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HASTY: a gene that regulates the timing of shoot maturation in *Arabidopsis thaliana*

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SUMMARY

In *Arabidopsis thaliana*, leaves produced at different stages of shoot development can be distinguished by the distribution of trichomes on the abaxial and adaxial surfaces. Leaves produced early in the development of the rosette (juvenile leaves) have trichomes on their adaxial, but not their abaxial surface, whereas leaves produced later in rosette development (adult leaves) have trichomes on both surfaces. In order to identify genes that regulate the transition between these developmental phases we screened for mutations that accelerate the production of leaves with abaxial trichomes. 9 alleles of the *HASTY* gene were recovered in this screen. In addition to accelerating the appearance of adult leaves these mutations also accelerate the loss of adaxial trichomes (a trait typical of bracts), reduce the total number of leaves produced by the shoot, and have a number of other effects on shoot morphology. The basis for this phenotype was examined by testing the interaction between *hasty* and genes that affect flowering time (*35S::LEAFY*, *35S::APETALA1*, *terminal flower1*), gibberellin production (*ga1-3*) or perception (*gai*), and floral morphogenesis (*leafy*, *apetala1*, *agamous*). We found that *hasty* increased the reproductive competence of the shoot, and that its does not require gibberellin or a gibberellin response for its effect on vegetative or reproductive development. The phenotype of *hasty* is not suppressed by *leafy*, *apetala1* and *agamous*, demonstrating that this phenotype does not result from the inappropriate expression of these genes. We suggest that *HASTY* promotes a juvenile pattern of vegetative development and inhibits flowering by reducing the competence of the shoot to respond to *LEAFY* and *APETALA1*.

Key words: Phase change, *HASTY*, *Arabidopsis thaliana*, Shoot development

INTRODUCTION

The primary shoot apical meristem of a plant progresses through several distinct phases during its development (Allsopp, 1967; Poethig, 1990; Hackett and Murray, 1993; Lawson and Poethig, 1995). After its initiation during embryogenesis, the shoot meristem begins its development in a juvenile phase, which is usually marked by the production of small, morphologically simple leaves that have a characteristic pattern of cellular differentiation. This is followed by an adult phase, during which the shoot meristem produces larger, more complex leaves that possess a different pattern of cellular differentiation than the one found in juvenile leaves. During the adult phase, the shoot also acquires the ability to undergo reproductive development, becoming competent to respond to floral inducers. The transition to the reproductive phase – which may occur spontaneously, or require one or more environmental stimuli – often involves dramatic changes in the structure of the shoot and results in the production of yet another leaf type (the bract) as well as the initiation of specialized reproductive structures, such as a flower or cone.

The transition from vegetative to reproductive growth has been studied extensively in many different species (Zimmerman et al., 1985; Bernier, 1988; Weigel, 1995). These studies have shown that floral induction is regulated both by diffusible factors produced by organs outside the shoot apex (e.g. cotyledons, leaves, roots), and by the competence of the shoot apex to respond to these factors (McDaniel, 1996). Information about the genes involved in this process has come primarily from genetic analyses of floral induction in *Pisum* (Reid et al., 1996) and *Arabidopsis* (Martínez-Zapater et al., 1994; Coupland, 1995; Weigel, 1995). Genes that act to promote flowering are defined by loss-of-function mutations that delay flowering, whereas genes that normally act to repress flowering are represented by loss-of-function mutations that accelerate flowering. Many such mutations have been identified and have been assigned to functionally related groups based on double mutant analyses, the sensitivity of mutant plants to gibberellin (GA), and their sensitivity to environmental stimuli such as photoperiodic conditions and low temperature (Dennis et al., 1996; Hicks et al., 1996; Peeters and Koornneef, 1996; Reid et al., 1996; Simon and Coupland, 1996; Koornneef and Peeters, 1997). These and other studies suggest that floral induction is regulated by a large number of factors that act in parallel, with no single factor being absolutely essential for the initiation of this process (Amasino, 1996). Several genes that affect flowering time have been cloned recently (Lee et al., 1994; Putterill et al., 1995; Amasino, 1996; Bradley et al., 1997; Macknight et al., 1997; Ohshima et al., 1997), and are providing new clues about the

molecular mechanism of this important developmental phenomenon.

