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Differential Regulation of Trichome Formation on the Adaxial and Abaxial Leaf Surfaces by Gibberellins and Photoperiod in *Arabidopsis thaliana* (L.) Heynh.[1]

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In wild-type (WT) Columbia and Landsberg *erecta* ecotypes of *Arabidopsis thaliana* (L.) Heynh., trichomes are present on the adaxial surfaces of all rosette leaves but are absent from the abaxial surfaces of the first-formed leaves. We have determined that both long-day (LD) photoperiod and gibberellin (GA) stimulate trichome formation. WT plants grown in LD conditions produce the first abaxial trichome on earlier leaves than plants grown in short-day (SD) conditions. Photoperiod sensitivity of abaxial trichome formation on WT plants develops gradually over time, reaching the maximum sensitivity about 24 d after germination. Application of gibberellic acid to WT plants growing in SD conditions accelerates the onset of abaxial trichomes. Conversely, application of 20 to 80 mg L^{-1} paclobutrazol, a GA biosynthesis inhibitor, to wild-type plants suppresses trichome initiation on the abaxial epidermis. The GA-deficient mutants *ga1–5* and *ga4–1* and the GA-insensitive mutant *gai–1* exhibit delayed onset of abaxial trichomes when grown in LD conditions. The null mutant *ga1–3* produces completely glabrous leaves when grown in SD conditions. Application of gibberellic acid to glabrous *ga1–3* plants consistently induces earlier formation of trichomes on the adaxial epidermis than on the abaxial epidermis, demonstrating a difference between the adaxial and abaxial surfaces in their response to GA with regard to trichome formation.

A common feature of the leaves of dicotyledonous species is their dorsiventral asymmetry. If a leaf is cut through its midrib along the long axis of the leaf, the two sides separated by the incision are typically mirror images of each other. However, if the cut is made through the center of the mesophyll layer, parallel to the plane of the leaf surface, the two portions separated by the incision are dorsiventrally asymmetrical. This is because palisade mesophyll occurs immediately below the adaxial epidermis, whereas spongy mesophyll occurs immediately above the abaxial epidermis. Superficial features such as stomata, wax deposition, and trichomes are also often differentially distributed on the two leaf surfaces. Despite the widespread occurrence of dorsiventrality in leaf development, relatively little is known about the genes and the mechanisms that control differential cell identity on the two leaf surfaces.

Mutants with abnormal dorsiventrality aid in the analysis of how dorsiventral asymmetry becomes established. In *Zea mays*, ligules are normally produced from the adaxial epidermis, but a dominant mutant, *ROLLED* (*RLD*), produces ligules from the abaxial epidermis (Hake et al., 1985). In *Antirrhinum majus*, the most severe alleles of the *phantastica* (*phan*) mutant have radially symmetrical needle-like leaves, bracts, and petals with only abaxial cell identity; the less severe alleles show varying degrees of reduction in adaxial tissue, indicating that PHAN is required for establishing adaxial cell identity (Waites and Hudson, 1995). The development of radially symmetrical leaves has also been observed in surgically incised leaf primordia (for review, see Steeves and Sussex, 1989), suggesting that the shoot apical meristem may exert an effect on the adaxial side of the leaf primordium that results in adaxial identity.

Additional information about the physiological differences between the two leaf surfaces will improve our understanding of the potential downstream genes regulated by the gene products, such as PHAN, that establish dorsiventral asymmetry. In *Arabidopsis thaliana* (L.) Heynh., a model plant for genetic and molecular studies, trichomes are differentially distributed on the leaf surfaces. Adaxial trichomes are present on all of the rosette leaves, whereas abaxial trichomes are absent from the first-formed leaves. The use of trichomes as a marker of dorsiventrality in *A. thaliana* has two advantages: first, trichomes are easily visible with the aid of a dissecting microscope without having to destroy the plant during observation, and second, it is easy to count the trichomes because there is only one type of trichome on the leaf epidermis; *A. thaliana* trichomes are all unicellular and nonglandular.

A number of genes are known to affect the time and position of trichome initiation in *A. thaliana*. Two genes that are necessary for the initiation of trichomes are *GLABRA1* (*GL1*) and *TRANSPARENT TESTA GLABRA* (*TTG*) (Hülskamp et al., 1994; Larkin et al., 1994), both of which must be active in the same cell. There is another group of genes that when mutated causes an unusually early onset of adaxial trichome initiation on cotyledons; these mutants

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Abbreviations: CL, continuous light; LD, long day; SD, short day; WT, wild type.

include *leafy cotyledon1* and *2* (*lec 1* and *lec 2*) and *fusca* 3 (Meinke et al., 1994). Cotyledons from these mutant plants also have other leaf-like features, suggesting that these trichome-bearing cotyledons may have some true leaf identity.

