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Gibberellin Is Required for Flowering in *Arabidopsis thaliana* under Short Days[1]

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ABSTRACT

Mutants of *Arabidopsis thaliana* deficient in gibberellin synthesis (*ga1-3* and *ga1-6*), and a gibberellin-insensitive mutant (*gai*) were compared to the wild-type (WT) Landsberg *erecta* line for flowering time and leaf number when grown in either short days (SD) or continuous light (CL). The *ga1-3* mutant, which is severely defective in *ent*-kaurene synthesis because it lacks most of the *GA1* gene, never flowered in SD unless treated with exogenous gibberellin. After a prolonged period of vegetative growth, this mutant eventually underwent senescence without having produced flower buds. The *gai* mutant and the "leaky" *ga1-6* mutant did flower in SD, but took somewhat longer than WT. All the mutants flowered readily in CL, although the *ga1-3* mutant showed some delay. Unlike WT and *ga1-3*, the *gai* mutant failed to respond to gibberellin treatment by accelerating flowering in SD. A cold treatment promoted flowering in the WT and *gai*, but failed to induce flowering in *ga1-3*. From these results, it appears that gibberellin normally plays a role in initiating flowering of *Arabidopsis*.

Exogenous GA[2] has been shown to promote the switch from vegetative growth to flowering in a variety of plants. Most species in which applied GA can induce flowering are long-day or cold-requiring plants, and many of these normally grow as rosettes under noninductive conditions (17). Exogenous GA fails to stimulate flowering in many other angiosperms (11). It is still unclear what role, if any, endogenous GA plays in floral induction. In a very few cases, such as *Samolus parviflorus*, an inhibitor of GA biosynthesis has been shown to prevent flowering in a GA-reversible manner (17). In other species, however, the application of GA synthesis inhibitors failed to block flowering (8). For these plants, it remains uncertain whether GA is normally involved in the induction of flowering.

Mutants that are specifically impaired in GA production have been obtained in a number of species. The GA-deficient mutants of rice (*Oryza sativa*), maize, *Arabidopsis*, pea (*Pisum sativum*), and tomato (*Lycopersicon esculentum*) all flower readily under normal growth conditions (11), although these flowers may show various structural defects, depending on the species (5, 10). A GA-deficient mutant of *Brassica rapa* takes somewhat longer to flower than normal (16), as does a GA-deficient mutant of *Thlaspi arvense* (9). A mutant of red clover never flowers without exogenous GA (2), but it is not clear whether this variant will prove to be defective primarily in GA metabolism.

It is possible that all the GA biosynthesis mutants that have been examined to date are "leaky" to some degree, and produce small amounts of active GAs, sufficient to induce flowering. To determine whether GA is necessary for flowering, it is essential to study a mutant that contains very little or no active GA. Therefore, we examined an extremely GA-deficient mutant of the quantitative long-day plant *Arabidopsis thaliana*, in which a major portion of the *GA1* gene is deleted (14). This gene is thought to encode a product necessary for carrying out the first committed step in GA biosynthesis, the formation of *ent*-kaurene (18). The availability of this apparently nonleaky mutant provided the opportunity for a definitive test of the role of GA in flowering of *Arabidopsis*. To gain a better understanding of GA action in flowering, we have also characterized the effect on flowering of the *gai* mutation, which impairs GA responsiveness. The results presented here indicate that under short photoperiods, GA is required for flowering in *Arabidopsis*.

MATERIALS AND METHODS

Plant Genotypes

The mutants of *Arabidopsis thaliana* (L.) Heynh. characterized here were derived from the line Landsberg (*erecta*), which we refer to as WT. The severely GA-deficient mutant, *ga1-3*, was induced by fast neutrons (6) and contains a deletion of a major portion of the *GA1* gene (14). The "leaky" mutant allele of the *GA1* locus, *ga1-6*, was obtained by ethylmethanesulfonate mutagenesis (6), and the GA-insensitive mutant (*gai*) was induced by x-rays (3). The *ga1-3* and *ga1-6* alleles were originally designated *ga1-31.89* and *gal-d352*, respectively, but have recently been renamed (14).

