Ethylene inhibits stem trichome formation in *Arabidopsis*

Susan I Gibson

Plant and Microbial Biology, University of Minnesota - Twin Cities, Saint Paul, Minnesota, United States

Corresponding Author: Susan I Gibson
Email address: gibso043@umn.edu

Trichomes, specialized cells that form on the above ground parts of plants, are useful model systems for studying cell differentiation. In this study, the plant hormone ethylene was found to strongly inhibit formation of trichomes on stems of *Arabidopsis thaliana*. Plants grown in the presence of high concentrations of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid fail to form trichomes on their primary inflorescences. In addition, plants carrying mutations in *CTR1* that confer a constitutive response to ethylene exhibit severe reductions in stem trichome numbers. In contrast, plants carrying mutations that confer ethylene insensitivity, and plants grown in the presence of an ethylene biosynthesis inhibitor, produce normal numbers of stem trichomes. Together, these results suggest that either excess ethylene or a constitutive ethylene response prevents the normal differentiation of cells that would otherwise form stem trichomes. Reduced ethylene levels and decreased ethylene response, in contrast, appear insufficient to cause cells that do not normally form trichomes to form trichomes. In contrast to ethylene, application of exogenous Glc results in increased stem trichome numbers. Besides affecting stem trichome numbers, ethylene may also affect branching of stem trichomes. In *Arabidopsis thaliana*, the vast majority of stem trichomes are unbranched. When wild-type *Arabidopsis thaliana* of the Col-0 ecotype are grown in the presence of an ethylene biosynthesis inhibitor, the percentage of stem trichomes that are branched increases significantly. However, growth in the presence of an ethylene biosynthesis inhibitor does not affect stem branching in wild-type *Arabidopsis thaliana* of the Ler-0 ecotype. Plants carrying the *etr1-1* and *ein2-1* mutations, which cause ethylene insensitivity, have an increased percentage of branched stem trichomes. In contrast, plants carrying the *ctr1-1* and *ctr1-12* mutations have a decreased percentage of branched stem trichomes. Growth in the presence of a precursor of ethylene biosynthesis also causes a substantial reduction in branching of *Arabidopsis* leaf trichomes, suggesting that ethylene has a negative effect on branching of both leaf and stem trichomes in *Arabidopsis*. 
Title: Ethylene inhibits stem trichome formation in Arabidopsis

Author: Susan I. Gibson

Affiliation: Department of Plant and Microbial Biology, University of Minnesota, Saint Paul, MN, USA

Corresponding author: Susan I. Gibson

Email address: gibso043@umn.edu
Abstract

Trichomes, specialized cells that form on the above ground parts of plants, are useful model systems for studying cell differentiation. In this study, the plant hormone ethylene was found to strongly inhibit formation of trichomes on stems of *Arabidopsis thaliana*. Plants grown in the presence of high concentrations of the ethylene precursor 1-aminocyclopropane-1-carboxylic acid fail to form trichomes on their primary inflorescences. In addition, plants carrying mutations in *CTR1* that confer a constitutive response to ethylene exhibit severe reductions in stem trichome numbers. In contrast, plants carrying mutations that confer ethylene insensitivity, and plants grown in the presence of an ethylene biosynthesis inhibitor, produce normal numbers of stem trichomes. Together, these results suggest that either excess ethylene or a constitutive ethylene response prevents the normal differentiation of cells that would otherwise form stem trichomes. Reduced ethylene levels and decreased ethylene response, in constrast, appear insufficient to cause cells that do not normally form trichomes to form trichomes. In contrast to ethylene, application of exogenous Glc results in increased stem trichome numbers. Besides affecting stem trichome numbers, ethylene may also affect branching of stem trichomes. In *Arabidopsis thaliana*, the vast majority of stem trichomes are unbranched. When wild-type *Arabidopsis thaliana* of the Col-0 ecotype are grown in the presence of an ethylene biosynthesis inhibitor, the percentage of stem trichomes that are branched increases significantly. However, growth in the presence of an ethylene biosynthesis inhibitor does not affect stem branching in wild-type *Arabidopsis thaliana* of the Ler-0 ecotype. Plants carrying the *etr1-1* and *ein2-1* mutations, which cause ethylene insensitivity, have an increased percentage of branched stem trichomes. In constrast, plants carrying the *ctl1-1* and *ctl1-12* mutations have a decreased percentage of branched stem trichomes. Growth in the presence of a precursor of ethylene biosynthesis also causes a substantial reduction in branching of *Arabidopsis* leaf trichomes, suggesting that ethylene has a negative effect on branching of both leaf and stem trichomes in *Arabidopsis*.

