Pak. J. Agri. Sci., Vol. 54(1), 15-20; 2017
ISSN (Print) 0552-9034, ISSN (Online) 2076-0906
DOI: 10.21162/PAKJAS/17.4439
http://www.pakjas.com.pk

DETERMINATION OF FLOWER BUD INITIATION IN ORIENTAL HYBRID LILIES 'SIBERIA' AND 'SORBONNE'

Juniel Galido Lucidos[1,2], Adnan Younis[3,*] and Ki-Byung Lim[2,4]

[1]Research, Extension and Training Office, Romblon State University, Odiongan, Romblon, Philippines;
[2]Department of Horticultural Science, Kyungpook National University, Daegu 41566, Korea;
[3]Institute of Horticultural Sciences, University of Agriculture, Faisalabad-38040, Pakistan.
[4]Institute of Agricultural Science and Technology, Kyungpook National University, Daegu, Korea
[*]Corresponding author's e-mail: adnan@knu.ac.kr

The flower initiation stage is one of the most critical stages in plant development. This study was conducted with an aim of identifying the precise flower bud initiation stage in oriental lily hybrids, 'Sorbonne' and 'Siberia', for the purpose of proper plant management during the flower development stage. The bulbs were micro-dissected 0, 10, 20, 30, and 40 days after planting. Five bulbs were randomly selected for dissection and flower bud initiation stage identification. Bulb circumference, shoot length, number of bulb scales, number of leafy scales, number of true/alternate leaves, and flower bud initiation stage were measured. The flower bud initiation stages were scaled as follows: 0 = no flower buds, 1 = starting stage, 2 = flower bud developing stage, and 3 = fully developed flower bud stage. In both the hybrids, flower bud initiation started 20 days after planting and was observed to be associated with shoot length; the initiation occurring in shoots 11–13cm long. It was also greatly affected by the cultural management during the growing of plants. Identification of the exact time of flower bud initiation will be useful in proper management of the plant materials with respect to their requirements of water, nutrient, environmental conditions, and pest control. This knowledge will help in developing quality flowers of the two studied oriental lily hybrids for breeding and commercial purposes.

Keywords: Bulb circumference, bulb scale, flower bud initiation stage, lily, dissection method, plant management

INTRODUCTION

Lilies are attractive economic flowering plants grown in pots or as cut flowers (Pobudkiewics and Treder, 2006; Younis *et al*., 2014). They belong to the genus *Lilium*, grouped in 7 sections comprising of 100–115 species, and can be cultivated under diverse climatic zones. In the flower trade, *Lilium* is recognized as a valuable cut flower and many breeding companies are working on the development of new cultivars of the species belonging to different sections of the genus. Among these, Oriental lilies, belonging to the section Archelirion, and their hybrids are one of the leading economically important groups with flowers having wide range of shape, size, color, and other morphological characteristics Younis *et al.*, 2014; Shimizu, 1987; Roh, 2011). Oriental hybrids like 'Siberia' and 'Sorbonne' are typically used for breeding research.

The transition from the vegetative stage to reproductive stage is a remarkable and dramatic event in flower development (Ram and Rao, 1984; Araki, 2001; Poethig, 2003). The vegetative- to reproductive-phase transition is controlled through a complex genetic system, an array of signal transduction pathways that regulate the developmental stage of the plants, and environmental cues viz., light (day length, photoperiod) and temperature (Simpson *et al.*, 1999; Araki, 2001; Amasino, 2010; Srikanth and Schmid, 2011). Morphological transitional phases have been extensively documented in various plant taxa (Bernier *et al.*, 1981; Lang, 1965). The investigational manipulations of plant meristem have revealed that the complete flower formation occurred in culture or either under non inductive environmental condition once the first floral organs are initiated (Soetiarto and Ball, 1969; Waterkcyn, 1965; Erwin, 2006). Different approaches have been undertaken to understand the complex mechanisms of flower development, growth, movement, and functions and have relied on optical and scanning electron microscopy, analytical tools, photography, and tissue culture (Ball, 1936; Blazquez *et al.*, 2006; Tan and Swain, 2006). For example, in *Agapanthus praecox* ssp. *orientalis*, flower buds were observed under dissecting and optical microscopes (Zhang *et al.*, 2011).