In contrast to what is known about floral induction, the mechanism of vegetative maturation is very poorly understood, particularly in herbaceous species (Wareing, 1987). We decided to investigate the regulation of vegetative maturation in *Arabidopsis thaliana* by identifying and characterizing genes that affect the timing of this process. Although *Arabidopsis thaliana* exhibits developmentally regulated changes in leaf morphology (Telfer and Poethig, 1994; Martínez-Zapater et al., 1995), the most useful marker of the vegetative phase of the shoot is the distribution of epidermal hairs (trichomes) on the leaf blade (Telfer et al., 1997). Rosette leaves produced during the juvenile vegetative phase develop trichomes on their upper (adaxial) surfaces but not on their lower (abaxial) surfaces. Rosette leaves produced during the adult vegetative phase develop both abaxial and adaxial trichomes. All bracts possess abaxial trichomes, but bracts that develop at apical positions within the inflorescence often have few or no adaxial trichomes. The development of abaxial trichomes is promoted by GA (Chien and Sussex, 1996; Telfer et al., 1997), a finding which is consistent with the effect of GA on phase change in other species (Zimmerman et al., 1985). However, GA does not appear to be the sole regulator of vegetative phase in *Arabidopsis* (Telfer et al., 1997).

Previous genetic analyses of shoot maturation in *Arabidopsis* have focused on mutations that affect flowering time. However, there is no *a priori* reason to expect that mutations that affect vegetative phase change will necessarily affect flowering time if vegetative phase change and the production of other factors that regulate flowering are regulated independently. Therefore, we sought to identify additional regulators of shoot maturation in *Arabidopsis* by screening for mutations that delay or accelerate the production of abaxial trichomes. Here we describe a new genetic locus, *HASTY (HST)*, which is defined by mutations that accelerate both vegetative phase change and flowering. We explore the interactions between *HST* and other genetic loci that affect vegetative and reproductive development and suggest a role for *HST* in regulating the attainment of reproductive competence.

MATERIALS AND METHODS

Genetic stocks and growth conditions

Mutant seed stocks were generously provided by the following individuals: *tfl1-10*, R. Meeks-Wagner (U. of Oregon, Eugene); *35S::LFY* (DW151.2.5) and *LFY::GUS* (DW150-209), D. Weigel (Salk Institute, San Diego); *35S::AP1*, M. Yanofsky (U. California, San Diego) All of these stocks were in the Columbia (Col) ecotype. *ag-1*, *ga1-3* and *gai* were obtained from the *Arabidopsis* Biological Resource Center at Ohio State University (Columbus, Ohio), and were in the Landsberg *erecta* (Ler) ecotype.

The original *hst-1* isolate was identified among the M_2 progeny of diepoxy butane-mutagenized seed. This material was generously provided by J. Ecker (U. of Pennsylvania, Philadelphia). *hst-2* was obtained from Ken Feldmann (U. of Arizona, Tucson) and *hst-5* was identified in stocks deposited by A. Kranz in the ABRC stock center (Norwich). *hst-1* was backcrossed 3 times to Col and outcrossed 5 times to Ler before phenotypic analysis was performed in each of these backgrounds. Seeds were grown on Metromix 200 (Scotts) in 10 cm pots (5-16 per pot) and placed at 4°C for two days before transfer to growth chambers (Conviron). Plants were grown under constant illumination (CL) at 18°C or 22°C, as indicated for each experiment. High humidity was maintained during germination and early growth by placing pots in covered flats. The lids were removed after about 2 weeks. In each experiment, pots containing the strains to be compared were interspersed in the same flats. Crosses were performed using *hst-1* plants grown at 18°C as the female parent.

Mapping

HST was mapped to chromosome 3 on the basis of linkage with *HY2* in the F_2 progeny of a cross of a *hst-1* mutant to the mapping line W100. The map position was then refined by analyzing linkage to chromosome 3 SSLP (Bell and Ecker 1994) and CAPS (Konieczny and Ausubel 1993) markers among the F_2 progeny of a cross between *hst-1* (Col) and Ler.

Genetic analysis

To isolate *hst-1 ga1-3* double mutants while circumventing the requirement of *ga1-3* seeds for exogenous GA for germination, F_1 seeds of known *hst-1/hst-1 ga1-3/+* and *hst-1/+ ga1-3/ga1-3* plants were treated as follows: seeds were imbibed overnight in petri dishes on water-soaked filter paper at 4°C in the dark. The embryos were then dissected from their seed coats by scraping the testa with dissecting needles and applying gentle pressure to pop out the embryos. The Petri dishes were then transferred to a growth chamber (22°C, CL). After 2 days undamaged seedlings with green cotyledons were transferred to soil and grown under CL at 22°C. Double mutants were identified as dark green, dwarf plants (a *ga1-3* phenotype) that had uprolled leaves and abaxial trichomes (a *hst-1* phenotype).