Introduction

Trichomes are specialized cells that form on above ground parts of plants, including leaves and stems. Trichomes serve a diverse array of functions, such as protecting plants against herbivores, UV radiation and dehydration (Levin, 1973; Ehleringer, 1984). Leaf trichomes have served as a very useful model system for the study of cell differentiation and pattern formation, particularly in *Arabidopsis*. Leaf trichome formation is controlled both spatially and temporally. In leaf primordia, the protodermal cells that will become trichomes stop cell division and undergo several rounds of DNA replication, typically reaching an average DNA content of 32C. Mature *Arabidopsis* leaf trichomes are unicellular and typically have two to four branches, with three branch trichomes being by far the most common (Plett, Mathur & Regan, 2009). Mutations that affect trichome branch number usually cause alterations either in endoreduplication or the structure of microtubules in the cytoskeleton (Mathur & Chua, 2000). The spacing of leaf trichomes is regulated by both positive and negative regulators of trichome formation. Among the best characterized positive regulators of trichome formation are TTG1, GL1, GL2 and GL3. Loss-of-function mutations in any of these genes causes a glabrous (reduced trichome density) phenotype. Additional transcription factors, including GIS, GIS2, ZFP5, ZFP6 and ZFP8, have
been shown to act in regulation of trichome formation on inflorescence organs (Gan et al., 2006; Gan et al., 2007; Zhou et al., 2011; Zhou et al., 2013).

Several phytohormones have been shown to affect trichome formation. Plants carrying mutations that decrease production of gibberellins have reduced numbers of leaf trichomes (Perazza, Vachon & Herzog, 1998) whereas plants carrying mutations that cause a constitutive gibberellin response have increased numbers of leaf trichomes (Chien & Sussex, 1996; Perazza, Vachon & Herzog, 1998). Gibberellins have also been shown to play a role in formation of stem trichomes. Expression of ZFP6 is induced by application of exogenous gibberellins (Zhou et al., 2013). ZFP6 is believed to act upstream of GIS (Gan et al., 2006), GIS2 (Gan et al., 2007), ZFP5 (Zhou et al., 2011) and ZFP8 (Gan et al., 2007) in the regulation of trichome formation on inflorescence organs. The expression of GIS2, ZFP5, ZFP6 and ZFP8 is also regulated by cytokinins (Gan et al., 2007, Zhou et al., 2013). In addition, cytokinins have been shown to stimulate trichome formation on leaves (Maes, Inzé & Goossens, 2008) and inflorescence stems (Greenboim-Wainberg et al., 2005; Gan et al., 2007). Jasmonic acid has also been shown to act as a positive regulator of trichome formation (Traw & Bergelson, 2003; Maes, Inzé & Goossens, 2008; Yoshida et al., 2009). brassinosteroids may also act as positive regulators of trichome formation. Arabidopsis bsl1 mutants have reduced leaf trichome numbers and an altered response to brassinosteroids, possibly due to a defect in brassinosteroid production. In addition, bsl1 mutant plants are hypersensitive to metabolizable sugars, such as Suc, suggesting that Suc may act as a negative regulator of leaf trichome formation (Laxmi et al., 2004). Salicylic acid has also been suggested to act as a negative regulator of trichome development, as exogenous salicylic acid causes a decrease in leaf trichome density (Traw & Bergelson, 2003).

Ethylene has not previously been reported to act in regulation of trichome formation. However, ethylene has been reported to affect trichome branching. ETR2 encodes an ethylene receptor (Sakai et al, 1998). The Arabidopsis etr2-3 loss-of-function mutation prevents ETR2 from activating CTR1 (Plett, Mathur & Regan, 2009). CTR1 is a member of the Raf family of serine/threonine protein kinases and acts as a negative regulator of ethylene response (Kieber et al., 1993). As a result, etr2-3 mutants exhibit a constitutive ethylene response. In addition, etr2-3 mutants produce unbranched leaf trichomes, suggesting that ethylene has a negative effect on Arabidopsis leaf trichome branching (Plett, Mathur & Regan, 2009). Ethylene has also been shown to affect the shape of trichomes on cucumber hypocotyls. Unlike Arabidopsis trichomes, cucumber trichomes are multi-cellular, typically consisting of three to four cells each. Transient exposure to ethylene causes an increase in the number of cucumber trichomes that are made up of more than four cells and increases trichome branching (Kazama et al., 2004).