We have examined the effects of daylength and the plant hormone GA on the distribution of leaf trichomes in *A. thaliana*. In LD conditions trichomes appear on the abaxial surfaces of cauline leaves at about the time of flowering, suggesting that floral induction signals may play a role in abaxial trichome formation. Both long days and GA are known to promote flowering in *A. thaliana* (Wilson et al., 1992). To test the effect of daylength on the onset of abaxial trichome formation, we compared LD- and SD-grown WT Columbia and Landsberg *erecta* plants and exposed SD-grown WT Columbia plants of different ages to a 32-h-long CL treatment. To study the influence of GA on trichome formation, we observed trichome onset and density on the leaves of GA-deficient mutants *ga1–3*, *ga1–5*, and *ga4–1* and on the leaves of a GA-insensitive mutant, *gai–1*. We also applied GA_3 to WT and *ga1–3* plants and applied paclobutrazol, a GA biosynthesis inhibitor (Hedden and Graebe, 1985), to WT plants.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Arabidopsis thaliana (L.) Heynh. WT Columbia and Landsberg *erecta* ecotypes and *ga1–3*, which was generated by fast neutron bombardment in the Landsberg *erecta* background (Koornneef and van der Veen, 1980), were obtained from Dr. Eva Huala (University of California, Berkeley). Plants of *ga1–5*, *ga4–1*, and *gai-1* were obtained from the Arabidopsis Biological Resource Center at the Ohio State University (Columbus). Plants were grown in growth chambers (Conviron, Asheville, NC) at 20°C and 50% RH. Because of the shortage of growth chamber space, different growth chambers were used for different experiments, and the settings were slightly different. An SD chamber was always set at 8-h light and 16-h dark cycles. An LD chamber (used for the photoperiod comparison experiment reported in Table I) was set for 8 h of normal irradiance and 8 h of low irradiance during the extended light period, followed by an 8-h dark period. A CL chamber had lights on all of the time. The photon flux density of the SD chamber used to grow *ga1–3* plants, paclobutrazol-treated Landsberg *erecta* plants, and GA-treated WT Columbia plants was 150 to 180 μmol m^{-2} s^{-1} provided by a mixture of cool-white and Gro-lux (Sylvania) fluorescent tubes. The photon flux density of the SD chamber used to grow WT Columbia and Landsberg *erecta* plants for comparing the effect of daylength on trichome formation was 175 to 200 μmol m^{-2} s^{-1} provided by a mixture of cool-white fluorescent tubes and incandescent bulbs. In that experiment (reported in Table I), the LD condition was set at the same photon flux density for the first 8 h, and then an extended 8-h light period was provided by only incandescent bulbs at a photon flux density of 2.5 μmol m^{-2} s^{-1}. To determine the photoperiod-sensitive phase of trichome formation (reported in Fig. 3), WT Columbia plants were grown under a photon flux density of 130 to 150 μmol m^{-2} s^{-1} provided by a mixture of cool-white fluorescent tubes and incandescent bulbs. Seeds were germinated every 3 d. Plants of different ages were induced at the same time by a 32-h-long light period without changing the photon flux density (130–150 μmol m^{-2} s^{-1}). Eight hours after the induction began, SD control plants were transferred to a dark room for 16 h. The CL chamber used to grow *ga1–3*, *ga1–5*, *ga4–1*, and *gai-1* plants had a photon flux density of 50 to 80 μmol m^{-2} s^{-1} provided by a mixture of cool-white fluorescent tubes and incandescent bulbs.

An all-purpose potting mix (Sunshine; Sun Gro Horticulture, Bellevue, WA) and vermiculite (1:1, v/v) were used to grow WT plants. GA-treated WT Columbia plants (Table II) were grown at a density of four plants per 10-cm pot. WT plants reported in Table I and Figure 3 were grown at a density of one plant per 5-cm pot. Paclobutrazol-treated WT Landsberg *erecta* plants were grown at one plant per cell in 4-cm, six-cell packs. A few mature embryos of *ga1–3* were germinated by removing the seed coats. Most seeds of *ga1–3* were germinated on 0.8% (w/v) agar-solidified medium containing 4.3 g L^{-1} Murashige-Skoog salts (JRH Biosciences, Lenexa, KS), B_5 vitamins (1 mg L^{-1} nicotinic acid, 10 mg L^{-1} thiamine HCl, 10 mg L^{-1} pyridoxine HCl, and 100 mg L^{-1} myoinositol), and either 1 μM GA_4 (Sigma) or 20 μM GA_3 (Sigma). After 2 d seedlings were transferred to a mixture of all-purpose mixture:vermiculite:sand (1:1:1, v/v/v), at a density of 16 plants per 10-cm pot. Seeds of *ga1–5*, *ga4–1*, and *gai–1* were germinated directly in soil without GA supplement. Seeds of WT plants were germinated on agar plates with Murashige-Skoog salts and B_5 vitamin and no GAs before transfer to soil. WT and GA mutant plants were watered regularly with tap water without any fertilizer.