The exact time of the transition from vegetative to flowering stage is of great importance in all flowering plants because it strongly impacts growth synchronization (Ramzan *et al*., 2014). The growth phase stages act as developmental landmarks and stimuli for collecting morphological data of interest at any specific stage of plant development (Boyes *et al.*, 2001). Moreover, knowledge about the timing of phase transition is important in ensuring proper flower development. For example, early premature flowering leads

to a lower market value of flowers, whereas, an elongated vegetative phase increases the plant biomass but decreases flowering (Demura and Ye, 2010). In this context, the present study was designed to identify the exact timing of flower initiation stage in two oriental lily hybrids, Siberia and Sorbonne. These varieties are important because they are used in breeding and physiology-related research. Understanding of the flower initiation stage of these cultivars will help researchers properly manage the plant, particularly during the critical stage of flower initiation. As successful breeding research starts with a healthy flower, identifying the relationship between the exact timing of flower initiation and requisites of culture management, such as water, nutrients, and pest control, would be of significance.

MATERIALS AND METHODS

***Plant material*:** The bulbs of oriental hybrid lilies 'Sorbonne' and 'Siberia' were imported from the Netherlands. Prior to planting, the bulbs were stored at 4℃ for 30 days. For spring planting season, the bulbs were planted in the greenhouse of Kyungpook National University, Daegu, Korea, in which the temperature was maintained between 20 and 30°C.

***Sampling and dissection*:** Bulbs with uniform circumference (~15–16 cm for 'Siberia' and 13 cm for 'Sorbonne') were randomly selected. Five bulbs were dissected before planting and served as controls. For other bulbs, sampling was conducted 10, 20, 30, and 40 days after planting to check for the formation of flower buds. At the time of dissection, the bulb circumference and shoot length was measured. The bulb scales, leafy scales, and true or alternate leaves were counted separately, and flower bud development was observed.

***Identification of flower bud stage*:** For measuring the flower bud initiation stage of the plant we used the following metrics: 0 = no flower bud formation yet, 1 = starting stage, 2 = developing stage, and 3 = fully formed flower bud stage. The observations were made under a light microscope (Olympus SZX16, Tokyo, Japan).

***Statistical analysis*:** The data was analyzed using Duncan's Multiple Range Test (DMRT) using the SPSS (version 19.0) software (SPSS Inc., USA). A value of $p<0.05$ was considered statistically significant.

RESULTS

To identify the exact flower bud initiation stage in the two oriental hybrid lilies 'Siberia' and 'Sorbonne' and its relation to proper crop management, different parameters were considered such as bulb circumference (cm), shoot length (cm), number of bulb scales, number of leafy scales, number of true/alternate leaves, and flower bud initiation stages (days).

***Bulb circumference*:** The average bulb size, in terms of its circumference, for cultivar 'Siberia' was ~15.9 cm at the time of planting; it increased to 16.4 cm after 30 days and up to 17.2 cm after 40 days of planting (Fig. 1). Unlike in the cultivar 'Siberia', the bulb circumference of 'Sorbonne', which was 13.6 cm at the time of planting, did not increase significantly even after 40 days of planting.

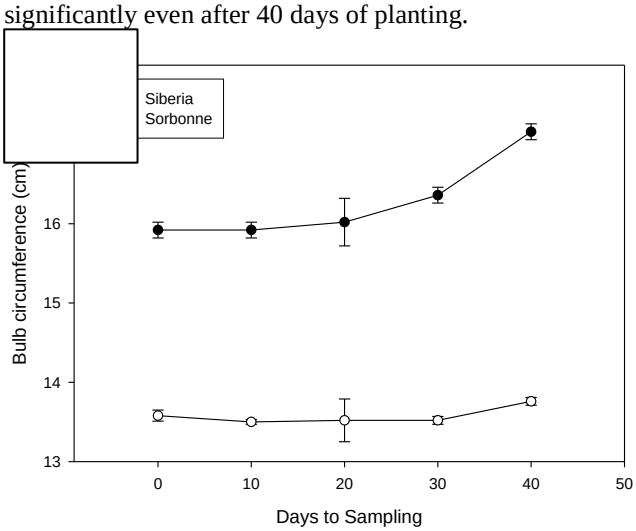

Figure 1. Bulb circumference of cultivar 'Siberia' and 'Sorbonne' in different sampling days.