Materials & Methods

Plant materials and chemicals

Wild-type seeds of Arabidopsis thaliana var. Columbia (Col-0) and Landsberg erecta (Ler-0) were obtained from Dr. Chris Somerville (University of California, Berkeley, CA). The ctrl-12/sis1-1 mutant was isolated from the Col-0 background (Gibson, Laby & Kim 2001). The ctrl-1, etr1-1 and ein2-1 mutations, in the Col-0 background, and the ttg-1, gl1-1, gl2-1 and gl3-1
mutations, in the Ler-0 background, were obtained from the Arabidopsis Biological Resource Center at Ohio State University. Arabidopsis minimal media was prepared as described (Kranz & Kirchheim, 1987). Aminoethoxyvinylglycine (AVG) and 1-aminocyclopropane-1-carboxylic acid (ACC) were obtained from Sigma (St. Louis, MO).

Analyzing trichome numbers and branching

To analyze trichome number and branching, the basal two cm of the primary inflorescence of each plant was removed. Magni-focusers that magnify 2.5 to 3 fold (Carolina Biological Supply Company) were used to aid in counting the number of trichomes on each stem section. First, the number of trichomes that were visible to an observer holding the stem section in a fixed position were counted. Next, the number of trichomes that would not have been visible to an observer on the opposite side of the stem section (i.e. those trichomes that would have been completely blocked from sight by the stem) were counted. The number of trichomes that were visible and the number of trichomes that would not have been visible to someone viewing the stem from the opposite side were then added together to get an estimate of the total number of trichomes on each stem section. In other words, the number of trichomes that would not have been visible to an observer looking at the stem from the opposite side were estimated to be similar to the number of trichomes that were not visible to the observer due to being on the opposite side of the stem from the observer. A dissecting scope was used to aid in counting branched versus unbranched trichomes on stem segments.

Scanning electron microscopy

Stems and leaves were placed in vials with 3% glutaraldehyde and left on a shaker at room temperature overnight. Tissue samples were post-fixed using osmium tetroxide, dehydrated in ethanol and critical point dried. The samples were mounted and sputter coated with gold and palladium. Tissues were then visualized using a Jeol JSM-6100 scanning electron microscope.

Results

ctr1 mutants have greatly reduced numbers of stem trichomes

In the course of studying plants homozygous for the ctr1-12 mutation (Gibson, Laby & Kim, 2001), it became apparent that these plants have greatly reduced numbers of visible trichomes on their primary stems (Fig. 1). To determine whether the reduced trichome number is due to the presence of the ctr1-12 mutation or to a mutation in a different gene in the genetic background of the ctr1-12 plants, an independent ctr1 mutant, ctr1-1 (Kieber et al., 1993), was obtained. The ctr1-1 plants also produce greatly reduced numbers of stem trichomes, indicating that this effect is due to mutations in the CTR1 gene (Fig. 1). All of the lines tested produce a greater density of stem trichomes when grown in soil in pots than when grown on synthetic media in Magenta boxes (Fig. 1)

Exogenous ethylene phenocopies the effects of ctr1 mutations on stem trichome density
The above results indicate that loss-of-function mutations in \textit{CTR1} inhibit trichome formation on Arabidopsis stems. As \textit{CTR1} is known to act as a negative regulator of ethylene response (Kiefer et al., 1993), these results suggest that ethylene plays a negative role in stem trichome formation. However, alternative models, such as \textit{CTR1} acting on trichome formation via a different response pathway, cannot be ruled out on the basis of the above results. Therefore, it was of interest to determine whether exogenous ethylene affects stem trichome formation. Towards this end, wild-type \textit{Arabidopsis thaliana} of the Col-0 ecotype were grown on minimal Arabidopsis media (Kranz & Kirchheim, 1987) supplemented with 1% Glc and varying amounts of ACC, a precursor of ethylene biosynthesis (Adams & Yang, 1979). Quantification of visible trichomes on the lowest two cm of the primary stem of these plants revealed that, although 0.5 µM ACC has no apparent effect on stem trichome density, 5.0 µM ACC causes a statistically significant reduction in stem trichome number and growth on 50 µM ACC results in almost complete elimination of visible stem trichomes (Fig. 2).

In the experiments described above, stem trichomes were characterized using Magni-focusers that magnify 2.5 to 3.0 fold. At this level of magnification, trichomes may not be visible either because they do not form or because they do not enlarge (develop) normally. To distinguish between these possibilities, sections from the bottom 2 cm of the primary inflorescences of \textit{ctr1-1}, \textit{ctr1-12}, and wild-type plants grown in the presence and absence of ACC were characterized by scanning electron microscopy. The stems of plants that were grown on ACC or that carry a loss-of-function mutation in \textit{CTR1} show no signs of trichome formation (Fig. 3). These results suggest that ethylene inhibits initiation and/or very early development of stem trichomes.