GA Solutions and Application

GA solutions used in the experiment reported in Table II and for germinating *ga1–3* contained 1% (v/v) ethanol; the control solution contained only 1% (v/v) ethanol. A 5-μL drop of GA_3 or control solution was applied to the adaxial epidermis of each leaf below the apical bud of each WT Columbia plant on d 21 and then twice more at 3-d intervals. Solutions used in the experiment reported in Figure 4 also contained 0.1% (v/v) Triton (Sigma). When *ga1–3* plants produced completely glabrous leaves, either a GA solution or the control solution was applied twice at 3-d intervals to the 10 youngest, reflexed leaves below the apical bud of each plant. Each of these leaves received a 5-μL drop of GA_3 or control solution on the adaxial epidermis. Each pot received a different GA concentration. Cross-contamination of different GA concentrations during watering was prevented by placing a small plastic tray under each pot.

Paclobutrazol Solution and Application

Paclobutrazol solutions of 20, 40, and 80 mg L^{-1} were made from a tap water dilution of Bonzi (Uniroyal Chem-

ical, Middlebury, CT), which contains 0.4% (w/v) paclobutrazol as the active ingredient. Two six-cell packs were placed in each aluminum tray, and 700 mL of paclobutrazol or water (control) were added to the bottom of each tray and poured out after 2 d.

Measurement of Trichome Formation and Flowering Time

Trichomes were observed using a dissecting microscope at ×60 magnification. The first leaf to bear trichomes was determined by counting the leaves from the base of the plant; leaf 1 was the first-formed true leaf on the plant. Flowering time of the WT plants was determined by counting the total number of leaves (rosette and cauline) at plant maturity. Because older leaves may senesce, each newly expanded leaf was marked with a tiny dot of red nail polish to identify leaf numbers. In the experiment reported in Figure 5, all of the reflexed leaves of *ga1–3* plants were marked before GA treatment to facilitate identification of leaves that matured afterward. The first leaf to bear trichomes after GA treatment was identified among unmarked leaves. In this experiment, leaf 1 was the oldest unmarked leaf.

Scanning Electron Microscopy

WT Columbia leaves and *ga1–3* leaves were fixed in buffered (pH 7.0) 4% (v/v) glutaraldehyde (Polysciences, Warrington, PA), dehydrated through an ethanol series, and then critical point dried in Samdri-PVT-3B (Tousimis Research, Rockville, MD). Dried leaves were sputter coated with gold (Polaron SE coating system; Watford, UK). An ISI-DS 130 (Top Con, Paramus, NJ) electron microscope was used to examine WT Columbia leaves, and a JEOL JSM 35C was used to examine *ga1–3* leaves due to the availability of the microscopes.

RESULTS

Ontogenetic Progression of Trichome Initiation in WT Plants

In WT Columbia and Landsberg *erecta* plants the adaxial epidermis of later-formed, mature rosette leaves bore several hundred regularly spaced trichomes per leaf (Fig. 1A). Adaxial trichomes were present on all of the rosette leaves. Adaxial trichome initiation became suppressed during cauline leaf differentiation, leading to a decline in the total number of adaxial trichomes arising on each successive cauline leaf. In addition, the spatial distribution of trichomes on the cauline leaves varied with leaf number: trichomes became restricted to the tip of the later-formed leaves (Fig. 2) because of the basipetal direction of trichome development on a differentiating leaf primordium (Fig. 1B).

Trichomes were not formed on the abaxial surfaces of early rosette leaves but were formed on the abaxial surfaces of later rosette and cauline leaves. The first-formed abaxial trichomes were usually found on the midrib near the petiole. Spatial distribution of abaxial trichomes progressed toward the leaf tip and margin of later-formed leaves. Eventually, abaxial trichomes were evenly distributed over the entire surface of the latest leaves (Fig. 2). Clonal studies by Larkin et al. (1996) indicated that trichome patterning in *A. thaliana* is not dependent on cell lineage.

LD Conditions Stimulate Trichome Formation

Altering the daylength while keeping the total irradiance constant had an effect on the appearance time of abaxial trichomes on both Columbia and Landsberg *erecta* WT plants. The onset of abaxial trichomes was accelerated in LD-grown Landsberg *erecta* and Columbia plants by approximately three and two leaves, respectively (Table I).

To determine the photoperiod-sensitive phase of trichome formation and of flowering, WT Columbia plants were germinated in an SD growth chamber every 3 d, switched to a 32-h-long CL photoperiod when the oldest group of plants was 33 d old, and switched back to SD conditions afterward (Fig. 3). Trichome formation was somewhat sensitive to CL treatment from d 6 to 21. During this period, older plants were more responsive to CL than younger plants. The photoperiod-sensitive phase spanned from d 24 to 33. On d 24 each plant had an average of about 9 mature leaves and many immature ones, and on d 33 each had about 14 mature leaves. When 24-d-old plants were exposed to CL, abaxial trichomes first appeared on leaf 10, whereas those transferred on d 33 did not bear abaxial trichomes until leaf 15. Thus, between d 24 and 33, plants were equally responsive to CL trichome induction.