***Shoot length*:** Flower bud development is also closely associated with the increase in shoot length. As shown in Figure 2, in the cultivar 'Siberia', the shoot length before planting was 1.59 ± 0.1 cm and it increased as the plant matured. At the start of flower initiation stage, 20 days after planting, the shoot length was 11.5 ± 0.1 cm, which increased to 37.8 ± 0.3 cm 40 days after planting, with the maturing of the flower buds. The elongation was initially slow, starting 10 days after planting, but was rapid after 30 days of planting. In the case of cultivar 'Sorbonne', the shoot length at planting was 1.5 ± 0.1 cm, which continuously increased with maturity. As with the cultivar 'Siberia', flower initiation occurred 20 days after planting when the shoot length was 12.8 ± 0.7 cm and mature flower buds were observed 40 days after planting with shoots attaining a length of 33.9 ± 0.2 cm. Further, the stem elongation was observed 10 days after planting with rapid shoot development occurring only 20 days after planting. The transition from vegetative to reproductive stage in both the cultivars was observed 15–20 days after planting.

***Bulb and leafy scale*:** The number of bulb scales and leafy scales was also measured and the data is provided in Figs. 3 and 4. The cultivar 'Siberia' had 20.6 ± 0.7 bulb scales and 2.8 ± 0.4 leafy scales at the time of planting. Ten days after planting, the number of bulb scales decreased to 19.6 ± 0.5, while the number of leafy scales increased to 3.2 ± 0.4. At the flower initiation stage, the number of bulb scales increased until 40 days after planting, while the number of leafy scales increased 20–30 days after planting but suddenly decreased after 40 days. In the cultivar 'Sorbonne', the number of bulb

scales at the time of planting was 22.6 ± 0.4, while the number of leafy scales was 2.6 ± 0.2. As in the case of 'Siberia', the number of bulb scales first decreased and then increased 20–40 days after planting, while the number of leafy scales increased starting from 20–30 days and then suddenly decreased after 40 days of planting.

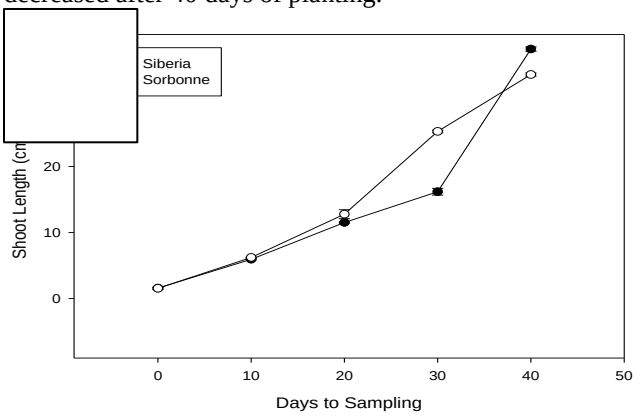

Figure 2. Shoot length of cultivar 'Siberia' and 'Sorbonne' in different sampling days.

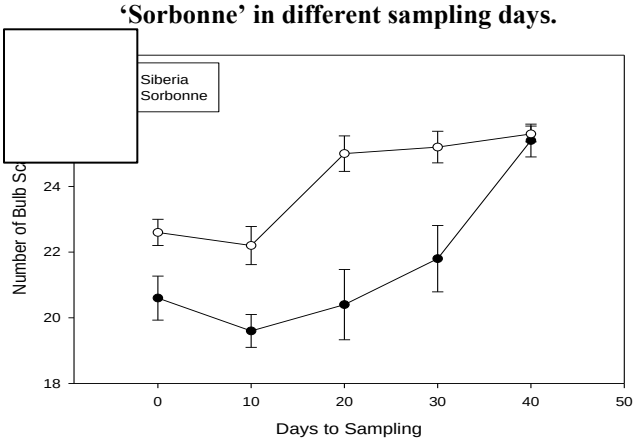

Figure 3. Number of bulb scales of cultivar 'Siberia' and 'Sorbonne' in different sampling days.