**Stem trichome numbers are not affected by decreased ethylene or ethylene response**

As increases in ethylene or ethylene response lead to reduced stem trichome numbers, it was of interest to determine whether decreased ethylene or ethylene response lead to increased stem trichome numbers. AVG is an inhibitor of ACC synthase, a key enzyme in ethylene biosynthesis (Schaller & Binder, 2017). Growth on media containing 1 µM or 8 µM AVG does not result in statistically significant alterations in stem trichome numbers in wild-type Col-0 or Ler-0 plants (Fig. 4). In addition, neither of two mutations that confer ethylene insensitivity, \textit{etr1-1} (Bleecker et al., 1988) and \textit{ein2-1} (Alonso et al., 1999), alter stem trichome densities (Fig. 5). The results of these experiments do indicate that the Col-0 ecotype produces a higher density of trichomes on the bottom two cm of the primary inflorescence than does the Ler-0 ecotype (Fig. 4).

Growth on 8 µM AVG does not result in production of stem trichomes by any of the mutants defective in trichome production that were tested. The \textit{ttg-1}, \textit{gl1-1} and \textit{gl2-1} mutants produced no visible stem trichomes on the bottom two cm of the primary inflorescence when grown on media containing 0 or 8 µM AVG. Similarly, addition of 8 µM AVG to the growth media does not lead to increases in stem trichome numbers in the \textit{gl3-1} mutant (data not shown).

**Exogenous Glc has a positive effect on stem trichome density**

Mutations that affect ethylene response may also affect sugar response (Zhou et al., 1998; Gibson, Laby & Kim, 2001). Therefore, it was of interest to determine whether growth on exogenous Glc affects stem trichome densities. Wild-type plants of the Col-0 ecotype have...
significantly higher trichome densities on the bottom two cm of their primary inflorescences when grown on media supplemented with 0.1 M Glc than when grown on media supplemented with 0.02 M Glc (Fig. 6). The effects of 0.1 M Glc cannot be mimicked by growth on an equimolar combination of Glc and sorbitol. In addition, *ctr1-1* and *ctr1-12* mutants produce greater numbers of stem trichomes when grown on media containing 0.1 M Glc than when grown on media with 0.02 M Glc (Fig. 6).

**Effect of ethylene on branching of stem and leaf trichomes**

The effects of altering ethylene levels and ethylene signaling on trichome branching were also examined. The vast majority of Arabidopsis stem trichomes are unbranched, with only a small percentage having two branches. Growth of wild-type Arabidopsis of the Col-0 ecotype on 0 to 50 µM ACC revealed no statistically significant differences in the percentages of branched stem trichomes (Fig. 7A). However, the very low rate (0.7%) of stem trichome branching observed in Col-0 plants growing on 0 µM ACC may make it difficult to detect any inhibition of stem trichome branching by ACC. To test the effects of decreased ethylene levels, plants were grown on media supplemented with different concentrations of AVG. Growth of wild-type Col-0 Arabidopsis on 1 or 8 µM AVG causes statistically significant increases in the percentages of stem trichomes that are branched relative to plants grown on media lacking AVG (Fig. 7B). In contrast, growth of wild-type Arabidopsis of the Ler-0 ecotype on the same AVG concentrations causes no statistically significant alterations in the percentages of branched stem trichomes. Instead, Ler-0 plants produce branched stem trichomes at a frequency of approximately 5% regardless of the AVG concentration. To further examine possible effects of ethylene signaling on stem trichome branching, several ethylene response mutants were characterized. The Arabidopsis *etr1-1* (Bleeker et al., 1988) and *ein2-1* (Alonso et al., 1999) mutations confer ethylene insensitivity whereas the *ctrl-1* (Kieber et al., 1993) and *ctrl-12* (Gibson, Laby & Kim, 2001) mutations confer a constitutive ethylene response. Plants carrying the *etr1-1* mutation exhibit a statistically significant increase in the percentage of branched stem trichomes. Plants carrying the *ein2-1* mutation also exhibited a slight increase in the percentage of branched stem trichomes, but this difference was not statistically significant (Fig. 8). Both the *ctrl-1* and *ctrl-12* mutations cause a statistically significant decrease in the percentages of stem trichomes that are branched.