In contrast, the photoperiod-insensitive phase for floral induction spanned from d 6 to 12. Plants exposed to CL when they were 6, 9, or 12 d old produced as many leaves (an average of 70 rosettes and cauline leaves) as the SD control plants upon flowering (Fig. 3). Between d 15 and 18, plants became more sensitive to the CL treatment but not as sensitive as the later period (from d 21 to 33), which was the photoperiod-sensitive phase for floral induction. Plants exposed to CL on d 15 and 18 showed a great variation in their response to floral induction; some plants had about 18 leaves (rosette plus cauline) due to quick induction, some had about 30 leaves due to slow or incomplete induction, and some had nearly as many leaves as the SD control.

Table I. *Comparison of abaxial trichome appearance and flowering time between WT Columbia and Landsberg erecta plants in LD and SD conditions*

Genotype	First Leaf Bearing Abaxial Trichomes (leaf no.)		Total No. of Leaves at Flowering	
	LD	SD	LD	SD
Columbia	8.2 ± 0.7[a,b]	9.7 ± 1.3[b]	12.5 ± 1.4	54.2 ± 5.2
Landsberg *erecta*	6.8 ± 0.4	10.1 ± 1.7	8.7 ± 0.7	39.1 ± 2.0

[a] Averages ± SD (n = 30). [b] Student's t test indicated that the difference between the calculated means is significant ($P < 0.01$).

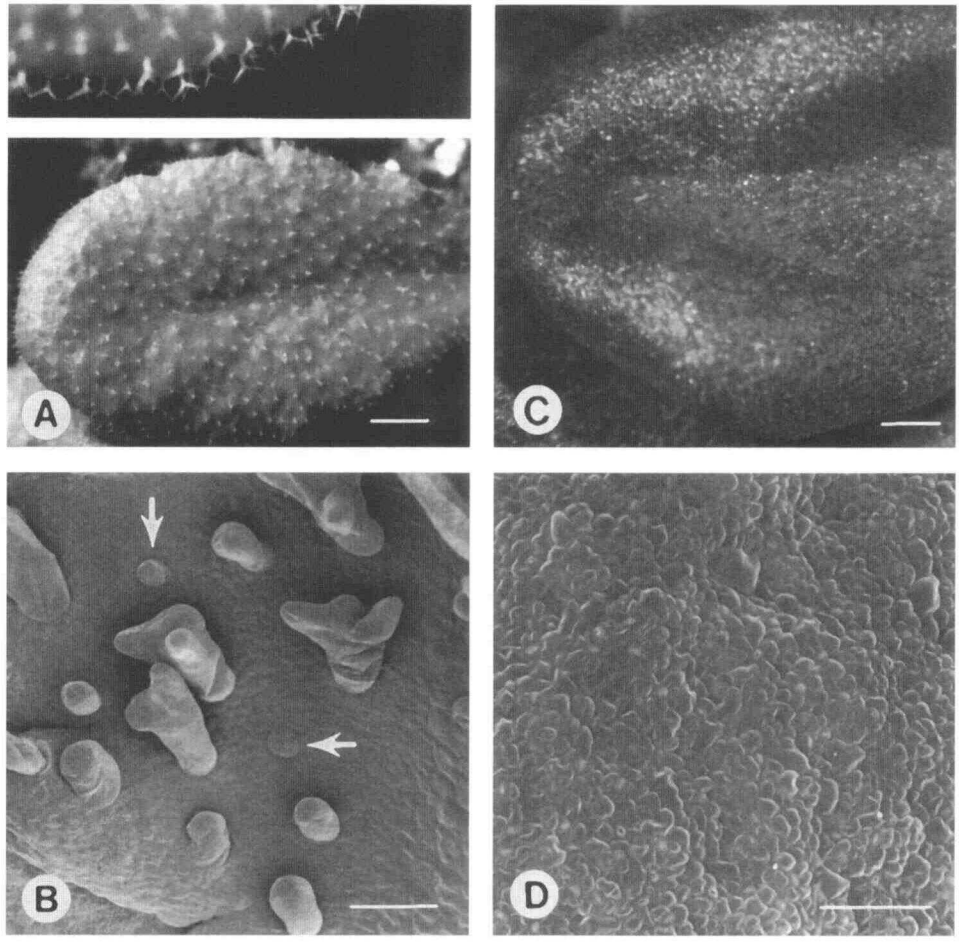

Figure 1. Comparison of leaf surfaces between *A. thaliana* WT Columbia and GA-deficient *ga1–3* (Ler) plants grown in SD conditions. A, WT Columbia leaf. Several hundred regularly spaced trichomes are present on the adaxial epidermis of a rosette leaf. Bar = 2.4 mm. Inset, Trichomes at a magnification of ×3.3 of the lower picture. The trichomes are nonglandular and have several spikes at the tip. B, Scanning electron micrograph of trichome initials. Developing trichome initials at different stages can be observed on the adaxial surface of a differentiating WT Columbia leaf. Arrows point to the youngest trichome initials. Bar = 35.1 μm. C, GA-deficient *ga1–3* leaf. No trichomes appear on the adaxial surface of *ga1–3* leaves that are initiated 3 months after germination. Bar = 0.36 mm. D, Scanning electron micrograph of *ga1–3* leaf. No trichome initials can be observed on the adaxial surface of a mature *ga1–3* leaf. Bar = 100 μm.