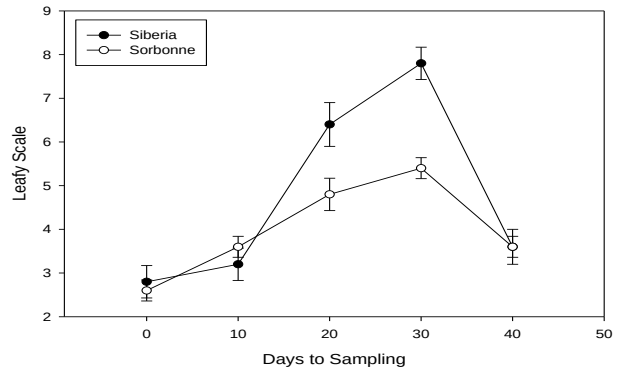

Figure 4. Number of leafy scales of cultivar 'Siberia' and 'Sorbonne' in different sampling days.

***True/alternate leaves*:** The number of true or alternate leaves that developed in the two hybrids was also measured (Fig. 5). In the case of 'Siberia', the number of true leaves was ~37.8 ± 1.2; at the flower initiation stage, the number was 42.4 ± 0.5 and it continued to increase till 40 days after planting when the observed number of leaves was 48.4 ± 0.5. The cultivar 'Sorbonne' initially had 26.4 ± 0.2 leaves and this number did not increase until the flower bud initiation stage. After flower bud initiation (~40 days after planting), the number of leaves increased to 28.2 ± 0.4.

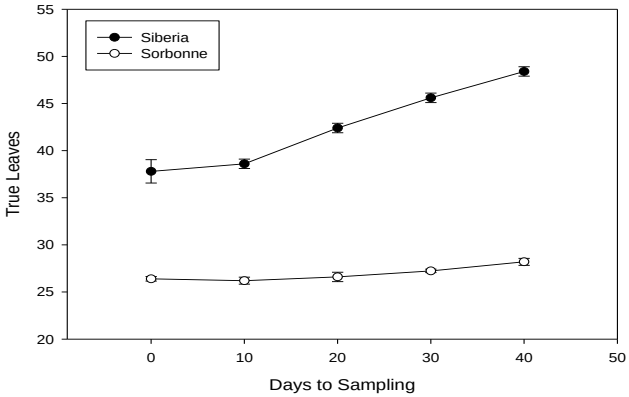

Figure 5. Number of true/alternate leaves of cultivar 'Siberia' and 'Sorbonne' in different sampling days.

***Flower initiation stage*:** In all flowering plants, the flower bud initiation stage is the most crucial in the transition from the vegetative to reproductive phase of plant development. In the case of oriental hybrid lilies 'Siberia' and 'Sorbonne', the flower bud initiation stage started 20 days after planting, continued to develop until 30 days after planting, and a fully formed flower bud was observed 40 days after planting. Flower bud initiation was also associated with shoot length and occurred in plants having 11–13-cm-long shoots. The different stages of flower bud development in the studied hybrid lilies are summarized and illustrated, respectively, in Table 1 and Figure 6 and 7.

Table 1. Flower bud initiation stage in Oriental hybrid lilies 'Siberia' and 'Sorbonne'.

Cultivar	Days to sampling	[Z]Flower initiation stage
Siberia	0	0
	10	0
	20	1
	30	2
	40	3
Sorbonne	0	0
	10	0
	20	1
	30	2
	40	3

[Z]Flower initiation stage scale- 0= no FB initiation yet, 1=starting stage, 2=developing stage,