The fact that ethylene has dramatic effects on formation of stem trichomes raised the question of whether ethylene also affects leaf trichomes. Scanning electron micrographs of plants grown in the presence of ACC reveal that, unlike with stem trichomes, the presence of even 50 µM ACC does not eliminate formation of leaf trichomes (Fig. 9). However, 50 µM ACC does have dramatic effects on leaf trichome branching. Normal leaf trichomes produced by *Arabidopsis thaliana* of the Col-0 ecotype have three branches. In contrast, the leaf trichomes present on ACC-grown plants show an almost complete lack of branching (Fig. 9).

**Discussion**

In this work, ethylene was found to have a profound inhibitory effect on the development of stem trichomes in Arabidopsis. Both application of a precursor of ethylene biosynthesis and mutations...
that cause a constitutive ethylene response result in severe reductions in stem trichome numbers. In contrast, neither an inhibitor of ethylene biosynthesis nor mutations that reduce ethylene response significantly affect stem trichome numbers. These results suggest that increasing ethylene levels or response prevents some cells that would normally form stem trichomes from differentiating properly. The findings that ethylene biosynthesis inhibitors and ethylene insensitive mutants do not produce increased numbers of stem trichomes suggest that neither increased ethylene levels nor response are sufficient to induce cells that would not normally form trichomes to form trichomes.

Ethylene has not previously been reported to affect trichome numbers. This may be due to the fact that most previous studies on trichome differentiation and development have focused on leaf trichomes. In this study, the effects of ethylene on stem trichome numbers were profound, whereas no dramatic effects of ethylene on leaf trichome numbers were observed. Although leaf trichome numbers were not quantified in this study, leaving open the possibility that ethylene might have minor but still statistically significant effects on leaf trichome numbers, the lack of dramatic effects of ethylene on leaf trichome numbers may explain why ethylene has not previously been reported to affect trichome numbers. Alternatively, it is possible that ethylene affects stem trichome numbers via gene(s) that are not needed for leaf trichome formation or that are regulated differently during formation of leaf versus stem trichomes. Potential precedent for this second possibility comes from studies showing that gibberellins and cytokinins regulate the expression of several genes involved in formation of stem trichomes (Gan et al., 2006; Gan et al., 2007; Zhou et al., 2011; Zhou et al., 2013).

Although ethylene has not previously been reported to affect trichome numbers, ethylene has been postulated to act as a positive regulator of root hair formation. Treatment with ACC causes formation of greater numbers of root hairs, whereas treatment with inhibitors of ethylene formation causes a decrease in root hair numbers (Tanimoto, Roberts & Dolan, 1995). In addition, some mutations that affect ethylene perception cause alterations in root hair differentiation (Masucci & Schiefelbein, 1994). However, the role of ethylene in root hair formation remains unclear, as the ethylene-insensitive mutants etr1 and ein2 exhibit wild-type root hair densities, whereas ctrl constitutive ethylene response mutants exhibit increased root hair densities (Masucci & Schiefelbein, 1996). Both trichomes and root hairs form from epidermal cells and their differentiation has been shown to be regulated by similar molecular mechanisms (Ishida et al., 2008). The results of this work suggest that ethylene has opposite effects on stem trichome numbers as on root hair numbers. Application of high concentrations of the ethylene biosynthesis precursor ACC leads to almost complete elimination of stem trichome formation but increases in root hair numbers. Elucidation of the molecular mechanisms by which ethylene affects stem trichome formation will require further experimentation.

Glc was found to have a positive effect on stem trichome numbers. The positive effects of 0.1 M Glc on stem trichome numbers are not mimicked by treatment with an osmotic control, indicating that the effects of Glc on stem trichome numbers cannot be due solely to osmotic stress. The finding that ethylene and Glc have opposite effects on stem trichome numbers is consistent with the results of previous studies showing that ethylene and sugars (Glc and Suc) have opposite effects on some pathways. For example, high concentrations of exogenous Glc or Suc inhibit early seedling development of Arabidopsis, whereas this inhibition is relieved by
treatment with ACC or by mutations conferring a constitutive ethylene response (Zhou et al., 1998; Gibson, Laby & Kim, 2001).

Previous evidence suggesting that sugars might a role in trichome formation comes from studies of the Arabidopsis bsl1 mutants. These mutants have reduced leaf trichome numbers and are hypersensitive to Suc, suggesting that Suc may act as a negative regulator of leaf trichome formation (Laxmi et al., 2004). The fact that sugars were shown to have a positive effect on stem trichome numbers in this study, but a negative effect on leaf trichome numbers in the study by Laxmi et al, could have multiple possible explanations. One possibility is that the differences in these studies could be due to differences in the molecular mechanisms leading to the formation of stem versus leaf trichomes. Another possibility is that the effects of mutations in BLS1 on leaf trichome numbers could be due to the role of BLS1 in brassinosteroid, rather than sugar, signaling.

