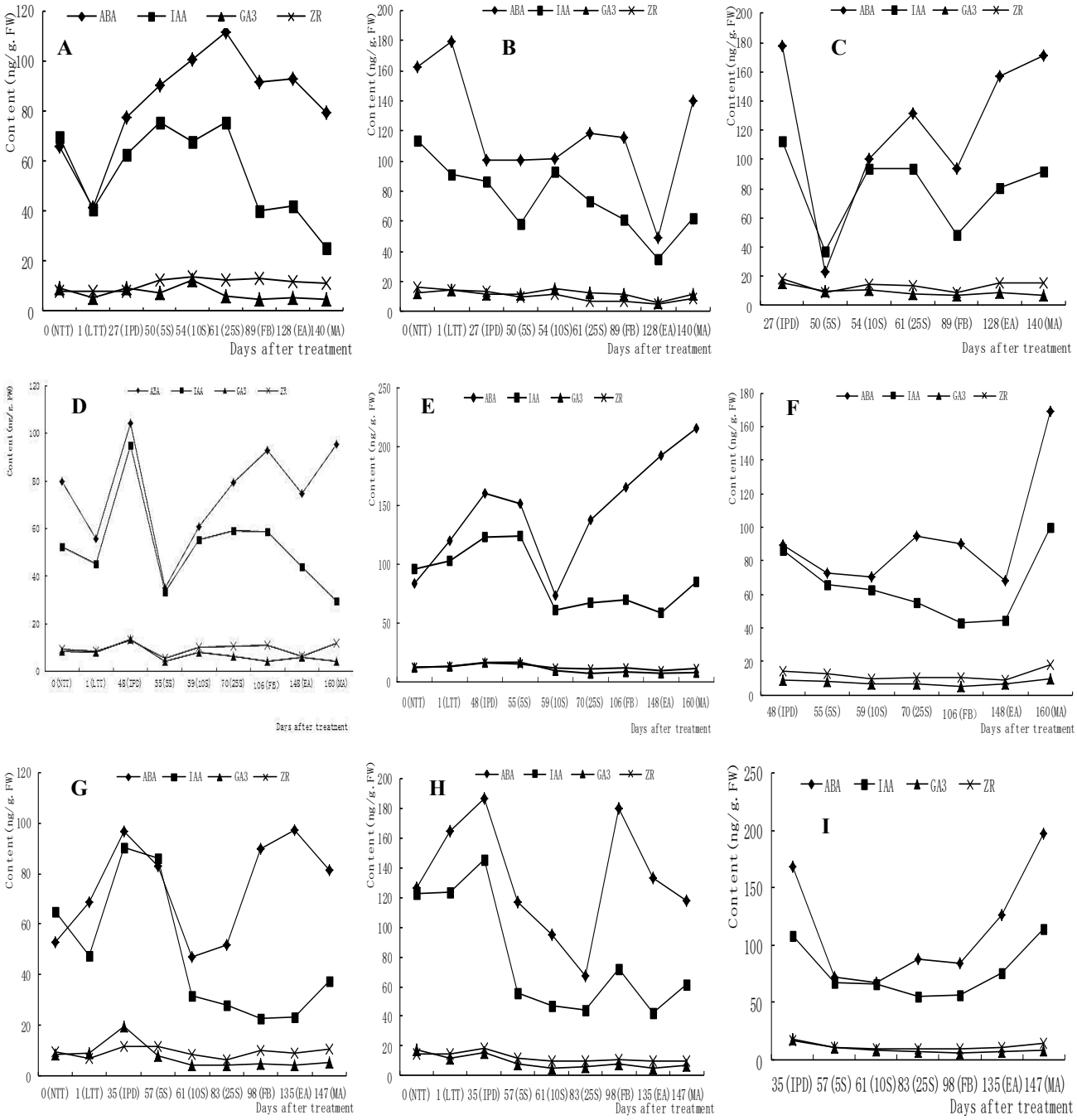

Fig. 6. The influence of abscisic acid (ABA), indoleacetic acid (IAA), gibberellic acid (GA_3) and zeatin riboside (ZR) on the flower bud differentiation in *Phalaenopsis* cultivars.
a. Leaves of 'Red Swan'; b. Roots of 'Red Swan'; c. Apical meristems of 'Red Swan'; d. Leaves of 'Big Chili'; e. Roots of 'Big Chili'; f. Apical metistems of 'Big Chili'; g. Leaves of 'Fuller's Sunset'; h. Roots of 'Fuller's Sunset'; i. Apical metistems of 'Fuller's Sunset'; NTT-normal temperature treatment; LTT-low temperature treatment; IPD-inflorescence primordium differentiation; 5S-5cm stalk; 10S-10 cm stalk; 25S-25 cm stalk; FB-flower bud; EA-early anthesis ; MA-middle anthesis