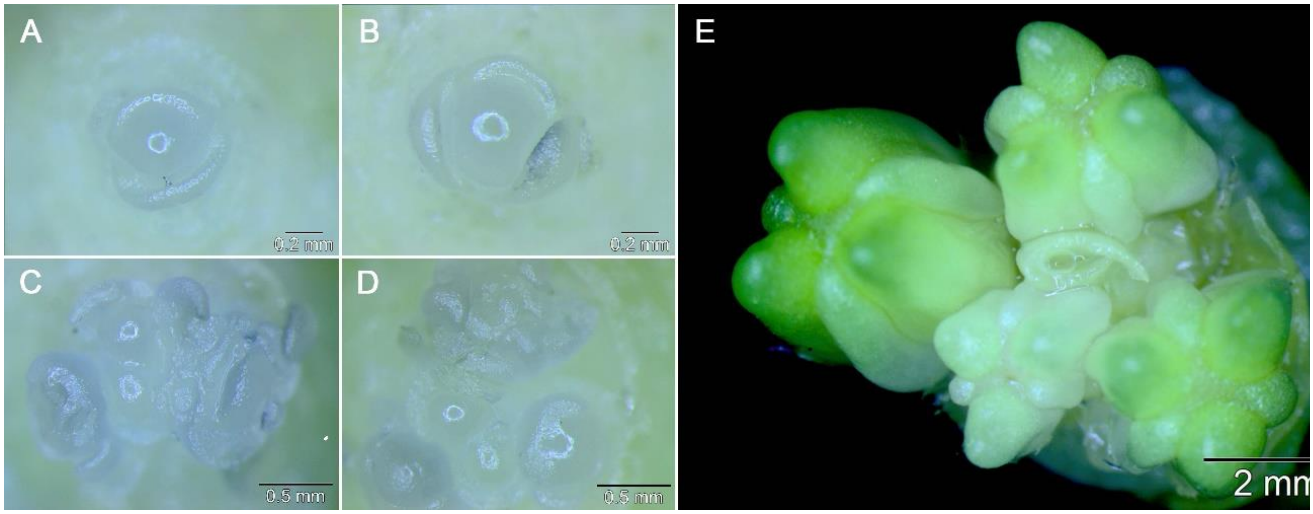

Figure 6. Flower bud development stages in oriental hybrid lily 'Siberia'. A. before planting, B. 10 DAP (Day's after planting), C. 20 DAP, D. 30 DAP, E. 40 DAP.

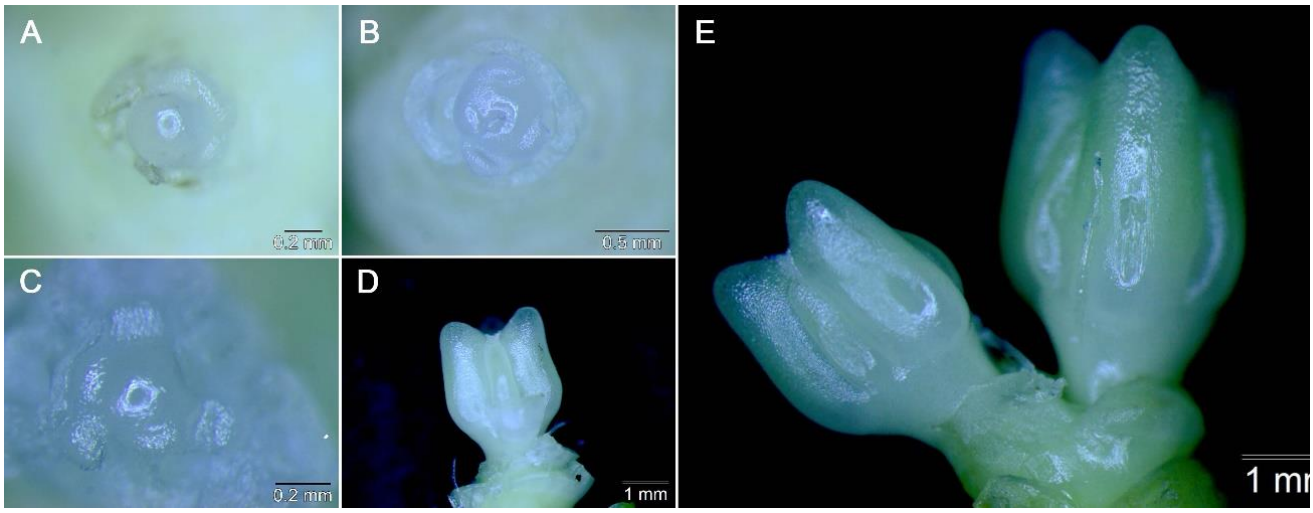

Figure 7. Flower bud development stages in oriental hybrid lily 'Sorbonne'. A. before planting, B. 10 DAP, C. 20 DAP, D. 30 DAP, E. 40 DAP.

DISCUSSION

To produce good quality bulbs throughout the year, proper handling of bulbs, the nature of bulb dormancy and maturity, and floral initiation and development should be clearly understood (Roh, 1989; Lucidos *et al.,* 2014). Production of *Lilium* involves three distinct phases, namely bulb production, bulb programming, and greenhouse forcing. The greenhouse forcing phase is further divided into three stages: stage I from potting to shoot emergence or flower bud initiation, stage II from shoot emergence or flower bud initiation to the visible bud phase, and stage III from visible bud to anthesis stage (Roh, 1999). The time and development of flower initiation vary greatly between *Lilium* species and can be divided into subclasses (Wilkins and Dole, 1997). In the present study, we considered stage I, i.e., the transition period from vegetative to reproductive phase, which is crucial in all flowering plants. Before entering the reproductive phase, proper crop management should be conducted to ensure optimal conditions of water, nutrients, and environmental conditions (such as temperature and photoperiod) as well as pest control (Lucidos *et al.,* 2013). Upon the onset of flower initiation stage, the plants should be supplied with sufficient water and nutrients in preparation for flower development, and there should be close monitoring of insects and pathogens that attack the plant during its reproductive stage. Inappropriate temperature and light conditions during storage, planting, development, and flowering often result in leaf aberrations and flower bud abortion (De Hertogh and Le Nard, 1993). According to Anderson *et al.* (2010), in *Lilium*, flower