To assess the effects of *hst* on *LFY* and *AP1* overexpression, we crossed *hst-1* homozygotes to *35S::LFY* and *35S::AP1* transgenic plants. F_1 plants carrying the transgenes were selected on the basis of their truncated inflorescences and the presence of axillary flowers in the rosette. These plants were self-pollinated and their progeny were analyzed. Plants homozygous for *hst-1* and homozygous or heterozygous for*35S::LFY* or *35S::AP1* were easily identified in the F_2 by their early trichomes and curled leaves (traits typical of *hst*) and truncated inflorescences and axillary flowers (traits typical of *35S::LFY* and *35S::AP1*). No attempt was made to determine the dosage of *35S::LFY* or *35S::AP1* in these plants.

To examine the effect of *hst-1* on *LFY* transcription, seeds homozygous for both *hst-1* and *LFY::GUS* and control seeds homozygous for *LFY::GUS* were germinated on soil under continuous illumination at 22°C. Seedlings were collected at 3 and 5 days after planting and stained overnight at 37°C in a solution containing 1 mg/ml X-gluc, 50 mM sodium phosphate buffer (pH 7), 3 mM potassium ferricyanide, 3 mM potassium ferrocyanide, and 0.1% Triton X-100. After staining, they were decolorized in 70% ethanol and examined with an Olympus BHS microscope using bright-field illumination.

RESULTS

Mutant isolation

By screening for mutations that result in the precocious production of abaxial trichomes on rosette leaves, we identified at least 9 loci that are capable of mutating to give this phenotype. Four of these genes have been previously identified. These include the structural gene for the phytochrome B apoprotein (*PHYB*) (Reed et al., 1993), genes involved in the production of the phytochrome chromophore (*HY1* and *HY2*) (Parks and Quail, 1991), and *SPY*, a component of the GA response pathway (Jacobson and

Fig. 1. The phenotype of *hst-1* in Col. (A) Four-week old wild-type Col (left) and *hst-1* (right) plants. *hst-1* plants produce fewer leaves than wild-type plants, and the leaves of mutant plants are curled upwards. (B) A wild-type (left) and *hst-1* (right) inflorescence. *hst-1* flowers are semi-sterile and are arranged irregularly in the inflorescence. (C) Wild type flower. (D) *hst-1* flower. (E) *hst-1* bract. Adaxial trichomes are absent from the tip of the bract. In wild-type plants, adaxial trichomes disappear first from the base of bract.

Olszewski, 1993). Because *spy* mutations cause a constitutive GA response (Jacobson and Olszewski, 1993) and *phyB* mutations cause an increased sensitivity to GA (Reed et al., 1996), the precocious appearance of abaxial trichomes in these mutants is consistent with the observation that GA is a positive regulator of this trait (Chien and Sussex, 1996; Telfer et al., 1997). In the case of a fifth locus, *PAUSED*, the production of abaxial trichomes at an unusually basal leaf position (leaf 1 or 2 instead of leaf 5 or 6) is associated with a delay in leaf production; the timing of the juvenile-to-adult transition is not affected by mutations of this locus (Telfer et al., 1997). Mutations of four other genes identified in this screen cause abaxial trichomes to be produced at an earlier time in shoot development than in wild-type plants.

Plants carrying alleles of *hst* are unique in that they have curled leaves, lack adaxial trichomes on at least part of the last rosette leaf, and flower early (Fig. 1). The F_1 progeny from a cross of the original mutant plant (*hst-1*) to Col were wild type and the F_2 progeny from these plants segregated wild-type and mutant plants in a ratio of approximately 3:1 (126 wild-type:39 mutant; $\chi^2 = 0.16$, $P > 0.5$); these results demonstrate that *hst-1* is a recessive mutation in a single nuclear gene. Using visible and molecular markers, *hst-1* was mapped to chromosome 3, approximately 0.8 cM to the south of GAPC (see Materials and Methods).