Growth Conditions

Seeds of the *ga1-3* mutant generally require exogenous GA to germinate. To minimize the effects of residual hormone on plant development, the seeds were treated for the minimum time necessary to induce germination. Dry seeds of all genotypes were soaked in 0.1 mM GA_3 (approximately 1 ml per 100 seed) in a loosely capped centrifuge tube for 2 to 7 d

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[2] Abbreviations: GA, gibberellin (in this context, any biologically active structure); SD, short days; CL, continuous light; WT, wild type.

at 4°C in darkness, and then for 30 to 32 h at 20°C under fluorescent lights (100 μmol m^{-2} s^{-1} photon flux). At this point the seeds had not yet visibly germinated. The seeds were rinsed with four 10-mL changes of water and then resuspended in 0.1% agar and pipetted into plastic pots 7 cm in diameter (round) or 4 cm wide (square). These pots contained a mixture composed of equal parts by volume of potting soil (BACTO), perlite, and vermiculite, which had been soaked with a mineral nutrient solution (13) and topped with approximately 4 mm of fine vermiculite. The pots were covered with plastic wrap and moved to growth chambers. After 4 d, the wrap was removed and the seedlings were thinned to one per pot. The plants were subirrigated with distilled water and after 2 months drenched with fresh nutrient solution.

Plants were grown in chambers illuminated by cool white fluorescent bulbs (120 ± 10 μmol m^{-2} s^{-1} photon flux). The spectrum of irradiation emitted by such bulbs has been described (7). The plants either received SD (8 h of light and 16 h of darkness) or CL. The growth temperature was 21°C except where indicated otherwise. For experiments in which a chilling treatment to promote vernalization was tested, seeds were treated with GA and rinsed as described above, and then planted in a 5°C SD chamber and incubated for 45 d. The seeds germinated and were thinned out during this period. The seedlings were then shifted to the 21°C SD chamber.

Measurements

Nine to 15 plants were assayed for each treatment. In leaf counting experiments, true leaves in the main rosette were counted once a week. Each time, the youngest leaf was marked with a small dot of pink nail polish (Maybelline). This made it possible to keep track of all the leaves on a plant despite loss of older leaves, due to senescence, during the course of the experiment.

Flowering time was defined as the number of days from placement in the growth chamber until flower buds became visible with the aid of a hand-held magnifying glass (×2). Plants were checked for flower buds every 2 to 3 d. Because leaf number and flowering time were measured in separate experiments, it is possible that some parameters, such as light quality, may have varied slightly from one experiment to another.

Hormone Application

Beginning 17 d after planting in SD, GA-treated plants were sprayed generously once a week with 0.1 mM GA_3 (Sigma) and 0.02% (v/v) Tween-20. Control plants were sprayed with a solution containing only the Tween-20 and 0.1% (v/v) dimethylformamide (the solvent for the GA_3 stock solution).

Microscopy and Photography

The shoot apical region was excised from a representative plant of each of the following types: the *ga1-3* mutant after 81 d of growth in SD, WT after 45 d of growth in SD, and the *ga1-3* mutant after 25 d of growth in CL. The apical regions were fixed in CRAF III fixative (1), under vacuum, for at least 24 h. The tissue was then serially dehydrated in an ethanol *tert*-butanol series prior to infiltration in paraffin. Serial longitudinal sections were cut at 10 microns, mounted on slides, and stained with hematoxylin (1). Median longitudinal sections were selected from these series and photographed under a Zeiss light microscope.

Table I. *Flowering Time in SD*

The mean and SE were calculated from observations on 10 to 14 plants per trial.