The effects of ethylene on branching of stem trichomes were also examined. It was not practical to determine if high concentrations of ACC have an effect on stem trichome branching as plants grown on high concentrations of ACC produce extremely few trichomes. Growth on the ethylene biosynthesis inhibitor AVG causes a statistically significant increase in the percentage of stem trichomes that are branched in the Col-0 ecotype, but not in the Ler-0 ecotype. Plants carrying the etr1-1 and ein2-1 mutations, which cause ethylene insensitivity, exhibited an increase in the percentages of stem trichomes that were branched, although the results for ein2-1 were not statistically significant. Plants carrying the ctr1-1 and ctr1-12 mutations, which cause a constitutive ethylene response, exhibit a decrease in the percentage of stem trichomes that are branched. Together, these results suggest that ethylene may have a negative effect on branching of stem trichomes.

Ethylene has previously been shown to have a negative effect on branching of Arabidopsis leaf trichomes. The etr2-3 mutation causes a constitutive ethylene response and a decrease in leaf trichome branching (Plett, Mathur & Regan, 2009). The results of this work also indicate that ethylene has a negative effect on branching of leaf trichomes in Arabidopsis. Treatment with high concentrations of an ethylene biosynthesis precursor results in almost complete elimination of branching of Arabidopsis leaf trichomes. In contrast, treatment with ethylene has been shown to increase branching in cucumbers (Kazama et al, 2004). The apparent discrepancy between the results for Arabidopsis versus the results for cucumber could be due to the fact that Arabidopsis trichomes are unicellular whereas cucumber trichomes typically consist of three to four cells each, which could lead to important differences in the mechanisms controlling branching.

Conclusions

Ethylene is a strong inhibitor of trichome formation on Arabidopsis stems. Treatments that increase ethylene levels and mutations that increase ethylene response both result in the almost complete absence of trichomes on the primary inflorescence. In contrast, treatments that decrease ethylene levels and mutations that decrease ethylene response do not alter stem trichome numbers, suggesting that reductions in ethylene response are not sufficient to trigger additional cells to differentiate to form trichomes. In contrast to ethylene, exogenous Glc has a positive
effect on stem trichome formation. Results presented here also suggest that ethylene affects
trichome branching in addition to trichome formation. Mutations that decrease ethylene response
tend to cause an increase in the percentage of stem trichomes that are branched whereas
mutations that increase ethylene response cause a decrease in the percentage of branched stem
trichomes. As has been shown previously, increased ethylene levels also cause a very large
reduction in branching of Arabidopsis leaf trichomes, suggesting that ethylene has a negative
effect on branching of both stem and leaf trichomes.

Acknowledgements

Carrie Readal is thanked for technical help in counting trichome numbers. Jim Barrish is thanked
for technical help with scanning electron microscopy.

References

Adams DO, Yang SF. 1979. Ethylene biosynthesis: Identification of 1-aminocyclopropane-1-
carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proceedings of
the National Academy USA 76:170-174.

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR. 1999. EIN2, a bifunctional

Bleecker AB, Estelle MA, Somerville C, Kende H. 1988. Insensitivity to ethylene conferred by a

Chien JC, Sussex IM. 1996. Differential regulation of trichome formation on the adaxial and
abaxial leaf surfaces by gibberellins and photoperiod in Arabidopsis thaliana (L.) Heynh.
Plant Physiology 111:1321-1328. DOI: 10.1104/pp.111.4.1321.


INFLORESCENCE STEMS modulates the regulation by gibberellins of epidermal

Arabidopsis transcription factors GIS, ZFP8 and GIS2 in the regulation of epidermal cell fate.
Development 134:2073-2081.

Gibson SI, Laby RJ, Kim D. 2001. The sugar-insensitive1 (sis1) mutant of Arabidopsis is allelic
to ctr1. Biochemical and Biophysical Research Communications 280:196-203. DOI:
10.1006/bbrc.2000.4062.

Greenboin-Wainberg Y, Maymon I, Borochov R, Alvarez J, Olszewski N, Ori N, Eshed Y,
inhibitor SPINDLY plays a positive role in cytokinin signaling. The Plant Cell 17:92-102.


Kazama H, Dan H, Imaseki H, Wasteneys GO. 2004. Transient exposure to ethylene stimulates
cell division and alters fate and polarity of hypocotyl epidermal cells. Plant Physiology
134:1614-1623. DOI: 10.1104/pp.103.031088.


Figure 1

Mutations in CTR1 have a negative effect on stem trichome numbers.