development that initiated during or just after the storage period must be completed after planting.

In both the studied cultivars, the bulb circumference increased 40 days after planting and was related to the development of bulb scales as the plant matured. Proper crop management could promote the formation of superior and healthy bulbs for the next planting season. According to Le Nard and De Hertogh (1993), light deficiency, high soil temperature, and other adverse environmental factors weaken the bulbs formed.

Our results demonstrate that the shoot length is highly associated with the flower bud initiation stage. In both the cultivars, the flower initiation stage started 20 days after planting when the average shoot length was 11–13 cm. According to previous reports, in *L. longiflorum*, *L. speciosum*, and LA (hybrids of *longiflorum* and asiatic lilies) hybrids, flower buds initiated when shoots were about 10–15 cm long, depending on the species/cultivars and environmental conditions. In *L.* × *elegans* hybrids, flower buds started to initiate after harvesting of the bulbs. In general, early flowering and short-stem cultivars initiated flowers before shoot emergence, while late flowering and long-stem cultivars had flower initiation after shoot emergence (Ohkawa *et al.*, 1990). As the selected oriental hybrid lilies are late-flowering cultivars (Wilkins and Dole, 1997) the flower bud initiation occurred after shoot emergence, specifically 20 days after emergence or when the shoots were 11–13 cm long. However, Lucidos *et al*. (2014) mentioned flower initiation and development 10–15 days after the planting of bulbs in the Orientals. This variation may be due to storage conditions/handling or environmental settings during planting.

***Conclusion*:** Based on the results obtained in the present study, it can be concluded that the flower initiation stage in the oriental hybrid lilies 'Siberia' and 'Sorbonne' occurs 20 days after planting when the average shoot length is 11–13 cm. After determination of flower initiation stage previous research findings emphasized on proper crop management practices, such as appropriate administration of water and nutrients, pest and disease monitoring, and optimum conditions of temperature, humidity, and light intensity in the greenhouse, that adhered to for development of healthy and good quality flowers that can be used in breeding or for commercial lily production.

***Acknowledgements*:** This work was supported by a grant from Regional Subgenebank Support Program of Rural Development Administration, Republic of Korea.(No.PJ012177).

REFERENCES

Amasino, R. 2010. Seasonal and developmental timing of flowering. Plant J. 61:1001-1013.

Anderson, N.O., A. Younis and Y. Sun. 2010. Intersimple sequence repeats distinguish genetic differences in Easter lily 'Nellie White' clonal ramets within and among bulb growers over years. J. Amer. Soc. Hort. Sci.135:445-455.

Araki, T. 2001. Transition from vegetative to reproductive phase. Curr. Opin. Plant Biol. 4:63-68.

Ball, N.G. 1936. The effect of nocturnal illumination by different regions of the spectrum on the subsequent opening of flower buds. New Phytol. 35:101-116.

Bernier, G., J.M. Kinet and R.M. Sachs. 1981. The Physiology of Flowering. CRC Press, Boca Raton, Florida, USA.

Blazquez, M.A., C. Ferrandiz, F. Madueno and F. Parcy. 2006. How floral meristems are built? Plant Mol. Biol. 60:855-870.

Boyes, D.C., A.M. Zayed, R. Ascenzi, A.J. McCaskill, N.E. Hoffman, K.R. Davis and J. Gorlach. 2001. Growth stage-based phenotypic analysis of *Arabidopsis*: a model for high throughput functional genomics in plants. Plant Cell 13:1499-1510

De Hertogh, A.A. and M. Le Nard. 1993. Physiological disorders. In: A.A. De Hertogh and M. Le Nard (eds.), The Physiology of Flower Buds. Amsterdam: Elsevier, pp.155-160.