Nine recessive *hst* alleles have been identified in four different ecotypes (Table 1). All of the alleles produce a similar array of phenotypes. Because *hst* alleles affect traits that also differ between ecotypes (e.g. the timing of abaxial trichome development, adaxial trichome loss and flowering) (Larkin et al., 1996; Peeters and Koornneef, 1996; Telfer et al., 1997), we have determined the relative severity of only those alleles isolated in, or introgressed into, the same background (data not shown). *hst-1*, *hst-2*, *hst-4*, *hst-6*, *hst-7*, *hst-8*, and *hst-9* are similar to each other in severity. *hst-3* is less severe than these seven alleles.

Table 1. Mutant alleles of *HST*

Allele	Mutagen	Ecotype
hst-1	Diepoxybutane	Columbia
*hst-2**‡	T-DNA	Wasilewskija
hst-3	Fast neutron	Landsberg *erecta*
hst-4	Ethyl methane sulfonate (EMS)	Columbia
hst-5†	unknown	Enkheim
hst-6	EMS	Columbia
hst-7	X-ray	Columbia
hst-8‡	T-DNA	Wasilewskija
hst-9	Fast neutron	Columbia

*Isolated by K. Feldman.
†Isolated by A. Kranz = N314/F-22.
‡Segregates independently of kanamycin resistance.

The *hst* phenotype

Vegetative morphology

The number of juvenile leaves, adult leaves and bracts produced by *hst-1* and wild-type plants grown at two different times is shown in Fig. 2. Like wild-type plants (Telfer et al., 1997), *hst-1* plants grown under similar conditions can produce slightly different numbers of juvenile and adult leaves. Nevertheless, *hst* mutants consistently produce fewer juvenile leaves than wild type and may produce fewer adult leaves as well. In Col, *hst-1* plants usually produce 2 juvenile leaves, although occasionally they produce only one. The number of adult leaves on *hst-1* plants is much more variable, and is particularly sensitive to light quality and to photoperiodic conditions. *hst-1* has a more extreme effect on shoot morphology in the Ler genetic background, an ecotype that normally produces fewer juvenile and adult leaves than Col. As in the case of wild-type plants, floral initiation in *hst -1* mutants is dramatically delayed by short day conditions; for example, under short days (8 hrs light: 16 hrs dark), *hst-1* (Col)

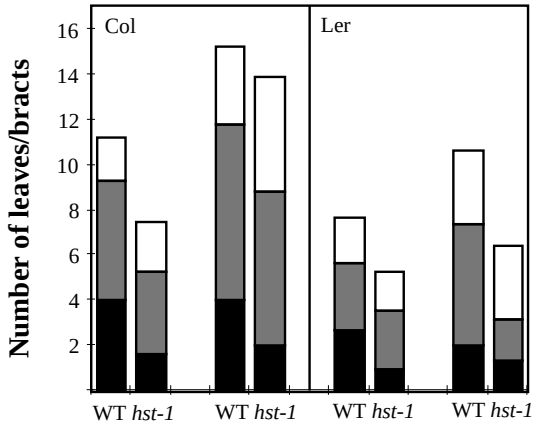

Fig. 2. The number of juvenile leaves, adult leaves and bracts in wild-type and *hst-1* plants. The left panel shows the phenotype of *hst-1* in Col, the right panel shows the phenotype of *hst-1* in Ler. For each ecotype, the phenotype of two sets of plants grown at different times in the same growth chamber is presented. Juvenile leaves (■), adult leaves (▩), bracts (□).

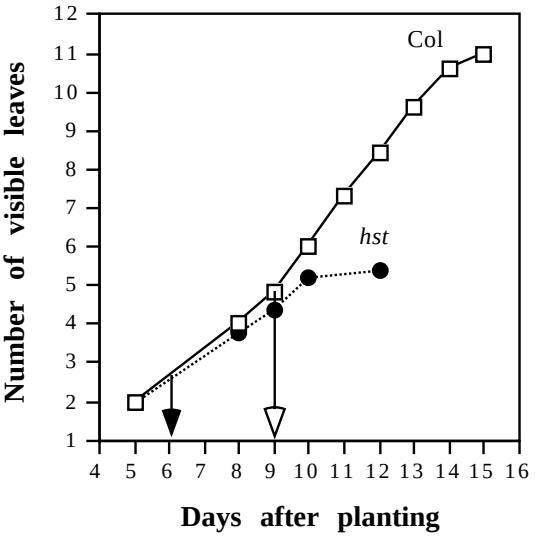

Fig. 3. The rate of leaf initiation of Col and *hst-1* plants. The first point represents the time at which the first two leaves were visible with a stereomicroscope. The time of emergence of the first adult leaf in *hst-1* (leaf 2.6) and Col (leaf 4.8) is indicated by an arrow. The last point on each curve is the time a which floral buds were first observed in each genotype. Standard error bars are obscured by the plot symbols.

mutants may produce more than 30 adult leaves (data not shown).