Seeds of the indicated mutant or Col-0 wild-type (WT) lines were surface sterilized and then incubated in the dark at 4°C for two days prior to sowing. (A) Seeds were sown in pots containing Metro-Mix 200 soil and grown for 40 days at room temperature under 54 to 75 µmol photons m⁻² s⁻¹ continuous fluorescent light. Trichomes were counted on the basal two cm of the primary inflorescence of each plant. Results are means +/- SD (n = 12). (B) Seeds were sown on Petri plates with minimal Arabidopsis media and grown for six days at 21°C under 40 to 58 µmol photons m⁻² s⁻¹ continuous fluorescent light. Seedlings were then transferred to Magenta boxes containing Arabidopsis minimal media supplemented with 0.02 M Glc and grown for an additional 34 days at room temperature under 51 to 72 µmol photons m⁻² s⁻¹ continuous fluorescent light. Trichomes were counted on the basal two cm of the primary inflorescence of each plant. Note that the results for Col-0 wild-type grown on Arabidopsis minimal media supplemented with 0.02 M Glc are the same as those depicted in Figure 6. Results are means +/- SD (n = 16). Mutant and wild-type results differed with *** p value < 0.001, according to a Student’s t-test.
(A) Plants grown on soil

(B) Plants grown on synthetic media
Figure 2

Exogenous ethylene biosynthesis precursor has a negative effect on stem trichome numbers.

Col-0 wild-type seeds were surface sterilized and then incubated in the dark at 4°C for four days. Seeds were then sown on Petri plates containing minimal Arabidopsis media plus 1% (w/v) Suc and grown under continuous fluorescent light for 16 days. The seedlings, which had not bolted, were then transferred to Magenta boxes with minimal Arabidopsis media plus 1% (w/v) Glc and the indicated concentration of ACC and grown for an additional 43 days under 44 to 53 µmol photons m⁻² s⁻¹ continuous fluorescent light. Trichomes were counted on the basal two cm of the primary inflorescence of each plant. Note that the results for Col-0 wild-type seeds on 0 µM ACC are the same as those depicted for Col-0 grown on 0 µM AVG in Figure 4. Results are means +/- SD (n = 12-18). Results on media lacking ACC differed from those on media with ACC with * p value < 0.05, ** p value < 0.01 and *** p value < 0.001, according to a Student’s t-test.
Figure 3

High concentrations of ACC or the \textit{ctr1-1} mutation inhibit visible signs of trichome formation.

Wild-type and \textit{ctr1-1} seeds were surface sterilized and then incubated in the dark at 4°C for two to three days. (A) Wild-type seeds were sown on Petri plates with minimal Arabidopsis media plus 1\% (w/v) Suc and grown under continuous fluorescent light for 7 days. Seedlings were then transferred to Magenta boxes with minimal Arabidopsis media plus 0.1 M Glc and 50 \textmu M ACC and grown for an additional 26 days under continuous fluorescent light. (B) Wild-type and \textit{ctr1-1} seeds were sown in pots containing Metro-Mix 200 and grown for 49 days under continuous light. Basal sections of primary inflorescences were collected from plants grown in Magenta boxes or in pots and used for scanning electron microscopy. No partially formed trichomes were observed on the stem sections from plants grown on 50 \textmu M ACC or from plants carrying the \textit{ctr1-1} mutations.
(A) Wild-type plants grown on synthetic media +/- ACC

0 µM ACC

50 µM ACC

(B) Plants grown on soil

Wild type

ctr1-1
Figure 4

An ethylene biosynthesis inhibitor does not alter stem trichome densities.

Col-0 and Ler-0 wild-type seeds were surface sterilized and then incubated in the dark at 4°C for four days. Seeds were then sown on Petri plates containing minimal Arabidopsis media plus 1% (w/v) Suc and grown under continuous fluorescent light for 16 days. The seedlings, which had not bolted, were then transferred to Magenta boxes with minimal Arabidopsis media plus 1% (w/v) Glc and the indicated concentration of AVG and grown for an additional 43 days under 44 to 53 µmol photons m⁻² s⁻¹ continuous fluorescent light. Trichomes were counted on the basal two cm of the primary inflorescence of each plant. Note that the results for Col-0 wild-type seeds on 0 µM AVG are the same as those depicted for Col-0 grown on 0 µM ACC in Figure 2. Results are means +/- SD (n = 11-12). Results between the indicated plant line and media combinations differed with * p value < 0.05, ** p value < 0.01 and *** p value < 0.001, according to a Student’s t-test.
Neither the *etr1-1* nor *ein2-1* mutations alter stem trichome densities.

