Exogenous Application of Gibberellic Acid Improves Flowering in Kalanchoë

Livia Lopes Coelho¹, Amalia Fkiara, Kathryn Kuligowska Mackenzie, Renate Müller, and Henrik Lütken
Crop Sciences, Department of Plant and Environmental Sciences, University of Copenhagen, Højbakkegård Allé 9-13, Tæstrup 2630, Denmark

Abstract. Kalanchoë is an economically important genus comprising numerous potted plants and recently is also emerging as cut flowers. However, the lack of information about flower-inducing factors limits the number of species that can be used in commercial production and breeding programs. Therefore, the aim of this study was to investigate the effects of exogenous application of gibberellic acid (GA3) on flower induction and flowering quality of Kalanchoë longiflora and Kalanchoë pinnata. The experiment was conducted under a short day (SD) photoperiod with a day temperature of 22 °C and a night temperature of 15 °C for 8 weeks. The treatments consisted of four applications of either 0.25 or 0.50 µg of GA3 per plant per week, providing a total of 100 µg or 200 µg GA3 per plant for the control. A lof GaG solution containing 1% agarose was applied to the shoot apex using a pipette. For both species, flowering was enhanced by the GA3 treatments compared with the control plants. Gibberellin-treated plants flowered earlier, produced more inflorescences, and exhibited an increased number of flowers compared with the control plants. Moreover, the GA3 treatments in K. longiflora delayed the appearance of wilted flowers. Plant height increased in plants that received GA3, but the number of nodes did not differ from the control plants. Thus, we conclude that the application of GA3 improves flowering of Kalanchoë species and can be a useful tool for the production of cut flower cultivars.

Floriculture is a very dynamic industry, and besides being able to produce plants throughout the year, the development of new cultivars is one of the most important aims of this sector (Kuligowska et al., 2016; Noman et al., 2017). Control of flowering time is crucial to guarantee that the plants will flower at the same time, and thus fulfill the market demand, as well as for cross-pollination programs.

Plants from the Kalanchoë genus have been cultivated as ornamental plants in Europe since the beginning of the 20th century (van Voorst and Arends, 1982), and Kalanchoë is presently the second most economically important potted plant in Europe with a turnover of 67 million Euros in 2016 (Royal Flora Holland, 2016). The Kalanchoë genus comprises around 139 species (Descoings, 2005), with a great variety of morphological traits. However, mainly Kalanchoë blossfeldiana and its interspecific hybrids are the popular ornamental plants of economic importance (Kuligowska et al., 2015). According to Currey and Erwin (2010, 2011), flower induction and the control of flowering time are difficult in several Kalanchoë species, and the lack of knowledge on the flower-inducing factors can limit the use of new plants by breeding companies.

Flowering is a complex process that assures successful plant reproduction. The flowering time needs to be tightly controlled by the environment to assure effective use of energy and available resources for the developing plant (Bratzel and Turk, 2015). In many plants, the flowering response is closely linked to endogenous hormonal levels, which is directly or indirectly associated with their developmental stage (Bratzel and Turk, 2015). Gibberellins are a class of hormones that play multiple roles in plant growth and development, such as seed germination, stem elongation, leaf expansion, shortening of juvenile phase and floral transitioning, and fruit patterning (Hedden, 2016). Gaskin et al. (1973) observed that the level of gibberellins increased considerably in Kalanchoë daigremontiana when flower formation was induced. Moreover, high concentration of GA3 in the leaves of K. daigremontiana was a prerequisite for the production of the floral stimulus (Gaskin et al., 1973). In general, Kalanchoë plants are responsive to SD or long SD (LSD) photoperiods, i.e., flowering will occur after a minimum number of SD cycles or when a period of long days (LDs) precedes the SDs (LSD plants) (Currey and Erwin, 2010, 2011; Zeevaart and Lang, 1962). However, other factors, combined with the photoperiodic requirement, such as cold night temperature can enhance flowering in Kalanchoë (Kroon et al., 1989; Sharma, 1970, 1973; Spear and Thimmann, 1954).

Kalanchoë longiflora is a succulent shrub from South Africa, up to 40 cm tall with attractive light bluish-gray leaves and quadrangular stem. In nature, the flowers appear during autumn to winter (Notten, 2014). The color of the foliage, length and shape of the stem, and the resistance to drought are very desirable traits that could be introduced to new Kalanchoë cultivars. Kalanchoë pinnata is a perennial succulent naturalized in many tropical and subtropical parts of the world. It grows up to 2 m height and exhibits dark green leaves. The bell-like pendant flowers exhibit a reddish-purple color, which is the main ornamental trait of this species (Descoings, 2005). Furthermore, K. pinnata can also be explored for medical purpose as the leaf extract exhibits antimicrobial activity (Quazi et al., 2011).