Genotype	Temperature	Days to Flowering
	°C	
WT	21	57 ± 2
gai	21	77 ± 12
ga1-6	21	70 ± 7
ga1-3	21	Never flowered (>117 d)
WT	25	40 ± 3
gai	25	62 ± 4
ga1-6	25	55 ± 2
ga1-3	25	Never flowered (>117 d)

RESULTS

Effect of Daylength in Flowering Time and Leaf Number

In SD, the extremely GA-deficient mutant, *ga1-3*, never flowered during any of four independent experiments, unless treated with exogenous GA_3 (Table I; Fig. 1). However, in CL the mutant flowered readily, although later than WT (Table II). Thus, extreme GA deficiency converts *Arabidopsis* from a

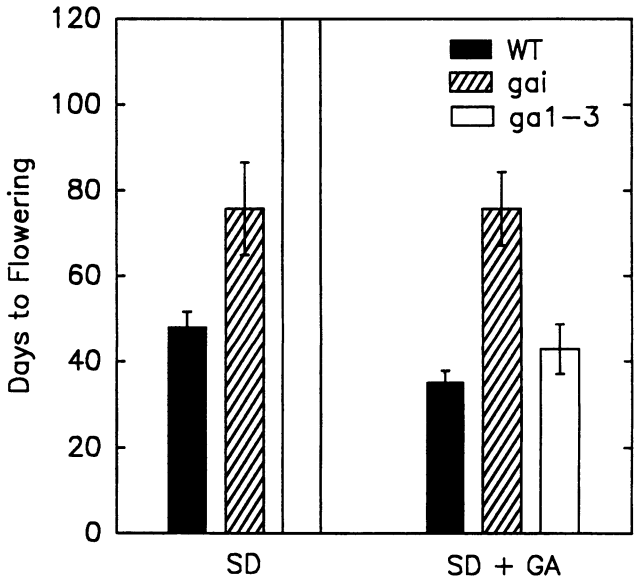

Figure 1. The effect of exogenous GA_3 on flowering time in SD. Each bar represents the mean of observations on 7 to 11 plants ± SE. GA-treated plants were sprayed weekly with the hormone as described. Untreated *ga1-3* mutant plants failed to flower, but the plants began to senesce at the indicated time.

Table II. *Flowering Time and Leaf Number in CL*

The mean and SE were calculated from observations on 9 to 15 plants per trial. The growth temperature was 21°C. Flowering time and leaf number data were obtained in separate experiments.

Genotype	Days to Flowering	Leaf Number
WT	18 ± 0	8.2 ± 0.7
gai	18 ± 0	11.6 ± 0.7
ga1-6	18 ± 0	-
ga1-3	29 ± 2	11.7 ± 0.7

facultative long-day plant to an obligate long-day plant. After a growth period of 5 to 6 months in SD, all untreated *ga1-3* mutant plants eventually died without flowering. In three separate experiments, the mean time to senescence of the *ga1-3* mutant in SD was found to be 190 ± 9, 178 ± 13, and 168 ± 17 d (±SE, n = 7–12).

The leaky GA-deficient mutant, *ga1-6*, did flower in SD, although it took somewhat longer to do so than WT (Table I; Fig. 1). Like *ga1-6*, the GA-insensitive (*gai*) mutant also flowered somewhat late in SD (Table I; Figs. 1 and 2). This result seems consistent with a general picture of *gai* as a mutant in which GA response is greatly reduced but not abolished (3). In SD at 25°C, all genotypes flowered more rapidly than at 21°C, but at both temperatures, the *gai* and *ga1-6* mutants flowered later than WT. In contrast, in CL, both the *gai* mutant and the *gal-6* mutant flowered at the same time as WT (Table II).

Like many annual plants, when *Arabidopsis* begins to produce flowers, it stops making true leaves. Thus, generally, a plant that fails to flower but continues to produce new nodes at a normal rate will accumulate more leaves than one that flowers early (7). In SD, the *ga1-3* mutant plants produced

