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SHORTENING THE JUVENILE PHASE FOR FLOWERING IN *KALANCHOE PINNATA* PERS.

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Summary. Plants of *Kalanchoe pinnata* flower normally at the end of 2 years. Flowering in the juvenile phase (3- and 9-month-old plants) has been induced by application of gibberellin (GA) either to the shoot tip and the youngest pair of leaves, or to the third leaf. Three-month-old plants required more exogenous GA (50 μg/plant) than 9-month-old plants (5 μg/plant). The simultaneous application of the growth retardant (2-chloroethyl)trimethylammonium chloride = CCC *via* the roots did not interfere with GA-induced flowering but overcame the inhibitory effects produced by a high concentration of GA (150 μg/plant) when applied alone.

Introduction

Flowering plants pass through a series of distinct phases during their development. That phase during which a shift from the vegetative to the reproductive state cannot be induced (even under conditions otherwise favourable for flower formation) is the juvenile phase. It is also called minimum vegetative stage, ripeness-to-flower stage, immature condition, or sterile stage.

In some plants the juvenile phase can be shortened either by increasing the light intensity (HIGAZY, 1962), or by application of auxin (DE ZEEUW and LEOPOLD, 1955). There is evidence that applied gibberellins may also shorten the juvenile phase (KATO *et al.*, 1958; RESENDE and VIANA, 1959; PENNER, 1960; PHARIS *et al.*, 1965).

Under the conditions of Delhi, plants of *Kalanchoe pinnata* Pers. (syn. *Bryophyllum calycinum* Salisb.) flower at the end of the second year, after having borne at least 37 pairs of leaves. The objective of this investigation was to reduce the juvenile phase in this plant by gibberellin treatment and to correlate the flowering response with the amount of gibberellin used. An attempt was also made to study if gibberellin-induced flowering in juvenile plants was in any way affected by the growth retardant, (2-chloroethyl)trimethylammonium chloride (CCC).

Material and Methods

Mature leaves of *K. pinnata*[1] were planted in pots and were maintained under field conditions of light and temperature prevailing from February, 1964 to March, 1965 in the Botanical Garden, University of Delhi. By proper manipulation of the

[1] This plant is easily propagated by leaf cuttings.

propagation time, plants in the juvenile phase (3 and 9 months old) were obtained for treatment in the first week of November, 1964. The 3-month-old plants were 3.6 ± 0.5 cm tall with 6 pairs of leaves; the 9-month-old plants were 36.7 ± 1.6 cm tall with an average of 12 leaf pairs.

In one set of experiments aqueous solutions of gibberellin A_3 (gibberellic acid; GA)[2], containing Tween 80 (0.05%) as wetting agent, were applied to the shoot tip and the youngest leaf pair of 3- and 9-month-old plants. Four concentrations of GA (1, 3, 10 and 30 µg/0.1 ml) were used and 0.1 ml applied per treatment. Five treatments were given, on alternate days, the total amount of GA received by each plant thus being 5, 15, 50 or 150 µg respectively. Controls were treated with distilled water containing Tween 80.

In another set, GA was applied to 9-month-old plants through the third leaf (from the apex). The leaf was first washed with a dilute solution of a detergent, Teepol B-300, and rinsed with distilled water. An aqueous solution of GA (15, 20, 25 and 30 mg/l) was applied to either surface and the leaf was enclosed within a polythene bag into which 10 ml of GA of the corresponding concentration were poured. The bag was tied around the petiole by a thread, with the lamina dipping into the liquid. Leaves of control plants were washed with Teepol B-300 and were enclosed in bags containing distilled water. In the first and second sets of experiments, 10 plants were used per treatment.

In the third set of experiments 9-month-old plants were given simultaneously 100 ml of CCC[3] at a concentration of 2000 mg/l, applied through the soil, and 0.1 ml of GA solution (1, 3, 10 and 30 µg/0.1 ml), applied to the shoot tip and the youngest pair of leaves enclosing it. Five treatments were given on alternate days and 5 plants were used per treatment. Thus, each plant received 1000 mg of CCC and 5, 15, 50 or 150 µg of GA.

