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The flower development and photoperiodism of native *Kalanchoe* spp. in Taiwan

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ABSTRACT

Flower initiation and development of *Kalanchoe* spp. were investigated in this study. The sequence of events in the differentiation process was divided into 8 stages: vegetative growth, flower initiation, bract primordium, sepal primordium, petal primordium, stamen primordium, pistil primordium, and visible floret stages. Under the short-day condition, *Kalanchoe spathulata*, *Kalanchoe blossfeldiana* 'Tenorio', and *Kalanchoe garambiensis* meristems initiated differentiation after 5, 10, and 15 days, respectively. *K. spathulata*, *K. garambiensis*, and *K. blossfeldiana* 'Tenorio' differentiated from the vegetative stage into the visible flower bud stage in 45, 50, and 55 days, respectively. The development from flower initiation to the visible flower bud stage was most rapid in *K. spathulata*, followed by *K. garambiensis* and *K. blossfeldiana* 'Tenorio'. *Kalanchoe gracilis* differentiated from the vegetative growth stage into the visible flower bud stage in 105 days. Therefore, *K. spathulata* and *K. garambiensis* exhibited flower characteristics relatively early. The main differences in flowering between species/cultivars were initiation timing and the time for subsequent development. The sepal primordial stage was the most sensitive stage to the short-day condition during flower development in *Kalanchoe* spp. The minimum number of short-days needed for flowering of *K. spathulata*, *K. garambiensis*, *K. blossfeldiana* 'Tenorio', and *K. gracilis* were 15, 15, 25, and 56 days, respectively. Therefore, *K. spathulata* or *K. garambiensis* can be used to produce hybrids in relatively fewer short-days.

1. Introduction

Initiation of flowering is a highly regulated process. The switch from vegetative to reproductive development is caused by floral induction, which depends on endogenous signals as well as environmental signals such as day length (Noy-Porat et al., 2009; Tan and Swain, 2006; Erwin, 2006; Nilsson and Weigel, 1997). Time of floral initiation is determined by photoperiod and hence the anthesis date is important (Bertero et al., 1999). Differences in the time of flower initiation and subsequent development resulted in different data regarding flowering of dahlias and chrysanthemums (Barrett and De Hertogh, 1978; Doorenbos and Kofranek, 1953). Marc and Palmer (1981) found that there was no significant difference in the time of flower initiation between 2 cultivars of sunflower. Postinitiation sensitivity to day length may account for some of the variation during anthesis. Nell et al. (1982) reported that differences in the number of long nights required for flower induction were related to delayed floral initiation rather than organogenesis or maturation.

Kalanchoe cultivars are produced commercially year-around through photoperiodic manipulation. Early meristem dimensional changes had been reported to occur after photoperiodic induction (Stein and Stein, 1960). Most kalanchoe require only 2 long nights for flower initiation (Pertuit, 1992). However, meristem dimensional changes in some *kalanchoe* cultivars occur 8–10 days after photoinduction (Pertuit, 1992; Schwabe, 1985). Commercial cultivars have different responses to short-days (Carlson et al., 1979). For the production of most pot kalanchoe, Pertuit (1977) recommended 21–42 long nights for flower initiation and development.

Flower reversion refers to the phenomenon wherein the floral meristem reverts to producing leaves instead of developing into floral organs (Battey and Lyndon, 1990). Seidlova and Opatrna (1978) indicated that if the number of inductive cycles is insufficient, leaf organogenesis is restored and a correlated initiation of bud primordium resumes along with a reversal to the vegetative state. Incomplete floral initiation of *Kalanchoe* spp. results in inflorescences with less bifurcation and florets with large, more developed bracts. Reversion flowers of soybean develop with 2–5 weeks of long-day treatment (Jiang et al., 2011). Ison (1985) suggested that mature plants of *Stylosanthes guianensis* var. *guianensis* 'Schofield' require 20 short-day cycles for irrevocable commitment to floral initiation if there are going to be returned to the long-day condition.

The minimum number of inductive photoperiodic cycles necessary for floral initiation can be determined by limiting the inductive photoperiod or transferring varying numbers of plants back to

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the non-inductive condition (Damann and Lyons, 1993). Short-day plants transferred to the long-day condition after floral induction and initiation under the short-day condition can be inhibited from floral development, particularly if the transfer occurs early in floral development (Adams et al., 1998). A single photoperiodic cycle is sufficient to induce flower formation for some short-day plants, including *Pharbitis nil* 'Violet', *Chenopodium rubrum*, and *Xanthium strumarium* (Thomas and Vince-Prue, 1997; Seidlova and Opatrna, 1978). Warner (2009) indicated that between 6 and 9 short photoperiods beginning 9 days after seedling emergence are needed in order to commit *Celosia argentea* var. *plumose* plants to flowering. Eight photoinductive cycles are needed to induce *Suaeda salsa* flowering (Kefu et al., 2002). In contrast, strawberry required between 7 and 23 short-days for floral induction, depending on genotype, temperature condition, and plant age (Verheul et al., 2006). Some reports suggest that photoperiodic cycles of floral induction vary among *Kalanchoe* cultivars (Pertuit, 1992; Khoury and White, 1980; Carlson et al., 1979).

In Taiwan, there are some endemic *Kalanchoe* spp., which can potentially be used to breed new cultivars. For examples, *Kalanchoe garambiensis* is a dwarf, free-branching plant, and *Kalanchoe spathulata* is an early flowering plant. However, little information is available regarding the flower initiation and development of these species. This study was conducted to follow the process of flower initiation and development in endemic species of *Kalanchoe*. Scanning electron microscopy was used to compare the development of these species and monitor when they achieved anthesis. The minimum number of short-days needed to induce full flowering was determined. This information will enhance understanding regarding flowering regulation and contribute to breeding projects.