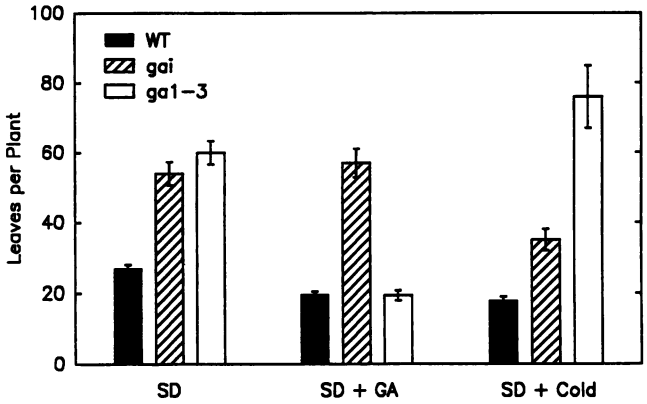

Figure 3. Total leaf number in SD. Each column represents the mean of observations on 9 to 10 plants ± SE. Leaf counting was terminated at the time of flowering for plants that did flower. Untreated and cold-treated *ga1-3* plants never flowered, so counting continued on these plants until complete senescence occurred. Cold-treated plants were chilled at the seed/seedling stage at 5°C for 45 d under SD prior to the start of the experiment.

more than twice as many leaves as WT on the main stem rosette and appeared to produce additional leaves (uncounted) on lateral rosettes (Figs. 3 and 4). Leaf production slowed in these plants eventually, although they never switched to making flowers. In SD, *gai* plants produced about twice as many leaves as WT (Fig. 3). Flowering time showed more variability than leaf number for the *gai* mutant (Table I; Figs. 1 and 3), perhaps reflecting variability in the growth rates of individual plants.

In CL, *ga1* and *ga1-3* plants produced somewhat larger number of leaves than WT plants (Table II). The *ga1-3* mutant

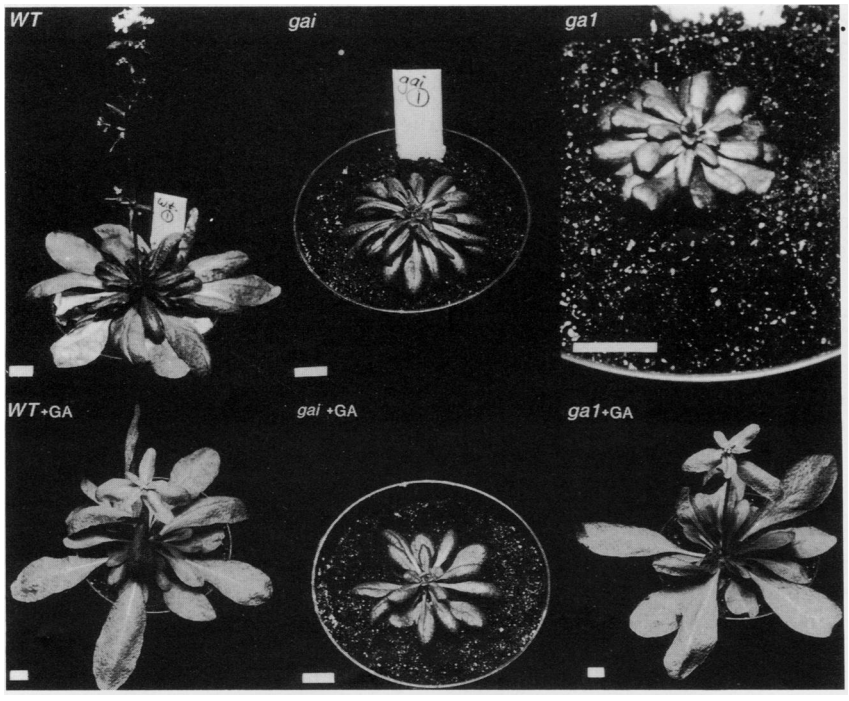

Figure 2. Phenotypes of *Arabidopsis thaliana* mutants in SD. The top three panels show *WT*, *gai*, and *ga1* (*ga1-3*) after 60 d of growth under SD conditions at 21°C. The lower panels show 45-d-old plants of the above genotypes that were grown under the same conditions, but that received GA_3 treatments. The white bar equals 1 cm.