At the beginning of the experiment the youngest visible leaf pair was marked as leaf pair No. 0. At the end of the experiment the number of leaf pairs and bracts developing above the marked pair were counted. The number of flowers produced was also recorded.

Results

Under the conditions existing in Delhi, plants of *Kalanchoe pinnata* start flowering by the end of November when the day length averages 11 hours[4]. During this period the average day temperature is nearly 26°C and night temperature goes down to 9°C. Flowering continues through the middle of March. Flower development is normal up to anthesis and pollination but ovaries and ovules remain arrested, not developing into fruits and seeds.

Application of GA to Shoot Tix and Youngest Leaf Pair. Treatment with GA produced the usual vegetative responses, that is, lighter leaf colour, increase in height and internode length, and also an increase in leaf-pair number.

[2] GA was obtained from the Imperical Chemical Industries Ltd., Welwyn, England.

[3] This substance was kindly supplied by American Cyanamid Co., Stamford, Conn., USA.

[4] We have not done any experiments to establish its precise photoperiodic requirement. It may be either a short-day or a long-short-day plant.

Three-month-old controls and plants treated with 5 μg and 15 μg of GA per plant did not enter the reproductive phase but some of the plants treated with 50 μg and 150 μg formed flowers after 6 or 7 weeks (Fig. 1 A). More flowers were produced on plants treated with 150 μg than with 50 μg of GA (Table 1). The flowers were morphologically similar to those normally found in 2-year-old plants.

Table 1

Influence of GA on stem growth and flowering in 3-month-old Kalanchoe pinnata plants*
Height at treatment: 3.6±0.5 cm. Number of leaf pairs at treatment: 6. Growth period: Three months after treatment.

Total GA per plant (μg)	Total height (cm)	No. of leaf pairs** after treatment	No. of plants flowering†	No. of flowers per plant
0***	14.8±0.8	5.2±0.3	0	0
5	27.0±2.0	6.2±0.6	0	0
15	28.4±2.2	7.3±0.9	0	0
50	29.8±4.0	9.9±0.9	5	25
150	38.5±3.6	11.2±0.9	6	33

*Applied five times to shoot tip and youngest pair of leaves.
** Including leaves and bracts.
*** Received distilled water containing Tween 80.
† Out of ten plants.

In 9-month-old plants, as little as 5 μg/plant of GA were sufficient to induce flowering 4 weeks after treatment (Fig. 1 B). The number of plants flowering at this concentration was 7 but at the other concentrations all 10 plants came to flower. The controls remained vegetative throughout (Table 2).

The number of flowers produced per plant increased with an increase in the GA dosage (Table 2), the greatest number developing at 50 μg/plant (Fig. 2 A). At 150 μg/plant, the flower buds showed symptoms of misdevelopment (Fig. 2 B); probably in connection with this, the number of flowers was lower than that with 50 μg of GA. Otherwise, the development of the flowers was comparable to that in 2-year-old plants.

Foliar Application of GA. The flowering response of 9-month-old plants in which GA was supplied through the leaf was comparable to that in 9-month-old plants treated with GA at the shoot tip. The optimum

Fig. 1 A—C. Induction of flowering in 3-month-old (A) and 9-month-old (B) plants of *Kalanchoe pinnata* by *GA* application to shoot tip and youngest pair of leaves, and in 9-month-old plants by foliar application of *GA* (C). Photographed 2 months after treatment. A, × 0.1; B, × 0.07; C, × 0.08