The factors underlying flowering in K. longiflora and K. pinnata are not well understood. Therefore, this study aimed to evaluate the effect of GA3 on flowering in both species.

Material and Methods

The experiment was conducted from June to Sept. 2016 using 3-month-old plants of K. longiflora and K. pinnata, obtained from cuttings from mother plants from a single genotype kept under extended LD photoperiod. The cuttings with one pair of expanded leaves were treated with rooting powder containing 2% alpha-naphthyl-acetic acid (Floromann Pudder “B”); Novotrade, Herlev, Denmark), rooted in peat substrate with sphagnum, clay, and silica (Weibulls Hortal AB, Hammenhög, Sweden). The air temperature during rooting and establishment of the plants was 22.5 ± 3 °C. The plants were grown under natural LD photoperiod for 3 months before the start of the experiment. Three months after propagation, the plants were transferred to a climate chamber (VEPHQ 5/2000; Heraeus V'Lis, Chemical Industries) with white LED lamps (FL300 SUNLIGHT fixture from Fiona Lighting; Sennatic A/S, Sønderø, Denmark), with an irradiance of ≈250 μmol·s⁻¹·m⁻² simulating the SD photoperiod (8 h day/16 h night) with a day temperature of 22 °C and a night temperature of 15 °C.

Irrigation was performed twice a week by an ebb-and-flow system during the growth period and by a drip system during the SD period, with tap water containing 100 g·L⁻¹ of macronutrients (10.4% NO₃⁻, 3.6% NH₄⁺), 2.9% P₂O₅, 23% K₂O. Pioner NPK Makro 14–3–23 + Mg “BLÅ”-Azelis, Chemical Industries, Antwerp, Belgium) and 10 mL·L⁻¹ micronutrients (Pioner Mikro med jern; Aze- lis, Chemical Industries).

After 1 week of acclimatization period under SDs, 24 plants of each species were
divided into three groups of eight plants, corresponding to the total amount of GA₃ (Sigma-Aldrich, St Louis, MO) received (0, 100, and 200 µg/plant). The total amount of GA₃ per plant was divided in four applications, performed once a week. In each application, each plant received 0.25 or 0.5 µg·µL⁻¹ of GA₃ in a volume of 100 µL of 1% (w/v) agarose solution (VWR Life Science Amresco LLC, Solon, OH) to facilitate adhesion. The agarose was dissolved in ddH₂O by bringing the solution to boil.

The solution of GA₃ was added to the final concentration when the agarose solution cooled down. The control plants were treated with the agarose solution without GA₃. The solution was applied to the shoot apical meristem using a pipette. Before the application of the hormone solution, the meristem and first pair of leaves were sprayed with a surfactant 0.25% (v/v) (Tween 20, Sigma-Aldrich, Steinheim, Germany) to lower superficial tension of the leaves by removing the waxy cuticle, and thus increasing absorption.

After 8 weeks under SDs, the plants were transferred back to the greenhouse and kept under LDs for ≈6 weeks or until the appearance of the first wilted flower on each plant. Flowering was regularly monitored for the opening of the first flower, which was considered when the petals (for K. longiflora) or sepals (for K. pinnata) started to separate. The number of inflorescences and flowers were evaluated when the first flower wilted, i.e., when the flower tube began to shrink.

The experimental setup consisted of two independent repetitions placed in time, with eight plants per treatment in each. There was no significant difference between data obtained from the two repetitions; therefore, data were pooled for further statistical analysis (n = 16). The sample size of the flowering response parameters varied in the control treatment because not all plants flowered (n = 12). The analysis of percentage of flowering was performed by χ² test (P ≤ 0.05) using the software R (RStudio Team, 2015), and all other statistical analyses were performed using the two-tailed Student’s t test with 95% degree of confidence (P ≤ 0.05) using Excel Microsoft Office package.

### Results

The application of GA₃ promoted flowering in all plants of both species. In contrast, not all plants flowered in the control treatment (Table 1). In both species, the hormonal treatment promoted significantly earlier flowering compared with the control, but there was no significant difference between the plants treated with 100 and 200 µg of GA₃ (Fig. 1).