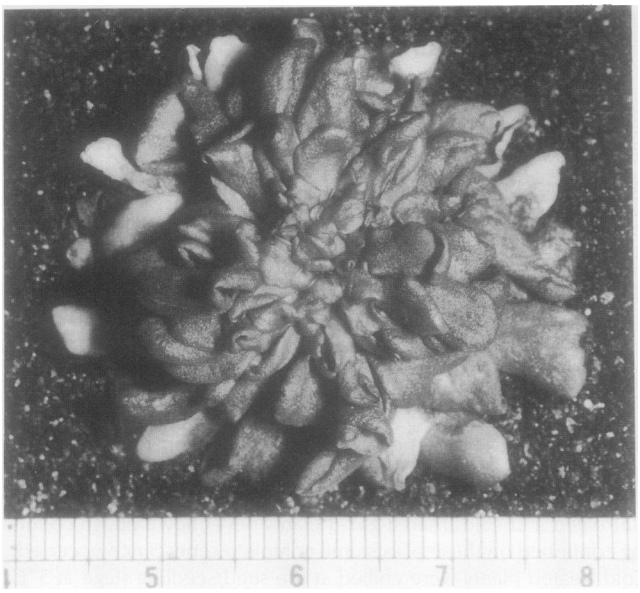

Figure 4. Mutant *ga1-3* after 113 d of growth in SD at 21 to 23°C. The scale is in centimeters.

showed retarded flowering in terms of absolute time as well as leaf number. The *gai* mutant produced a larger number of leaves than WT in CL in one experiment, whereas it flowered as rapidly as WT in another (Table II). Because the mutant does not seem to exhibit an unusually rapid rate of leaf production (*gai* actually appears to develop more slowly than WT), this discrepancy may have been caused by a slight variation in some parameter between the two experiments.

In contrast to WT, the *gai* mutant failed to respond to applied GA_3 by accelerating flowering or decreasing leaf number. The *ga1-3* mutant responded dramatically to regular spraying with GA_3 by producing many fewer leaves and flowering almost as promptly as GA_3-treated WT (Figs. 1–3).

Effect of Cold Treatment

Because a chilling treatment has been shown to accelerate flowering in some *Arabidopsis* genotypes (7), we tested whether low temperature would induce or promote flowering of GA mutants. A prolonged cold treatment (45 d) in SD prior to growth at 21°C succeeded in reducing leaf number and accelerating flowering in the *gai* mutant, but it also reduced leaf production by WT plants (Fig. 3). The net result was that *gai* still produced roughly 2-fold more leaves than WT. Cold treatment failed to stimulate the *ga1-3* mutant to flower, and likewise failed to cause this mutant to produce fewer leaves (Fig. 3).

Dominance of the Late Flowering Trait

To test whether the late-flowering phenotype of the *gai* mutant line was due to a single mutation, the F_2 progeny from a *gai* X WT cross were scored for late flowering in SD. The cross was scored at a time when all of 10 WT control plants had flowered and all of 10 *gai* control plants were vegetative. At this time, only 27 out of 100 F_2 plants had visible flower buds. Thus, under the growth conditions used, late flowering appears to be inherited as a single dominant nuclear mutation ($\chi^2 = 0.213$, $P > 0.5$). Because the *gai* mutation shows partial dominance for the dwarfing phenotype, and visually dominant mutations are generally rare, it seems likely that both the late-flowering and the dwarf phenotype are caused by the same mutation.

Examination of Shoot Apices

To try to determine whether the *ga1-3* mutant makes any progress toward flowering in SD, such as formation of flower primordia, shoot apices of the *ga1-3* mutant and WT that had grown in SD or CL were examined by light microscopy (Fig. 5). In SD, no flower primordia or buds could be discerned in the *ga1-3* mutant. Instead, the small, flattened apex appeared to be surrounded by leaf primordia (Fig. 5). In contrast, microscopic flower buds were apparent in the mutant grown in CL, and also in the WT grown in SD (Fig. 5).