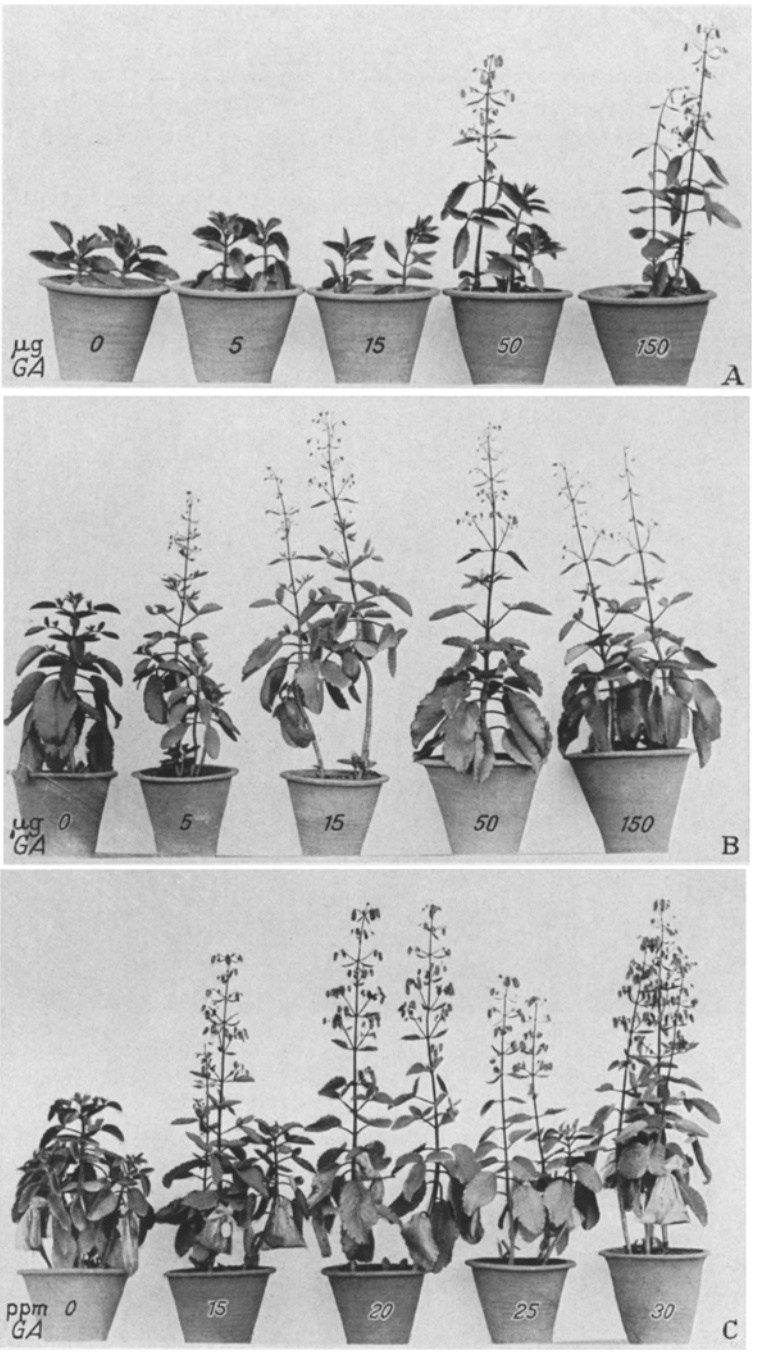

Fig. 1 A—C (legends see p. 30)

concentration of GA, as assayed by stem elongation, number of flowering plants and number of flowers per plant (Fig. 1C), was 15—20 mg/l. At 25 mg/l and 30 mg/l, both stem length and flower number tended to be lesser.

Effect of CCC and GA on Stem Growth and Flowering. Nine-month-old plants treated with CCC alone remained in the vegetative state and stem