Compared with control plants, GA₃ positively affected the number of inflorescences and flowers produced per plant (Figs. 2 and 5). The numbers of inflorescences in both species were significantly higher in plants treated with GA₃, but it did not differ between different hormone concentrations (Fig. 2A and B). The number of flowers per plant in K. longiflora increased significantly as the concentration of GA₃ increased (Fig. 2C). However, in K. pinnata, the number of flowers was significantly higher in the plants treated with 200 µg of GA₃ than the control, whereas plants treated with 100 µg GA₃ did not differ statistically from the control or from the 200 µg treatment (Fig. 2D).

In K. longiflora, the treatments with GA₃ delayed the wilting of flowers compared with the control plants, but no difference was observed between the 100 and 200 µg concentrations of GA₃ (Fig. 3). In K. pinnata, the use of GA₃ did not affect the flower longevity. The first wilted flower appeared ≈15 d after the first open flower (data not shown). After 8 weeks in SD, plant height and number of nodes were evaluated. Kalanchoe longiflora and K. pinnata treated with GA₃ were significantly taller compared with the control, but the heights of plants did not differ statistically between 200 and 100 µg (Fig. 4). The number of nodes did not differ between treatments in both species (data not shown). At the end of the SD period, the plants of K. longiflora and K. pinnata had ≈11 and 14 nodes, respectively.

Even though the two concentrations of the hormone did not produce statistical difference in most traits evaluated, the 200 µg/plant concentration promoted increased number of flowers; thus more pollen was available for cross-pollination.

### Table 1. Effect of different concentrations of gibberellin acid (GA₃) on the percentage of plants flowering (%) in Kalanchoe longiflora and Kalanchoe pinnata.

| GA₃ (µg/plant) | K. longiflora | K. pinnata |
|---------------|---------------|------------|
| 0             | 75 b          | 75 b       |
| 100           | 100 a         | 100 a      |
| 200           | 100 a         | 100 a      |

Data were compared using χ²-test (P ≤ 0.05), n = 16.

Fig. 1. Effect of different concentrations of gibberellin acid (GA₃) on the number of days until the appearance of the first open flower (A) Kalanchoe longiflora and (B) Kalanchoe pinnata. Values followed by different letters are significantly different (P ≤ 0.05) according to t test. Bars represent the se.

Fig. 2. Effect of different concentrations of gibberellin acid (GA₃) on the number of inflorescences of (A) Kalanchoe longiflora and (B) Kalanchoe pinnata, and number of flowers of (C) K. longiflora, and (D) K. pinnata. Values followed by different letters are significantly different (P ≤ 0.05) according to t test. Bars represent the se.
Grows and breeders of ornamental plants experience difficulties in controlling flowering in some species of the Kalanchoe genus (Currey and Erwin, 2010, 2011), including K. longiflora and K. pinnata. In this study, we aimed at inducing flowering in these species by providing SD conditions and to examine the effect of exogenous GA3 application on flower response.

The photoperiodic treatment combined with the cool night temperature was sufficient to promote flowering in both K. longiflora and K. pinnata when 3-month-old plants were subjected to SD treatment. In contrast, our previous study did not succeed to induce flowering in 6-week-old plants of K. longiflora under SD conditions and different night temperatures (Coelho et al., 2015). Thus, the differences in flower induction can be attributed to the age of the plants.

Gibberellin plays an important role in the process of flowering and flower development, and it can have an effect on shortening the juvenile stage of plants (Evans and Poethig, 1995; Matías-Hernández et al., 2016). In our study, not all plants flowered in the control group in both species (not treated with GA3) (Table 1), suggesting that they were in the end of the juvenile phase and GA3 might be involved in the shortening of juvenility rather than in the transition to flowering. However, to our knowledge, there is no information available regarding the length of the juvenile period in K. longiflora. Similar to our results, GA3 has been reported to reduce the juvenile period of Tagetes erecta (Kumar et al., 2014) and stimulate the rate of flower development of Vitis labrusca × Vitis vinifera cultivar Kyoho (Cheng et al., 2015).

In this study, the flowering of K. pinnata took place when the plants were 7 months old, contrasting with a previous report that described flowering in this species to occur when the plants were 2 years old (Wadhi and Ram, 1967). The same authors (Wadhi and Ram, 1967) reported that the application of GA3 on the shoot tips or juvenile leaves of 3-month-old plants of K. pinnata can have an effect on shortening the juvenile stage. Their results demonstrated that the plants treated with 5 or 15 μg of GA3 per plant did not enter the reproductive phase, but plants that received 50 or 150 μg of GA3 formed flowers after 6 or 7 weeks (Wadhi and Ram, 1967).