The levels of ABA and IAA levels were much higher than those of ZR and GA_3 at all flower bud development phases (Fig. 6). The levels of ABA at roots and apical meristems had a wide range, while that of ABA at leaves were relatively stable. It could be speculated that roots and apical meristems were the main places producing and using ABA. The level of ABA at roots increased to 160-190 ng/g·FW at first, and then decreased after the inflorescence primordium differentiation started. In contrast, the level of ABA at apical meristems was high at first, decreased when the stalk was 1-5 cm, and increased markedly after the stalk was 5-10 cm. ABA promotes flower bud differentiation by inhibiting vegetative growth or promoting CTK (cytokinin) accumulation in lychee (Chen, 1990). So the high level of ABA at apical meristems inhibited the stalk growth at first to promote the inflorescence primordium differentiation. Li (2008) researched on the changes in the endogenous hormones of

stem apices in tomatoes and speculated that ABA accelerated ovary locule formation through similar biosyntheses of ABA and GA_3. This result was also confirmed in *Citrus* (Zhang *et al*., 1994). Wan (2018) found ABA may play an important role in promoting flower bud differentiation in strawberry and relatively higher ABA and CTKs levels but relatively lower GAs and IAA levels required during flower bud differentiation were detected. Of all the four hormones studied, the level of ABA was the highest, decreasing at apical meristems after the stalk emerged, and increasing after the length of stalk was more than 5cm. So high level of ABA promoted inflorescence primordium differentiation at first. When the stalk emerged and grew to the length of more than 5cm, the level of ABA increased again and might play an important role in floral organ formation.

The average levels of IAA at roots and apical meristems were higher than that of IAA at leaves, like ABA. It could be speculated that roots and apical meristems were the main places producing and using IAA, too. IAA is the most common, naturally-occurring, plant hormone of the auxin class. Auxin, moving in the polar transport stream, mainly controls stem growth (Booker *et al*., 2003). IAA has many different effects, as all auxins do, such as inducing cell elongation and cell division with all subsequent results for plant growth and development. It can be seen from Fig. 6 that when the stalk emerged, IAA levels in the apical meristems were high, about 100 ng/g·FW, inhibiting the stem growth, to store energy in the apical meristems for flower bud initiation. High level of IAA decreased when the stalk began rapid elongation (longer than 5cm), resulting in apical dominance, promoting stalk growth. Xu (2008) *et al*., examined diffusible IAA from various parts of tulip plant during rapid elongation of the flower stalk and found that the top internode is probably the major source of auxins account for rapid elongation of the flower stalk. Žárský (1990) *et al*., found that lower IAA level and ethylene production may be directly correlated with a larger number of flower buds, while a higher IAA level is generally considered to act as a background inhibitor of flowering. Wilmowicz (2013) suggested that flowering inhibition evoked by IAA in *Pharbitis nil* results from its stimulatory effect on both ACC synthase and oxidase gene expression and, consequently, enhances ethylene production. So IAA controlled the elongation of stalk and the lower level of IAA might promote the flower formation directly. After anthesis, IAA level increased back, inhibiting the continuous elongation of the stalk.

The levels of GA_3 at apical meristems generally decreased after the stalk started to elongate. In sweet cherry, the level of GA_3 increased to the highest value at the end of flower bud differentiation (Wang *et al*., 2002), as the same as the result of this research. In Arabidopsis thaliana, GA_3 are known as an alternate pathway in flowering control (Yu *et al*., 2006), whereas in day-neutral plants, GA_3 was known as the internal factor controlling flowering (Lawson and Poethig, 1995). Marciniak (2018) found gibberellins (GAs) control the flower induction in the short-day plant *Ipomoea nil*. As a kind of exogenous hormone, GA_3 could reduce the flower drop % and increase the fruit set % (Khan *et al*., 2014). The level of GA_3 in 'Big Chili' was less than that in 'Red Swan' and 'Fuller's Sunset', and it took much longer time to complete the flower bud differentiation for ' Big Chili' and its anthesis was much later than 'Red Swan' and 'Fuller's Sunset'. This suggested a possible involvement of GA_3 in autonomous flower induction and the GA_3 level might impact the anthesis.