DISCUSSION

Results presented here indicate that the Landsberg *erecta* line of *A. thaliana* requires the hormone GA in order to initiate flowers in SD. In spite of the fact that it is missing a substantial portion of the *GA1* gene, the *ga1-3* mutant has been found to produce greatly reduced but still detectable levels of several GAs (18). This may be due to the action of a duplicate *GA1* gene that is poorly expressed in vegetative tissues (18), or some alternative means of *ent*-kaurene synthesis. Thus, one possible explanation for the photoperiodism exhibited by the *ga1-3* mutant might be that in this genotype, GA production drops to an even lower level in SD than in CL, a level below a critical threshold for flowering. It is also possible that *Arabidopsis* may be more sensitive to low levels of GA in CL than in SD. An alternative possibility for the photoperiod effect could be that there are two different flowering pathways in *Arabidopsis*, a GA-requiring pathway that operates in SD and a GA-independent pathway that functions in CL. A true null mutant that lacks all GA would be needed to test this hypothesis. The observation that the *ga1-3* mutant flowered somewhat late even in CL would seem to argue against the existence of a pathway that is completely GA independent (Table II).

The *gai* mutant flowered promptly when grown in CL, but took longer to flower than WT when grown in SD, whether or not it was sprayed with large quantities of GA_3. The *gai* mutant normally has greatly elevated endogenous levels of GAs (15), and is defective in every known GA response of *Arabidopsis*, including seed germination, stem elongation, and apical dominance (3). We can now add rapid flowering in SD to the list. The *gai* mutant appears to show impairment in a primary step in GA action. The behavior of this mutant suggests that some important change occurs in *Arabidopsis* when photoperiod is lengthened, other than a rise in GA levels, and this unknown change somehow accelerates flowering.

Like *gai*, the *ga1-6* mutant does flower in SD, although it shows some delay. In spite of the fact that GA production is