In comparison, abaxial trichome formation responded to CL treatment at an earlier developmental stage than floral formation (as early as d 6 for trichomes compared with d 15 for flowering). Like flowering, the photoperiod sensitivity of abaxial trichome formation developed gradually over time until the maximum sensitivity was reached on about d 24.

GA Stimulates Trichome Formation

Seeds of *ga1–3* were either sown on a medium containing 1 μM GA_4 for 2 d or broken out of the seed coat to induce germination. Plants of *ga1–3*, deficient in *ent*-kaurene synthetase A enzyme (Sun and Kamiya, 1994), were dwarfed and had dark-green, incompletely expanded leaves. Trichomes failed to form on the abaxial epidermis of any leaf throughout the life cycle of the plants in both the CL and SD groups. However, a few trichomes were formed on each plant on the adaxial leaf surfaces of CL-grown plants. These plants had approximately 10 leaves when the inflorescence appeared. In the SD group the successive leaves of *ga1–3* plants produced decreasing numbers of adaxial trichomes, and about 3 to 4 months after sowing (after more than 50 leaves had been produced), the later-formed leaves were completely glabrous (Fig. 1, C and D).

The formation of abaxial trichomes was delayed in the GA mutants with less severe phenotypes (*ga1–5*, *ga4–1*, and *gai–1*). Plants of *ga1–5* and *ga4–1* were GA responsive and semi-dwarfed (Koornneef and Van der Veen, 1980). Biochemical and molecular studies of the *ga4* mutant indicate that it is deficient in 3β-hydroxylase in the GA biosynthetic pathway (Talon et al., 1990a; Chiang et al., 1995). The partially dominant *gai–1* mutant resembles GA-deficient mutants but is insensitive to both applied and endogenous GA (Koornneef et al., 1985; Talon et al., 1990b). In fact, *gai–1* contains low levels of C_{20}-dicarboxylic acid GA and high levels of C_{19}-GA (Talon et al., 1990b). Unlike *ga1–3*, these three mutants did not require exogenous GA for germina-

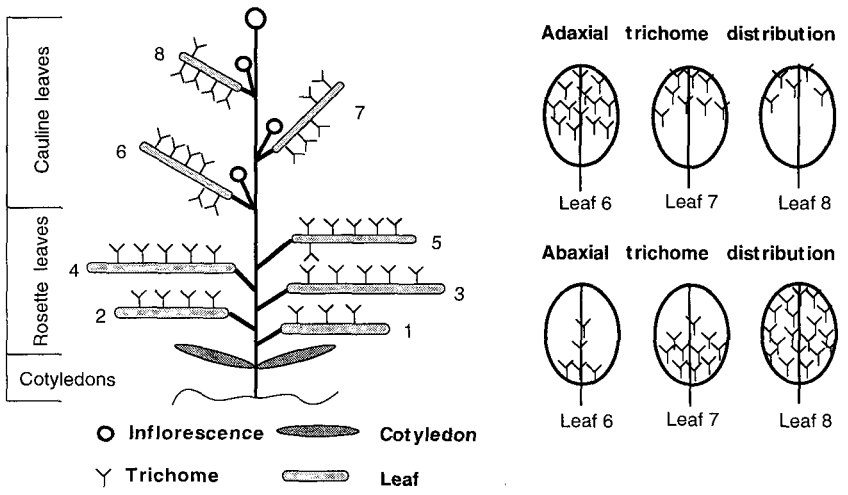

Figure 2. Schematic drawing of trichome distribution on a WT *A. thaliana* plant grown in CL. Leaves 1 to 4 lack abaxial trichomes. Leaves 6 to 8 are cauline leaves showing inhibition of adaxial trichome formation from the leaf base toward the tip on successively younger leaves. On the abaxial surfaces of these leaves, trichome distribution changes gradually on successive leaves: trichomes are limited to the base and midvein of leaf 6, the basal half of leaf 7, and cover the entire abaxial surface of leaf 8.

tion. Under CL conditions, all three mutants delayed abaxial trichome formation by one to two leaves (Fig. 4). All three of the mutants formed the first abaxial trichome on about leaf 5, whereas the WT plant formed the first abaxial trichome on about leaf 3. The delay of abaxial trichome formation was shown to be significant ($P < 0.01$) based on Student's t test. Similarly, the total number of leaves produced at flowering by each of the three mutants was about one leaf more than that produced by the WT.