ZR levels at apical meristems were higher than at leaves, which meant that there was more cell division at apical meristems than at leaves. Werner *et al*., (2001) suggested that the cytokinins are necessary to maintain cell division, and to promote the transition of stem cells from undifferentiated to differentiated state. Trivellini (2015) found that cytokinins seem to be strongly involved the regulation of the ABA response in flower tissues. In the present research, the levels of ZR at apical meristems were indeed higher when the inflorescence primordium differentiation started. So ZR might took part in autonomous flower induction.

Conclusions

Phalaenopsis cultivars were all coordinated by the cold-induced release of reproductive bud dormancy. The morphological development of flower bud differentiation in *Phalaenopsis* cultivars was non-synchronized but the sequence was generally similar: flower organ differentiation began with the formation of the outer tepal primordia, followed by the inner tetal primordia and finally, by the lip and column (stamen and carpel) primordia. The flower stalks in 'Red Swan' emerged first, while in 'Big Chili' emerged last. The earlier the flower bud started differentiation, the earlier the flower bloomed. The inflorescence primordium differentiation started under 26/18°C and 26/20°C (day/night) and no flower stalks emerged under 29/21°C within 50 d. Different temperature treatments did not affect significantly the length of flower stalk. But temperature played a possible important role in leaf growth. The temperature in CK (29/21°C, day/night) was more beneficial to vegetative growth than in T1 (26/20°C, day/night) and T2 (26/18°C, day/night). T1 and T2 displayed shorter length of leaves than CK. The timing of inflorescence primordium differentiation and flower bud development was controlled by plant hormones. It was found increasing ABA and GA_3 levels of apical meristems of stalk before flower bud differentiation was able to promote the flower induction and the inflorescence primordium differentiation. Levels of IAA and ZR controlled the vegetative growth phase and the floral inductive phase, like the elongation of stalk and leaves, and they might control the flower induction and flower formation directly.

Acknowledgments

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References

Booker, J., S. Chatfield and O. Leyser. 2003. Auxin acts in xylem-associated or medullary cells to mediate apical dominance. *Plant Cell*, 15: 495-507.

Chen, W.H., Y.C. Tseng, Y.C. Liu, C.M. Chuo, P.T. Chen, K.M. Tseng, Y.C. Yeh, M.J. Ger and H.L. Wang. 2008. Cool-night temperature induces spike emergence and affects photosynthetic efficiency and metabolizable carbohydrate and organic acid pools in *Phalaenopsis aphrodite*. *Plant Cell Rep.*, 27(10): 1667-1675.

Chen, W.S. 1990. Endogenous growth substances in xylem and shoot tip diffusate of lychee in relation to flowering. *Plant Physiol*, 25(3): 314-315.

Guo, W.J. and N. Lee. 2006. Effect of leaf and plant age, and day/night temperature on net CO^2 uptake in *Phalaenopsis amabilis* var. *formosa*. *J. Amer. Soc. Hort. Sci.*, 131(3): 320-326.

He, J., S.L. Yu and C. Ma. 2009. Effects of plant growth regulator on endogenous hormone levels during the period of the red globe growth. *J. Agric. Sci*., 1: 92-100.

Hsu, C.C., H.H. Chen and W.H. Chen. 2018. *Phalaenopsis*. In: *Ornamental Crops.* (Eds.): Johan Van Huylenbroeck, *Belgium*, pp. 567-625.

Jin, J.K., H.L. Chen, C.W. Yu and C.H. Lin. 2005. Establishment of a plastochron index for growth assessment of *Phalaenopsis*. *Can. J. Bot*., 83: 47-53.

Khan, A.S, T. Shaheen, A.U. Malik, I.A. Rajwana and I. Ahmad. 2014. Exogenous applications of plant growth regulators influence the reproductive growth of citrus sinensis osbeck cv. blood red. *Pak. J. Bot*., 46(1) 233-238.

Kim, D.G., K.K. Kim and C.G. Been. 2015. Development of intergeneric hybrids between wind orchids (*Sedirea japonica* and *Neofinetia falcata*) and moth orchids (*Phalaenopsis alliances*). *Hort. Envir. & Biotechnol*., 56(1): 67-78.

Kwon, Y.E., H.J. Yu, S. Baek, G.B. Kim, K.B. Lim and J.H. Mun. 2017. Development of gene-based identification markers for *Phalaenopsis* 'KS Little Gem' based on comparative genome analysis. *Hort. Envir. & Biotechnol*., 58(2): 162-169.