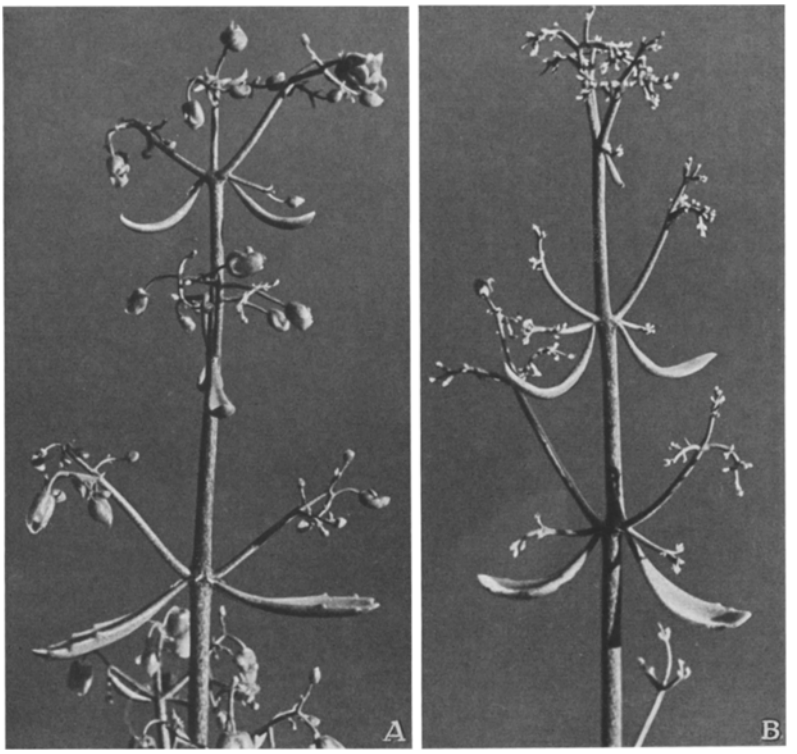

Fig. 2A and B. Portions of inflorescences developed on 9-month-old plants after application of 50 μg (A) and 150 μg (B) of *GA* to shoot tip and youngest pair of leaves. The development of flower buds is arrested in B. Photographed 2 months after treatment. × 0.5

length and leaf number were not affected (Table 3). Application of GA to CCC-treated plants resulted in marked internodal elongation, increase in leaf number, appearance of the characteristic light green colouration of the foliage, and initiation of flowering. Partial flowering occurred with as little as 5 μg of GA per CCC-treated plant. In the previous experiments, where GA alone had been applied to the shoot tip of 9-month-old plants, it was observed that 150 μg of GA proved toxic to the development of flower buds. In combination with CCC, flowering was normal and there was an increase in the number of flowers (Tables 2 and 3).

Table 2

Influence of GA on stem growth and flowering in 9-month-old Kalanchoe pinnata plants*

Height at treatment: 36.7 ± 1.6 cm. Number of leaf pairs at treatment: 12. Growth period: Three months after treatment.

Total GA per plant (µg)	Total height (cm)	No. of leaf pairs** after treatment	No. of plants flowering†	No. of flowers per plant
0***	39.5 ± 2.0	4.8 ± 0.8	0	0
5	50.3 ± 5.0	6.4 ± 0.9	7	30
15	82.0 ± 2.2	7.1 ± 0.1	10	51
50	76.1 ± 3.2	7.3 ± 0.1	10	69
150	64.4 ± 4.0	7.0 ± 0.1	10	59

* Applied five times to shoot tip and youngest pair of leaves.
** Including leaves and bracts.
*** Received distilled water containing Tween 80.
† Out of 10 plants.

Table 3. *Flowering response and stem growth of 9-month-old Kalanchoe pinnata plants treated with CCC and GA*

Height at treatment: 36.7 ± 1.6 cm. Number of leaf pairs at treatment: 12. Growth period: Four months after treatment.

Total amount of chemicals applied/plant		Total height (cm)	No. of leaf pairs** after treatment	No. of plants flowering†	No. of flowers per plant
CCC* (g)	GA (µg)				
0	0***	44.6 ± 5.6	0.6 ± 0.5	0	0
1	0***	46.2 ± 2.5	5.8 ± 0.4	0	0
1	5	70.0 ± 8.7	7.8 ± 0.2	3	26.8
1	15	80.6 ± 2.5	8.6 ± 0.2	5	57.0
1	50	80.4 ± 3.3	8.2 ± 0.4	5	76.0
1	150	95.6 ± 7.9	8.6 ± 0.2	5	104.0

* Supplied through roots in aqueous solution.
** Including leaves and bracts.
*** Received distilled water containing Tween 80.
† Out of 5 plants.