Seeds of the indicated Col-0 wild-type (WT) and mutant lines were surface sterilized and then incubated in the dark at 4 °C for four days. Seeds were sown on Petri plates with minimal Arabidopsis media supplemented with 1% (w/v) Suc and grown under continuous fluorescent light for 16 days. Seedlings were then transferred to pots containing Metro-Mix 200 soil and grown under 53 to 96 µmol photons m⁻² s⁻¹ continuous fluorescent light for an additional 42 days. Trichomes were counted on the basal two cm of the primary inflorescence of each plant. Results are means +/- SD (*n* = 17-20). Differences in trichome numbers between the indicated lines were not statistically significant (defined as having a *p* value of < 0.05, according to a Student’s *t*-test).
Exogenous Glc has a positive effect on stem trichome density. Seeds of the indicated mutant or Col-0 wild-type (WT) lines were surface sterilized and incubated in the dark at 4°C for two days. The seeds were then sown on Petri plates with minimal Arabidopsis media and grown for six days at 21°C under 40 to 58 µmol photons m⁻² s⁻¹ continuous fluorescent light. Seedlings were then transferred to Magenta boxes containing Arabidopsis minimal media supplemented with the indicated additives and grown for an additional 34 days at room temperature under 51 to 72 µmol photons m⁻² s⁻¹ continuous fluorescent light. Trichomes were counted on the basal two cm of the primary inflorescence of each plant. Note that the results for Col-0 wild-type grown on Arabidopsis minimal media supplemented with 0.02 M Glc are the same as those depicted in Figure 1. Results are means +/- SD (n = 12-16). Results for plants grown on 0.02 M Glc differed from results for plants of the same line grown on 0.02 M Glc + 0.08 M sorbitol or 0.1 M Glc with * p value < 0.05, ** p value < 0.01 and *** p value < 0.001, according to a Student’s t-test.
Figure 7

Effects of altering ethylene biosynthesis on stem trichome branching.

Col-0 and Ler-0 wild-type seeds were surface sterilized and then incubated in the dark at 4°C for four days. Seeds were then sown on Petri plates containing minimal Arabidopsis media plus 1% (w/v) Suc and grown under continuous fluorescent light for 16 days. The seedlings, which had not bolted, were then transferred to Magenta boxes with minimal Arabidopsis media plus 1% (w/v) Glc and the indicated concentrations of ACC or AVG and grown for an additional 43 days under 44 to 53 μmol photons m⁻² s⁻¹ continuous fluorescent light. Branched and unbranched trichomes were counted on the basal two cm of the primary inflorescence of each plant. Results are means +/- SD (n = 11-18). Results on media lacking ACC and AVG differed from those of plants of the same ecotype grown on media with ACC or AVG with *p value < 0.05, according to a Student’s t-test.
(A) Col-0 wild type grown on ACC

% branched trichomes vs [ACC] (0 µM, 0.5 µM, 5 µM, 50 µM)

(B) Col-0 and Ler-0 wild types grown on AVG

% branched trichomes vs [AVG] (0 µM, 1 µM, 8 µM)

Legend: Col-0, Ler-0
Figure 8

Effects of altering ethylene response on stem trichome branching.

Seeds of the indicated Col-0 wild-type (WT) and mutant lines were surface sterilized and then incubated in the dark at 4 °C for four days. Seeds were sown on Petri plates with minimal Arabidopsis media supplemented with 1% (w/v) Suc and grown under continuous fluorescent light for 16 days. Seedlings were then transferred to pots containing Metro-Mix 200 soil and grown under 53 to 96 µmol photons m⁻² s⁻¹ continuous fluorescent light for an additional 42 days. Branched and unbranched trichomes were counted on the basal two cm of the primary inflorescence of each plant. Results are means +/- SD (n = 13-18). Mutant and wild-type results differed with * p value < 0.05, ** p value < 0.01 and *** p value < 0.001, according to a Student’s t-test.
Figure 9

Treatment with an ethylene precursor inhibits branching of leaf trichomes.

Col-0 wild-type seeds were surface sterilized, incubated in the dark at 4°C for two to three days, sown on Petri plates with minimal Arabidopsis media plus 1% (w/v) Suc and then grown under continuous fluorescent light for 7 days. Seedlings were then transferred to Magenta boxes with minimal Arabidopsis media plus 0.1 M Glc and 0 or 50 µM ACC and grown for an additional 26 days under continuous fluorescent light. The adaxial surfaces of rosette leaves were then examined using a scanning electron microscope.
0.1 M Glc

0.1 M Glc + 50 μM ACC