In our study, the plants treated with 100 and 200 μg GA3 per plant in total produced flowers 8–9 weeks after the last application. The difference between the numbers of weeks until the appearance of the first flower can be attributed to the difference in the number of applications that plants received in each study because flowering response to GA3 depends on the concentration of the hormone, application time, and the number of applications (Boyle et al., 1994).

An old study in K. daigremontiana demonstrated that the application of GA3 to mature leaves was more efficient to trigger flowering than when the application was made to the youngest leaf pair and shoot tip (Zeevaart, 1969). However, in the present study, we chose to apply GA3 to the shoot apical meristem and youngest pair of leaves, based on the fact that the mature leaves of K. pinnata detached very easily and there was a risk of the GA3 not remaining on the plant enough time to produce an effect. It is known that GA3 induces flowering via different floral pathway components in the leaves and shoot apical meristem (Hyun et al., 2016). Furthermore, GAs induce the expression of the floral integrator gene SUPPRESSOR OF OVEREXPRESSION OF CONSTANS 1 and the floral meristem identity gene LEAFY, both expressed in the shoot apex, thus promoting flowering (Porri et al., 2012).

The treatments with GA3 promoted early flowering in K. longiflora and K. pinnata, which flowered 11–13 d and 4–5 d earlier than the controls, respectively (Fig. 1A and B). Earlier flowering due to GA3 treatment was also observed in gladiolus (Sarkar et al., 2014), where plants treated with GA3 flowered 3.74 and 5.87 d earlier than the control. The early flowering made it possible to achieve a desirable improvement in the production of ornamental plants; it not only reduces production costs by saving energy and resources, but also makes production more effective, allowing more production cycles per time frame. Even though the flowering was not highly accelerated in K. pinnata, it is worth to mention that each day in production is costly in terms of greenhouse space, light, and heating, especially in the Nordic countries, such as Denmark.

Moreover, our results demonstrated that GA3 treatment positively affected the number of inflorescences and flowers in K. longiflora (Figs. 2A and C, and 5A) and K. pinnata (Figs. 2B and D, and 5B). In addition, GA3 application in K. longiflora increased flower longevity (Fig. 3). These effects were also observed in other species; treatments with GA3 enhanced the number of flowers and flower longevity in different varieties of chrysanthemum (Dimpala et al., 2015) and increased the number of florets per inflorescence in gladiolus (Sarkar et al., 2014).

The plant height was increased in both species by the GA3 application (Figs. 4 and 5). The number of nodes did not differ from plants not receiving the treatments (data not shown). Gibberellins stimulate cell division and expansion in response to light, thus promoting stem elongation (Gupta and Chakrabarty, 2013). The stem elongation stimulated by the GA3 treatment is not directly related to flowering; however, in many species, transition to flowering triggers the stem elongation (Hedden, 2016). Even though Kalanchoe species are traditionally used as potted plants, lately, there is increasing interest in using them as cut flowers because of their outstanding vase life (Queen, https://www.queen.dk/en). Thus, GA application can also be applicable in cut flower production because long stems are a very desirable trait.

In the present study, we demonstrated that the exogenous application of GA3 under the SD photoperiod can significantly reduce time to flowering and enhance the flowering response in terms of the percentage of plants flowering, number of inflorescences, and number of flowers in K. longiflora and K. pinnata. Moreover, the GA3 treatment extended the flower longevity in K. longiflora. Similarly, Dong et al. (2017) demonstrated that the photoperiodic pathway plays a major role in regulating flowering.
role in determining flowering time in chrysanthemums, whereas the GA3 pathway acted as a subsidiary for flowering. In addition, this is the first study reporting successful induction of flowering in *K. longiflora*, whereas the GA3 pathway acted as a role in determining flowering time in chrysanthemums (Cardoso et al., 2012; Cheng et al., 2015; Kumar et al., 2014; Sarkar et al., 2014; Wadhi and Ram, 1967). However, the proposed methodology can be suitable for flowering induction in the breeding programs. The use of GA3 application in commercial production of *Kalanchoe* would require a more convenient hormone application such as spraying plants, which is more cost- and time efficient (Cardoso et al., 2012; Chen et al., 2003).

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