2. Materials and methods

2.1. Plant materials

Native *K. spathulata*, *K. garambiensis*, *Kalanchoe gracilis*, and a commercial cultivar *Kalanchoe blossfeldiana* 'Tenorio' were used in the experiment. Under natural conditions, *K. spathulata* and *K. garambiensis* anthesis occurred in late October in Taichung, Taiwan. *K. blossfeldiana* 'Tenorio' achieved anthesis in late November. However, anthesis in *K. gracilis* occurred in early February.

These plants were propagated using cuttings comprising 3 leaf pairs. The cuttings were stuck in a 2 in. plastic pot and placed in a greenhouse with a rain shelter in the experimental farm of National Chung Hsing University. The potting medium used was a mixture of 2 volumes of peat moss (Litfert; Poraisle Co., Lithuania) and 1 volume of perlite. All plants were grown under the long-day condition at 25–30 °C. Overhead watering was provided. Plants were watered twice a week. After 4 weeks, rooted cuttings were shifted to 3 in. plastic pots and grown in a greenhouse with the fan and pad cooling system. The plants were fertilized by watering with 200 mg L^{-1} soluble fertilizer (20N-8.9P-16.0K; Scotts-Sierra Horticultural Products Co., Marysville, OH, USA) twice a week and adding 0.5 g Osmocote (14N-14P_2O_5-14K_2O; Scotts-Sierra Horticultural Products Co., Marysville, OH, USA) fertilizer in each pot. Plants were watered twice a week to maintain good growth. Pesticide application and prevention treatments were provided once every 2 weeks. Prior to the experiments, the plants were illuminated using incandescent lamps (FL40D/38; China Electric Mfg. Corporation, Taiwan) between 10 PM and 2 AM every night between September 1 and March 22 to prevent flower initiation. The average day/night temperature was 30 °C/26 °C in the glasshouse with the fan and pad cooling system. Depending on the experimental condition, the plants were maintained under controlled light conditions or shifted to the natural short-day condition.

2.2. Observation of flower initiation and development

Five shoot tips of *K. spathulata*, *K. garambiensis*, and *K. blossfeldiana* 'Tenorio' were sampled at 5-day intervals. The shoot tips were taken from plants aged 5–55 days. Shoot tips of *K. gracilis* were sampled at 7-day intervals. The shoot tips were taken from plants aged 7–105 days. The leaves and leaf primordia were removed carefully under a binocular microscope in order to expose the shoot apex. Observation of the shoot tips continued until the plant reached the visible flower bud stage. The shoot apices were excised and fixed in a 2.5% glutaraldehyde solution (in 0.1 M phosphate buffer, pH 7.0) for 2 h. The specimens were rinsed with a 0.1 M phosphate buffer 3 times, followed by dehydration through an ethanol series (50%, 70%, 80%, 90%, and 100%; 10 min at each concentration). After the specimens were soaked 3 times in 100% ethanol for 10 min each time, they were dried with a critical point dryer (HCP-2; Hitachi, Tokyo, Japan). Each specimen was mounted on an aluminum stub and coated with gold using an ion coater (E1010; Hitachi, Tokyo, Japan) for 1 min. Specimens were observed and photographed at an accelerating voltage of 15 kV with a scanning electron microscope (SEM) (S-2250; Hitachi, Tokyo, Japan) (Chen et al., 2003).

2.3. Response on the number of short-days

Besides *K. gracilis*, the experimental species and cultivar were maintained under the natural short-day condition for 5, 10, 15, 20, 25, or 30 days and then transferred to the long-day condition until anthesis. *K. gracilis* plants were also maintained under the short-day condition for 7–84 days. During the long-day condition, the minimum irradiance supplied was 20 μmol m^{-2} s^{-1} PPFD between 10 PM and 2 AM *via* an incandescent light source. Data regarding days to flowering and flowering rate were recorded.

2.4. Experiment design

The completely randomized design was used in this research. Four replications were laid out for each treatment. Five plants were used in each replication. All data were analyzed with analysis of variance (ANOVA) and LSD analysis by COSTAT Software (CoHort Software, Minneapolis, USA).

3. Results

3.1. Morphology of flowers during initiation and development

On the basis of the SEM micrographs of *K. garambiensis* (Fig. 1), the sequence of events was divided into 8 stages of flower development (Table 1). In the vegetative growth stage, the apical meristem showed a typical globular shape with 2 leaf primordia at the sides (stage zero; Fig. 1A). The apex was flat, and the separated leaf primordia were obvious. The flat and wide base of the apical meristem made it easily distinguishable from the vegetative meristem (stage I; Fig. 1B). The observation indicated that the meristem had moved from the vegetative growth state to the reproductive state. Next, the apices became a dome shape. Then, the meristem developed a pair of bract primordium alone the side (stage II; Fig. 1C). In stage III, the first whorl of the floral meristem, the sepal primordia, were clearly visible after removal of the leaf and bract primordia (Fig. 1D). When the apex differentiated to this stage and the primordia continued to develop, the apex bloomed without abortion. At stage IV, petal primordia were visible in the second whorl of the floral meristem after removal of the sepal and bract primordia (Fig. 1E). Four petals developed in the right-angle position. Eventually, the stamen and pistil primordia developed in the third and fourth whorls of the floral meristem (stages V and VI; Fig. 1F and