Discussion

The juvenile phase of perennials may extend from a few months to several years; in annuals and biennials its duration is maximally a few months. In some annuals like *Pharbitis nil* (KUJIRAI and IMAMURA, 1958) the photoperiodic stimulus may be perceived even by the cotyledons. In *Chenopodium rubrum* (CUMMING, 1959) the minimum number of leaves necessary for the plants to flower is two. In *Bryophyllum daigremontianum* the juvenile phase may last for one year (PENNER, 1960) or

two years (see ZEEVAART, 1962) and the plants must bear at least 10—12 pairs of leaves before they are ready to flower (ZEEVAART, 1962). The plants of *Kalanchoe pinnata* flower in nature, under the conditions of Delhi, only when they are 2 years old and have produced at least 37 pairs of leaves.

ZEEVAART (1958) suggested the following alternative explanations for the existence of a juvenile phase: (1) The leaves on the juvenile plant are less sensitive to photoinduction and therefore either fail to produce the floral stimulus, or are producing it in insufficient quantities; (2) the terminal and axillary buds are unable to respond to the floral stimulus.

Evidence in support of the first proposal comes from work on *Perilla crispa* (ZEEVAART, 1958) and *Lolium temulentum* (EVANS, 1960). ZEEVAART (1962) showed that the second supposition was untenable in *Bryophyllum daigremontianum*. By grafting juvenile plants onto flowering donor stocks he demonstrated that the growing tips were fully capable of responding to the floral stimulus. We have found that juvenile plants of *K. pinnata* flower in response to GA treatment. Our experiments do not permit, however, a decision as to whether this flowering response is due to increased flower-stimulus production by the leaves or to increased sensitivity of the buds.

The demonstration of naturally occurring gibberellin-like substances in the plants (see KNAPP, 1963), induction of flowering by plant extracts containing gibberellins (see LANG, 1965), and increase in the endogenous levels of gibberellins during the change from the vegetative to the reproductive phase (HARADA and NITSCH, 1959; LANG, 1960; NICHOLLS and LANG, 1964; SKENE and LANG, 1964) suggest that there is a definite relationship between flowering and gibberellin metabolism. The failure of flower induction in the juvenile phase could be attributed either to a total absence or to an insufficient quantitiy of native gibberellins. In agreement with these ideas is the report of RESENDE and VIANA (1959) who obtained induction of flowering by GA application in seedlings of *Bryophyllum proliferum* which normally require 6 years to flower. Three-month-old plants of *B. daigremontianum* required as little as 0.15 µg of GA to enter the reproductive phase (PENNER, 1960). KATO *et al.* (1958) induced cone development (flowering) in seedlings and adult plants of *Cryptomeria japonica* by GA treatment. PHARIS *et al.* (1965) obtained similar results in *Cupressus arizonica* and found, moreover, that younger seedlings required more exogenous GA than older ones. The present results with *K. pinnata* also show clearly that younger plants (3-month-old) need more exogenous GA than older ones (9-month-old), namely, 50 µg as compared to 5 µg. These findings, therefore, suggest that younger plants have low endogenous levels of GA which are insuffi-

cient for flower formation. Thus it is possible to induce flowering in the juvenile phase by exogenous supply of GA.

The growth retardant CCC induces effects opposite to those of GA. In recent years KENDE *et al.* (1963) and NINNEMANN *et al.* (1964), working with cultures of *Fusarium moniliforme* have demonstrated that CCC does not destroy or inactivate preformed GA but blocks its biosynthesis. HARADA and LANG (1965) and BALDEV *et al.* (1965) came to the conclusion that the same mechanism is operative in higher plants and accounts for the effects of CCC and certain other retardants on growth and other developmental processes in these plants. Our results also indicate that CCC does not interfere with exogenously applied GA. They are in agreement with the conclusion that juvenile plants of *K. pinnata* are deficient in endogenous GA since in such plants application of an inhibitor of GA biosynthesis should have little if any effect on growth and development.