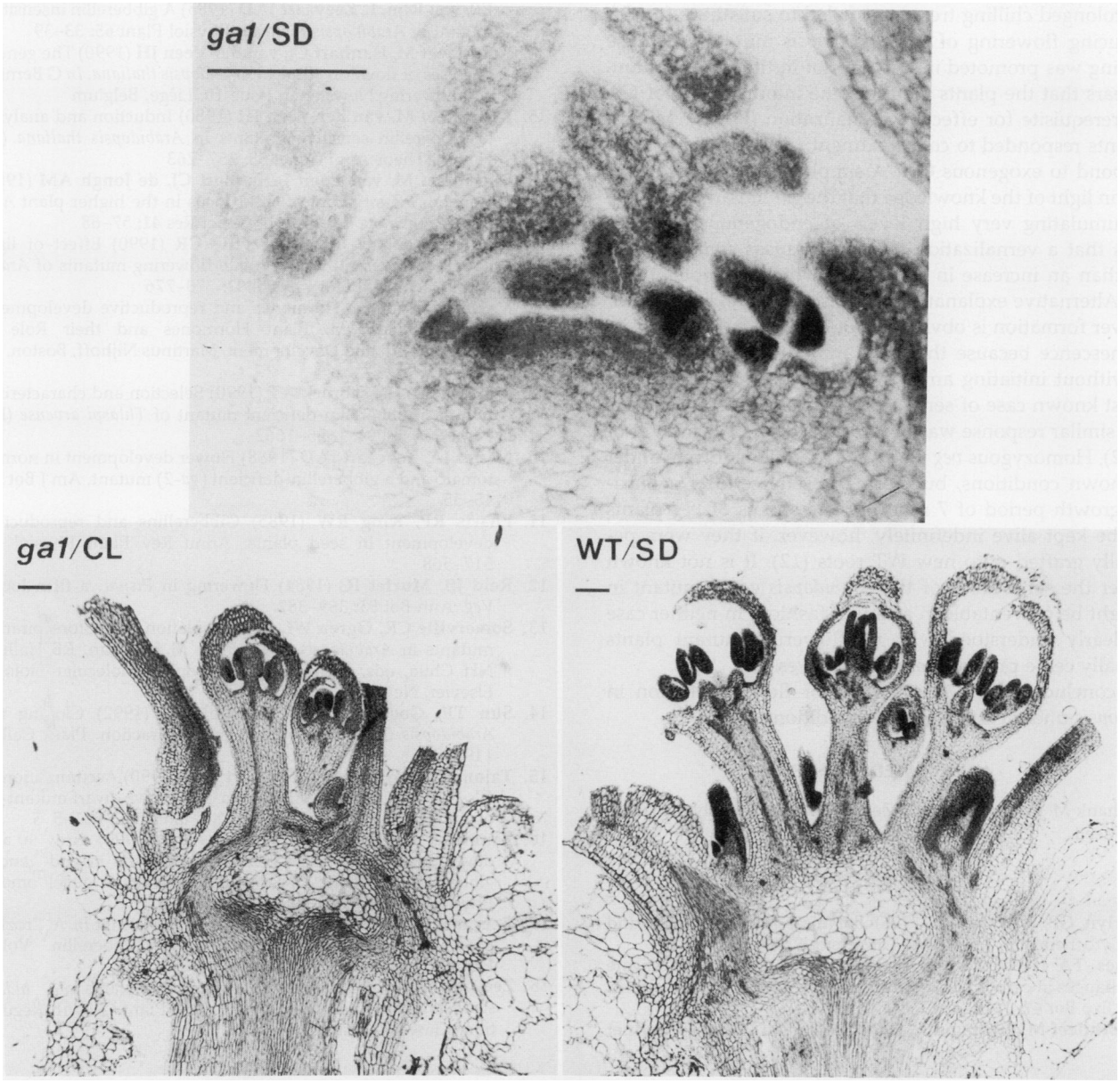

Figure 5. Median sections of shoot apices. The *ga1-3* mutant grown in SD was 81 d old. The WT grown in SD was 45 d old. The *ga1-3* mutant grown in CL was 25 d old. The black bar equals 0.1 mm.

reduced enough to cause dwarfing in this mutant (6), the plant can still synthesize enough GA to trigger SD flowering. Thus, it seems that higher GA levels are needed by *Arabidopsis* for elongation growth than for flowering in SD.

Apparently, leaky GA-insensitive or -deficient mutations exert a quantitative affect on the time to flowering in *Arabidopsis*, and a moderate reduction in GA levels or response delays, but fails to prevent, flowering in SD. The reason for this delay is not clear. It is possible that active GA must accumulate to a certain threshold level in order for flowering to occur, and that mutants impaired in hormone synthesis take longer than normal to accumulate sufficient GA in SD. Similarly, it might be proposed that the *gai* mutant, which is not totally insensitive to GA, flowers rapidly in continuous light by virtue of abnormally high levels of endogenous GA, but flowers slower under SD because of a dramatic drop in GA production. However, this theory does not explain why application of exogenous GA_3 failed to noticeably accelerate flowering of the *gai* mutant in SD. Perhaps, when either GA levels or the ability to perceive GA are low, the timing of development somehow becomes perturbed. Many of the monogenic flowering mutants that have been reported in *Arabidopsis* are late flowering types (4, 7). By analogy with the GA mutants, it seems possible that some of these might be leaky alleles of loci for which only a trace of function is needed to cause flowering.

A prolonged chilling treatment failed to substitute for GA in inducing flowering of the *Arabidopsis* mutants. Because flowering was promoted in WT but not in the *ga1-3* mutant, it appears that the plants require some minimal level of GA as a prerequisite for effective vernalization (Fig. 3). Mutant *gai* plants responded to cold treatment, although they failed to respond to exogenous GA. A simple explanation for this result, in light of the knowledge that the *gai* mutant is capable of accumulating very high levels of endogenous C_{19} GAs (15), is that a vernalization treatment causes some change, other than an increase in GA levels, which promotes flowering. Alternative explanations are also possible, however.

Flower formation is obviously not a necessary prerequisite for senescence because the *ga1-3* mutant plants eventually died without initiating any visible flower buds. This is not the first known case of senescence in the absence of flowering. A similar response was observed with the *veg* mutant of pea (12). Homozygous *veg* plants fail to initiate flowers under any known conditions, but normally senesce after a maximum growth period of 7 months. The shoots of *veg* plants could be kept alive indefinitely, however, if they were periodically grafted onto new WT roots (12). It is not known whether the senescence of the *Arabidopsis ga1-3* mutant in SD might be preventable in a similar fashion. In neither case is it clearly understood why nonflowering mutant plants eventually cease production of new leaves.