Demura, T. and Z.H. Ye. 2010. Regulation of plant biomass production. Curr. Opin. Plant Biol. 13:299-304.

Erwin, J. 2006. Factors affecting flowering in ornamental plants. In: N.O. Anderson (ed.), Flower Breeding and Genetics: Issues, challenges and opportunities for the 21st century. Dordrecht, Springer, The Netherlands.

Lang, A. 1965. Physiology of flower initiation. In: W. Ruhland (ed.), Encyclopedia of Plant Physiology, Vol. 15/1. Springer-Verlag, Berlin, pp.1380-1536.

Le Nard, M. and A.A. De Hertogh. 1993. Tulipa. In: A.A. De Hertogh and M. Le Nard (eds.), The Physiology of Flower Bulbs. Elsevier, Amsterdam, Netherland.

Lucidos, J.G., A. Younis, Y.J. Hwang and K.B. Lim. 2014. Determination of optimum conditions for breaking bulb dormancy in relation to growth and flowering in *Lilium hansonii*. Hort. Environ. Biotechnol. 55:257-262.

Lucidos, J.G., B.R. Kwang, A. Younis, C.K. Kim, Y.J. Hwang, B.G. Son and K.B. Lim. 2013. Different day and night temperatures responses in *Lilium hansonii* in relation to growth and flower development. Hort. Environ. Biotechnol. 54:405-411.

Ohkawa, K., A. Kano and A. Nakaya. 1990. Time of flower bud differentiation in Asiatic hybrid lilies. Acta Hort. 266:211-220.

Pobudkiewicz, A. and J. Treder. 2006. Effects of flurprimidol and daminozide on growth and flowering of oriental lily 'Mona Lisa'. Sci. Hortic.110:328-333.

Poethig, R.S. 2003. Phase change and the regulation of developmental timing in plants. Science 301:334-336.

Ram, H.Y.M. and R.I.V. Rao. 1984. Physiology of flower bud growth and opening. Proc. Indian Acad. Sci. (Plant Sci.). 93:253-274.

Ramzan, F., A. Younis, A. Riaz, S. Ali, M.I. Siddique and K.B. Lim. 2014. Pre-planting exogenous application of gibberellic acid influences sprouting, vegetative growth, flowering, and subsequent bulb characteristics of 'Ad-Rem' tulip. Hort. Environ. Biotechnol. 55:479-488.

Roh, M.S. 1989. Control of flowering in *Lilium*-A review. Herbertia 45:65-69.

Roh, M.S. 1999. Physiology and management of *Lilium* bulbs. Acta Hort. 482:39-46.

Roh, M.S. 2011. Controlled flowering in the genus *Lilium*-review of the past achievements and the future direction of research. Acta Hort. 900:189-204.

Shimizu, M. 1987. The lilies of Japan; species and hybrids (Japanese). Seibundo Shinkosha, Tokyo Japan.

Simpson, G.G., A.R. Gendall and C. Dean. 1999. When to switch to flowering. Ann. Rev. Cell Dev. Biol. 15:519-550.

Soetiarto, S.R. and E. Ball. 1969. Ontogenetical and experimental studies of the floral apex of *Portulaca gradiflora*. 2. Bisection of the meristem in successive stages. Can. J. Bot. 47:1067-1076.

Srikanth, A. and M. Schmid. 2011. Regulation of flowering time: all roads lead to Rome. Cell. Mol. Life Sci. 68:2013-2037.

Tan, F.C. and SM. Swain. 2006. Genetics of flower initiation and development in annual and perennial plants. Physiol. Plant. 128:8-17.

Waterkeyn, L., P. Martens and J.P. Nitsch. 1965. The induction of flowering in *Nicotiana*: Morphological development of the apex. Amer. J. Bot. 52:264-270.

Wilkins, H.F. and J.M. Dole. 1997. The physiology of flowering in *Lilium*. Acta Hort. 430:183-188.

Younis, A., Y.J. Hwang and K.B. Lim. 2014. Classical vs. modern genetic and breeding approaches for lily (*Lilium)* crop improvement: a review. Flower Res. J. 22:39-47.

Zhang, D., X. Shen and L. Zhuo. 2011. Flower development and anatomy of *Agapanthus praecox* ssp. orientalis. Agri. Sci. China. Sci. Direct. 10:1365-1373.