Application of four 5-μL drops of 1 mM GA_3 1 week after germination to *ga1–3* mutant plants grown in CL conditions restored leaf expansion and trichome formation on both surfaces of pre-existing and newly formed leaf primordia. Distribution of abaxial trichomes covered the basal portion of a leaf at first and then spread toward the leaf tip on successive leaves in a manner similar to that shown in Figure 2.

GA treatment also promoted the early onset of abaxial trichome initiation in WT plants. When WT Columbia plants grown in SD conditions were treated with GA_3 starting on d 21, abaxial trichomes were induced as early as leaf 6, whereas SD control plants initiated abaxial trichomes at approximately leaf 13 (Table II). GA treatment also caused the plants to flower early. Treated plants formed approximately 20 fewer leaves than control plants.

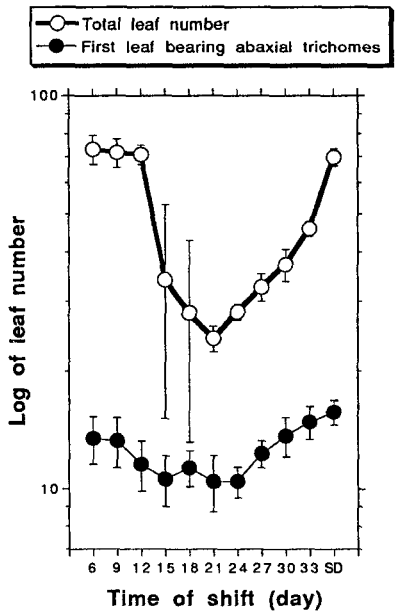

Figure 3. The photoperiod-sensitive and -insensitive phases of abaxial trichome formation and floral induction of WT Columbia plants. WT plants were germinated in an SD chamber every 3 d. When the oldest group of plants was 33 d old and the youngest group was 6 d old, all of the plants were exposed to a 32-h-long CL treatment without changing the light intensity. Afterward, the chamber was shifted back to the previous SD condition. SD control plants were placed in a dark room during part of the CL treatment to maintain the SD photoperiod. After plants bolted, the first leaf to bear abaxial trichomes and the total leaf number (rosette plus cauline) were measured. The mean leaf numbers are plotted (n = 16–26). Vertical bars represent the SD values.

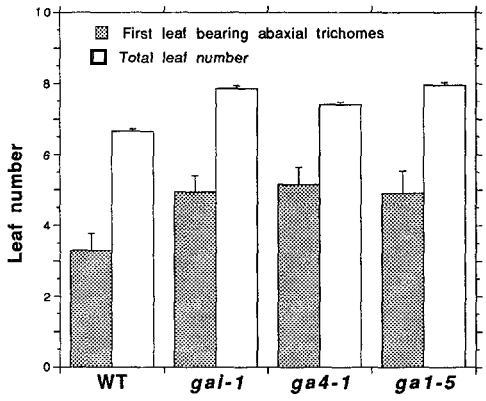

Figure 4. Comparison of abaxial trichome appearance and total number of leaves produced at flowering among WT (Ler), GA-insensitive (*gai-1*), and GA-deficient (*ga4–1, ga1–5*) mutants grown in CL. The mean leaf numbers are plotted (n = 14–20). Vertical bars represent the SD values. Student's t test indicates that the null hypothesis can be rejected at a significance level less than 0.01 for comparison between the means of the WT and each of the mutants.

GA was required for trichome formation on both leaf surfaces, even though trichome formation on the adaxial epidermis of *ga1–3* plants grown in SD conditions continued for 3 to 4 months. GA_3 application stimulated trichome formation on both surfaces of *ga1–3* plants and accelerated the appearance of abaxial trichomes on WT Columbia plants.

Paclobutrazol Suppresses Trichome Formation

To further explore the effects of GA on trichome formation, 35-d-old SD-grown Landsberg *erecta* plants were treated with 20 mg L^{-1}, 40 mg L^{-1}, and 80 mg L^{-1} paclobutrazol. Paclobutrazol inhibits GA biosynthesis at the three successive oxidative steps between *ent*-kaurene and *ent*-kaurenoic acid early in the GA biosynthesis pathway (Hedden and Graebe, 1985). In all three treatments, newly formed leaf primordia produced only a few or no abaxial trichomes, whereas control plants treated with tap water continued to produce several hundred abaxial trichomes per leaf. The number of adaxial trichomes produced by leaf primordia formed after the application of paclobutrazol did not appear to be reduced. No trichome initials were observed in the glabrous region of the abaxial epidermis using a dissecting light microscope. The trichome distribution on the affected abaxial surfaces was similar to that commonly observed on the adaxial surfaces of WT cauline leaves. Thus, the field of inhibition progressed from the base toward the tip of successive leaves. Leaf expansion was also inhibited by paclobutrazol treatment.