We conclude that GA is crucial for flower initiation in *Arabidopsis* under certain growth conditions.

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LITERATURE CITED

1. **Berlyn GP, Mikshe JP** (1976) Botanical Microtechnique and Cytochemistry, Iowa State University Press, Ames, IA
2. **Jones TA** (1990) Use of a flowering mutant to investigate changes in carbohydrates during floral transition in red clover. J Exp Bot **41:** 1013–1019
3. **Koornneef M, Elgersma A, Hanhart CJ, van Loenen-Martinet EP, van Rijn, L, Zeevaart JAD** (1985) A gibberellin insensitive mutant of *Arabidopsis thaliana*. Physiol Plant **65:** 33–39
4. **Koornneef M, Hanhart CJ, van der Veen JH** (1990) The genetic analyses of flowering time in *Arabidopsis thaliana*. *In* G Bernier, ed, Flowering Newsletter, Issue 10. Liège, Belgium
5. **Koornneef M, van der Veen JH** (1980) Induction and analyses of gibberellin sensitive mutants in *Arabidopsis thaliana* (L.) Heynh. Theor Appl Genet **58:** 257–263
6. **Koornneef M, van Eden J, Hanhart CJ, de Jongh AM** (1983) Genetic fine-structure of *GA-1* locus in the higher plant *Arabidopsis thaliana* (L.) Heynh. Genet Res **41:** 57–68
7. **Martinez-Zapater JM, Somerville CR** (1990) Effect of light quality and vernalization on late flowering mutants of *Arabidopsis thaliana*. Plant Physiol **92:** 770–776
8. **Metzger JD** (1987) Hormones and reproductive development. *In* PJ Davies, ed, Plant Hormones and their Role in Plant Growth and Development, Martinus Nijhoff, Boston, pp 431–462
9. **Metzger JD, Hassebrock AT** (1990) Selection and characterization of a gibberellin-deficient mutant of *Thlaspi arvense* (L.). Plant Physiol **94:** 1655–1662
10. **Nester JA, Zeevaart JAD** (1988) Flower development in normal tomato and a gibberellin-deficient (*ga-2*) mutant. Am J Bot **75:** 45–55
11. **Pharis RP, King RW** (1985) Gibberellins and reproductive development in seed plants. Annu Rev Plant Physiol **36:** 517–568
12. **Reid JB, Murfet IC** (1984) Flowering in *Pisum*: a fifth locus, *Veg*. Ann Bot **53:** 369–382
13. **Somerville CR, Ogren WL** (1982) Isolation of photorespiration mutants in *Arabidopsis thaliana*. *In* M Edelman, RB Hallick, NH Chua, eds, Methods in Chloroplast Molecular Biology, Elsevier, New York, pp 129–139
14. **Sun TP, Goodman HM, Ausubel FM** (1992) Cloning the *Arabidopsis GA1* locus by genomic subtraction. Plant Cell **4:** 119–128
15. **Talon M, Koornneef M, Zeevaart JAD** (1990) Accumulation of C_{19}-gibberellins in the gibberellin-insensitive dwarf mutant *gai* of *Arabidopsis thaliana* (L.) Heynh. Planta **182:** 501–505
16. **Zanewich KP, Rood SB, Williams PH** (1990) Growth and development of *Brassica* genotypes differing in endogenous gibberellin content. I. Leaf and reproductive development. Physiol Plant **79:** 673–678
17. **Zeevaart JAD** (1983) Gibberellins and flowering. *In* A Crozier, ed, The Biochemistry and Physiology of Gibberellins, Vol 2. Praeger, New York, pp 333–374
18. **Zeevaart JAD, Talon M** (1991) Gibberellin mutants in *Arabidopsis thaliana*. *In* CM Karssen et al., eds, Plant Growth Regulation. Kluwer, Amsterdam