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Literature

BALDEV, B., A. LANG, and A. O. AGATEP: Gibberellin production in pea seeds developing in excised pods: effect of growth retardant AMO-1618. Science **147**, 155—157 (1965).

CUMMING, B. G.: Extreme sensitivity of germination and photoperiodic reaction in the genus *Chenopodium* (Tourn.) L. Nature (Lond.) **184**, 1044—1045 (1959).

EVANS, L. T.: Inflorescence initiation in *Lolium temulentum* L. I. Effect of plant age and leaf area on sensitivity to photoperiodic induction. Aust. J. biol. Sci. **13**, 123—131 (1960).

HARADA, H., and A. LANG: Effect of some (2-chloroethyl)trimethylammonium chloride analogs and other growth retardants on gibberellin biosynthesis in *Fusarium moniliforme*. Plant Physiol. **40**, 176—183 (1965).

—, and J. P. NITSCH: Changes in endogenous growth substances during flower development. Plant Physiol. **34**, 409—415 (1959).

HIGAZY, M. K. M. T.: Shortening the juvenile phase for flowering. Meded. LandbHoogeschool Wageningen **62** (8), 1—53 (1962).

KATO, Y., N. FUKUHARU, and R. KOBAYASHI: Stimulation of flower bud differentiation of conifers by gibberellin. *In:* Abstr. 2nd Meeting Japan Gibberellin Research Ass. 1958, p. 67—68.

KENDE, H., H. NINNEMANN, and A. LANG: Inhibition of gibberellic acid biosynthesis in *Fusarium moniliforme* by AMO-1618 and CCC. Naturwissenschaften **50**, 599—600 (1963).

KNAPP, R.: Gibberelline. *In:* Modern methods of plant analysis (H. F. LINSKENS and M. V. TRACEY, eds.), vol. 6, p. 203—218. Berlin-Göttingen-Heidelberg: Springer 1963.

KUJIRAI, C., u. S. IMAMURA: Über die photoperiodische Empfindlichkeit der Kotyledonen von *Pharbitis nil* Chois. Bot. Mag. (Tokyo) **71**, 408—416 (1958).

LANG, A.: Gibberellin-like substances in photoinduced and vegetative *Hyoscyamus* plants. Planta (Berl.) **54**, 498—504 (1960).

— Physiology of flower initiation. *In*: Encyclopedia of plant physiology (W. RUHLAND, ed.), vol. XV/1, p. 1380—1536. Berlin-Heidelberg-New York: Springer 1965.

NICHOLLS, P. B., and A. LANG: Endogenous gibberellin changes and internode growth in the barley inflorescence. Plant Physiol. **39** (Suppl.), XXVI (1964).

NINNEMANN, H., J. A. D. ZEEVAART, H. KENDE, and A. LANG: The plant growth retardant CCC as inhibitor of gibberellin biosynthesis in *Fusarium moniliforme*. Planta (Berl.) **61**, 229—235 (1964).

PENNER, J.: Über den Einfluß von Gibberellin auf die photoperiodisch bedingten Blühvorgänge bei *Bryophyllum*. Planta (Berl.) **55**, 542—574 (1960).

PHARIS, R. P., M. D. E. RUDDAT, C. C. PHILLIPS, and E. HEFTMANN: Precocious flowering of Arizona cypress with gibberellin. Canad. J. Bot. **43**, 923—927 (1965).

RESENDE, F., and M. J. VIANA: Gibberellin and sex expression. Port. Acta biol. A **6**, 77—98 (1959).

SKENE, K. G. M., and A. LANG: Native gibberellins and flower formation in *Bryophyllum daigremontianum*. Plant Physiol. **39** (Suppl.), XXXVII (1964).

ZEEUW, D. DE, and A. C. LEOPOLD: Altering juvenility with auxin. Science **122**, 925—926 (1955).

ZEEVAART, J. A. D.: Flower formation as studied by grafting. Meded. LandbHoogeschool Wageningen **58** (3), 1—88 (1958).

— The juvenile phase in *Bryophyllum daigremontianum*. Planta (Berl.) **58**, 543—548 (1962).

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