Comparison of Adaxial and Abaxial Epidermal Response to GA_3 and CL

The previously described observations concerning differential trichome distribution on GA-deficient mutant plants and WT leaves treated with paclobutrazol suggest that the adaxial leaf epidermis may be more responsive to GA treatment than the abaxial epidermis. To test this hypothesis, we applied different concentrations of GA_3 ranging from 1 μM to 1 mM to glabrous *ga1–3* mutant plants grown in SD conditions and observed when adaxial and abaxial trichomes were formed. The experiment was carried out twice; data from the first experiment are presented in Figure 5. In general, the higher the GA concentration used, the earlier trichomes appeared on both leaf surfaces and the earlier the plants flowered. In the first experiment none of the plants treated with 1 μM GA_3 flowered or produced trichomes on the abaxial epidermis, although trichomes were induced on the adaxial epidermis. In the second experiment of eight plants treated with 1 μM GA_3, one flowered but failed to produce trichomes on the abaxial epidermis. The other seven plants flowered and produced trichomes on the abaxial epidermis after a significant delay. One consistent finding from all of the GA treatments was that the first GA-induced abaxial trichomes always appeared on later leaves than the first GA-induced adaxial trichomes. Control plants treated with 1% ethanol and 0.1% Triton remained glabrous and did not flower during the experiment.

Our earlier experiments suggested that the effect of photoperiod on trichome formation was mediated by GAs because *ga1–3* mutant plants grown in CL did not initiate trichomes on the abaxial epidermis. To determine whether

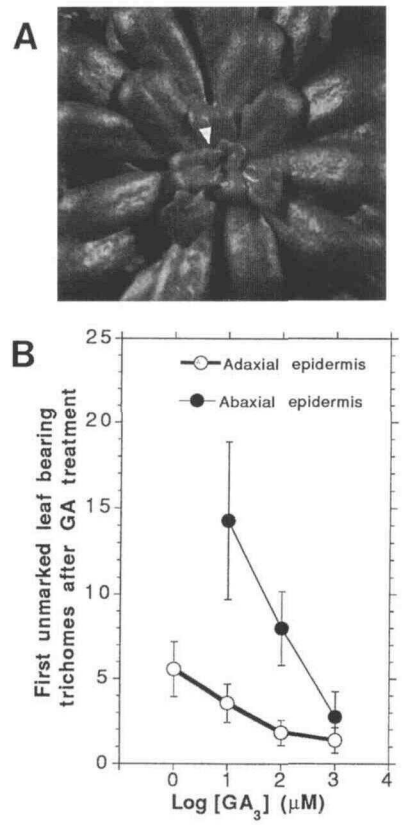

Figure 5. Comparison of adaxial and abaxial trichome initiation on *ga1–3* leaves in response to different concentrations of GA_3. Plants of *ga1–3* were grown in SD conditions for 3 months until the youngest leaves became completely glabrous. At that time all of the reflexed leaves were marked with a dot of red nail polish. A GA_3 solution was applied to the 10 youngest marked leaves of each plant. Each of these leaves received a 5-μL drop of GA_3 in 1% ethanol and 0.1% Triton. Control plants were treated similarly but received ethanol and Triton without GA_3. A, Arrowhead points to the youngest reflexed leaf; B, new formation of adaxial and abaxial trichomes after GA_3 treatment was measured by counting the first unmarked leaf bearing trichomes on either epidermis. The mean leaf numbers are plotted (n = 6–8). Vertical bars represent the SD values.

Table II. *The effects of GA_3 on abaxial trichome appearance and flowering time of WT Columbia plants*

Columbia plants grown in SD conditions were treated with a 5-μL drop of 1 mM GA_3 on each leaf below the apical bud starting on d 21. Plants were treated with GA_3 three times at 3-d intervals.

	Control	1 mM GA_3
First leaf bearing abaxial trichomes (leaf no.)	12.6 ± 0.8[a]	6.7 ± 0.7
Total leaf no. (rosette plus cauline)	64.0 ± 4.0	43.2 ± 3.3

[a] Averages ± SD (n = 9–12).

daylength altered the overall responsiveness of both leaf surfaces to GAs with regard to trichome formation, we performed a photoperiod shift experiment using *ga1–3* mutant plants whose seeds were soaked in 20 μM GA_3 for 2 d before being transferred to soil. Plants were grown in SD conditions and transferred to CL at either 2 or 3 months after germination. Before transfer, 2-month-old *ga1–3* plants had no trichomes on the abaxial epidermis and a few trichomes on the adaxial epidermis of the youngest leaves, whereas 3-month-old mutant plants had no trichomes on either epidermis of the youngest leaves. Three weeks after transfer, plants that were shifted when they were 2 months old produced trichomes on both surfaces of newly formed leaves, whereas plants that were shifted when they were 3 months old produced trichomes only on the adaxial surface. Control plants kept under SD conditions produced only glabrous leaves after the 3rd month. CL increased trichome production on both leaf surfaces of *ga1–3* plants.