Lawson, E.J.R. and R.S. Poethig. 1995. Shoot development in plants: time for a change. *Trends Genet*., 11(7): 263-268.

Lee, H.B., S.H. Lim, N.H. Lim, S.K. An and K.S. Kim. 2018. Growth and CO^2exchange in young *Phalaenopsis* orchids grown under different levels of humidity during the vegetative period. *Horticulture Envir. & Biotechnol.*, 59(1): 37-43.

Lee, H.B., S.K. An and K.S. Kim. 2015. Inhibition of premature flowering by intermittent high temperature treatment to young *Phalaenopsis* plants. *Horticult., Envir & Biotechnol.*, 56(5): 618-625.

Li, Y., T.L. Li and D. Wang. 2008. Correlation between endogenous hormones of stem apices and fruit locule numbers in tomatoes during floral bud differentiation stages. *Agri. Sci. China*, 7(4): 447-454.

Marciniak K., A. Kućko, E. Wilmowicz, M. Świdziński, J. Kęsy, J. Kopcewicz, K. Marciniak, A. Kućko, E. Wilmowicz, M. Świdziński, J. Kęsy and J. Kopcewicz. 2018. Photoperiodic flower induction in Ipomoea nil is accompanied by decreasing content of gibberellins. *Plant Growth Reg.*, 84(2): 395-400.

Tran, K. 1999. Floral and Vegetative differentiation in vitro and in vivo. *Morphogen. in Plant Tiss. Cult.*, 23(12): 215-233.

Wan, C.Y., M. Lin, B.Y. Chen, J.F.L.H.Z. Huo, J.T. Xu and X.P. Chen. 2018. Effects of nitrogen during nursery stage on flower bud differentiation and early harvest after transplanting in strawberry. *Brazil. J. Bot.*, 41(1): 1-10.

Wang, Y.H., C.H. Fan, X. Shen, G.M. Qu and J.D. Shi. 2002. Changes in endogenous hormones during the flower bud differentiation of sweet cherry. *Acta Agriculturae Boreali-occidentalis Sinica*, 11: 64-67.

Wei, L., F.R. Peng, S.B. Wang and P.P. Tan. 2010. Morphology and changes of several metabolite content during flower bud differentiation in *Phalaenopsis*. *Acta Horticulturae Sinica*, 37: 1303-1310.

Werner, T., V. Motyka, M. Strnad and T. Schmülling. 2001. Regulation of plant growth by cytokinin. *Proc.. Nat. Acad. Sci. Uni. States Amer.*, 98(18): 10487-10492.

Wilmowicz, E., K. Frankowski, J. Kęsy, P. Glazińska, W. Wojciechowski, A. Kućko and J. Kopcewicz. 2013. The role of PnACO1 in light- and IAA-regulated flower inhibition in Pharbitis nil. *Acta Physiologiae Plantarum.*, 35(3): 801-810.

Xu, R.Y., Y. Niimi, Y. Ohta and K. Kojima. 2008. Changes in diffusible indole-3-acetic acid from various parts of tulip plant during rapid elongation of the flower stalk. *Plant Growth Reg.*, 54(2): 81-88.

Yu, H., T. Dhavale and S. Yang. 2006. Molecular mechanisms of hormone functions in flowering. *Floricult. Ornament Plant Biotechnol*, 1: 25-32.

Žárský, V., L. Pavlová, J. Eder and I. Macháčková. 1990. Higher flower bud formation in haploid tobacco is connected with higher peroxidase/IAA-oxidase activity, lower IAA content and ethylene production. *Biol. Plant.*, 32(4): 288-293.

Zeng, X.P., Z.C. Liu, M.H. Su and C. Chen. 2008. Dynamic changes of endogenous hormones and polyamine in *Phalaenopsis* leaves during floral bud formation. *Subtrop. Plant Sci.*, 37(3): 1-5.

Zhang, S.J., X.F. Wang, X.H. Yang, F.J. Yang and H.M. Tian. 2011. Variation of endogenous phytohormone concentration in cotyledonary node calli during cucumber regeneration. *Acta Agricult. Boreali-occidentalis Sin*., 20: 153-157.

Zhang, S.L., K.S. Chen, Q.F. Ye, D.M. Chen and C.R. Liu. 1994. Changes of endogenous IAA, ABA and ZT in pollinated, non-pollinated and parthenocarpic ovary (fruitlet) of *Citrus*. *Acta Horticulturae Sinica*, 21: 117-123.

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