DISCUSSION

Our results show that trichome formation is regulated by GA and daylength (other aspects of light are likely to be involved as well, but this has not been fully studied). In addition, trichome formation on the adaxial leaf epidermis of *A. thaliana* is regulated differently from that on the abaxial leaf epidermis. There are physiological differences between the two leaf surfaces that result in the suppression of adaxial trichomes on cauline leaves and a delay in abaxial trichome formation on rosette leaves.

It has been shown previously that the genes *GL1* and *TTG* are required for trichome formation. Our findings indicate that there is an absolute requirement for GA for the formation of trichomes. The protein sequence of *GL1* from *A. thaliana* is homologous to the Myb family of transcription factors (Oppenheimer et al., 1991). Recent work by Gubler et al. (1995) indicates that in barley aleurone cells the RNA expression of a novel Myb transcription factor is up-regulated by GA. The function of the *TTG* gene in both anthocyanin production and trichome formation can be replaced by the *R* gene from maize in transgenic *A. thaliana ttg* mutant plants (Lloyd et al., 1992). The protein encoded by the *R* gene is a transcription factor that normally regulates anthocyanin biosynthesis in maize. Both *gl1* and *ttg* mutants have normal leaf expansion and stem elongation, thus lacking signs of GA deficiency. Therefore, it is likely that GA regulates events upstream of one or both of these genes or is required at the same developmental stage.

A stimulatory effect of GA on trichome formation has also been reported in *Zea mays* L., in which trichomes have been used as one of several markers of the juvenile to adult phase change (Evans and Poethig, 1995). The GA-deficient maize mutants, *d1*, *d3*, and *d5*, have delayed trichome formation in addition to altering the timing of other traits related to the vegetative and the reproductive phase change.

Although the effect of LD photoperiods on stem elongation and flowering time is well documented for LD rosette plants (Jones and Zeevaart, 1980; Koornneef et al., 1991; Talon et al., 1991), the effect of daylength on trichome initiation has not been reported. Long days may increase the rate of GA biosynthesis, GA activity, or the overall sensitivity to GA. LD induction may up-regulate any one of these persistently because once LD-induced trichome formation is initiated abaxial trichomes continue to be formed on plants shifted back to SD conditions. An example of an LD-induced increase in GA level has been reported in *Silene armeria* (Talon and Zeevaart, 1992). Stimulation of *ent*-kaurene biosynthesis by LD conditions has been reported in spinach and in *Agrostemma githago* (Zeevaart and Gage, 1993). Alteration of sensitivity to GA by LD photoperiods has been proposed as a possible regulating mechanism for stem elongation in several species (Jones and Zeevaart, 1980; Metzger, 1985).

Data from our experiments do not provide adequate information to clearly determine how GA may be regulated by daylength. In *A. thaliana*, the *ga1–3* mutant has a 5-kb deletion in the 5′ region of the gene encoding the *ent*-kaurene synthetase A enzyme (Sun and Kamiya, 1994) and is therefore considered to be a null mutant. Our experiment shifting *ga1–3* plants from SD conditions to CL when the plants were either 2 or 3 months old and presumably still contained residual amounts of the exogenous GA_3 used to induce germination resulted in the formation of trichomes on both leaf surfaces (2-month shift) or only on the adaxial epidermis (3-month shift). If we assume that GA biosynthesis is blocked, this suggests that CL may increase the overall sensitivity of leaves to GA_3 or the biological activity of GA_3. However, because we have not eliminated the possibility of weak *ent*-kaurene synthetase isoenzyme activity that may be stimulated by CL, we cannot unequivocally prove that the CL-induced trichomes on *ga1–3* plants are not due to increased GA concentration. Quantification of the levels of active endogenous GAs in the *ga1–3* mutant under both SD and CL conditions may help resolve this issue.

The general pattern of trichome distribution observed in WT Columbia and Landsberg *erecta* plants is dependent on numerous factors. In addition to the rates of GA biosynthesis and degradation, inhibitory factors must also be involved in the suppression of trichomes. Other regulatory elements determine the photoperiod sensitivity of trichome formation. Mozley and Thomas (1995) have reported that WT Landsberg *erecta* plants are only moderately sensitive to floral induction by LD conditions during early development. Our finding that WT Columbia seeds germinated on 1 mM GA_3 (data not shown) do not produce abaxial trichomes until leaf 4 also suggests that there is a GA-insensitive phase for abaxial trichome formation during early vegetative development. The suppression of adaxial trichomes on cauline leaves and sepals suggests that additional inhibitory factors are present in the adaxial epidermis during the later stages of development.

To summarize, the difference in trichome distribution on the leaves of *A. thaliana* is dependent on a number of factors, which may include the rate of GA biosynthesis and metabolism, the site of GA sequestration, or the sensitivity to GA. The leaf surfaces are differentially responsive to GA.

Moreover, there are developmental stage and epidermis-specific inhibitory factors.

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