The number of juvenile and adult leaves produced by a plant depends both on the temporal duration of the juvenile and adult phases of shoot development and the rate of leaf initiation during these phases (Telfer et al., 1997). *hst-1* seeds sometimes germinate slightly later than wild-type seed and may initiate leaves at a slightly slower rate than wild-type plants (Fig. 3). Nevertheless, the first adult leaf in *hst-1* emerges significantly earlier than the first adult leaf in wild-type plants, implying that the effect of *hst-1* on the number of juvenile leaves can be attributed primarily to a reduction in the duration of the juvenile phase of rosette development.

In wild-type plants, leaves produced in the inflorescence typically lack trichomes on all or part of their adaxial surface (Chien and Sussex, 1996; Telfer et al., 1997). The extent of this loss is at least partly dependent on the reproductive status of a plant, in that conditions that promote floral development accelerate the loss of adaxial trichomes. We found that adaxial trichome loss occurs more rapidly in *hst* plants than in wild type. Thus, the first bract of *hst-1* plants often has few or no adaxial trichomes and the second bract usually has none. Furthermore, the last leaf in a *hst-1* rosette frequently lacks adaxial trichomes at its apex, suggesting that the process of adaxial trichome loss has become uncoupled from inflorescence development. The pattern of trichome loss within a *hst-1* leaf or bract is unusual, however, in that it is opposite to the wild-type pattern. Normally, adaxial trichomes disappear first from the base of a bract and are lost in progressively more apical regions of bracts produced later in shoot development. In *hst* mutants, adaxial trichomes disappear first from the apex of the blade, and this glabrous region extends basipetally in successive bracts (Fig. 1E). This unusual pattern does not reflect a reversal of the normal gradient of epidermal cell differentiation because *hst-1* leaves exhibit a normal basipetal gradient of stomatal differentiation and a normal basipetal gradient of trichome differentiation in regions of the leaf blade that are capable of producing trichomes (data not shown).

In addition to its effects on shoot maturation, *hst-1* also has significant effects on leaf morphology. The leaf blades of leaves 1 and 2 in *hst* plants initially develop a tight upward curl, but usually flatten as the blade expands (Fig. 1A). Leaves produced later in development almost always remain tightly curled and are also narrower than wild-type leaves. As in the case of trichome production, this phenotype is more pronounced in an Ler genetic background.

Inflorescence and floral architecture

The first few nodes of the *Arabidopsis* inflorescence possess bracts and have elongated lateral branches (coflorescences); later (higher) nodes have individual flowers that are not subtended by bracts. These lateral structures are arranged in a spiral phyllotaxis and are separated by elongated internodes. In the Col background, the inflorescence architecture of *hst-1* plants is normal except in small regions in which phyllotaxis is disrupted and internodes undergo little or no elongation (Fig. 1B). These regions, which typically encompass up to half a dozen nodes, can occur anywhere in the inflorescence, resulting in clusters of bracts and branches, bracts, branches and flowers, or just flowers. Some plants have several such clusters, separated by regions of normal phyllotaxis and internode elongation. *hst-1* plants occasionally have an increased number of bracts and coflorescences, and usually produce many more flowers than in wild type, probably as a consequence of their reduced fertility (see below). *hst-1* reduces internode elongation to an even greater extent in an Ler background, resulting in the production of a short, compact inflorescence.

The number, position and morphology of floral organs is normal in *hst-1* plants, but like leaves, these organs are reduced in size (Fig. 1D). Seed set is reduced to about 5% of normal

in plants grown at 22°C and about 25% of normal at 18°C. Mutant plants produce a reduced amount of pollen, and crosses onto *hst* mutants routinely yield fewer seeds than crosses onto wild type. These observations suggest that the low seed set of mutant plants is due to a combination of low male and female fertility. We have seen no evidence of embryo lethality.

How does *hst* affect flowering time?

Physiological and genetic analyses indicate that flowering time in *Arabidopsis* is determined by complex interactions between environmental signals and regulatory genes that act to promote or repress flowering (Martínez-Zapater et al., 1994; Amasino, 1996). Many mutations affecting this process have been identified in *Arabidopsis*, but most previously characterized mutations have relatively little effect on vegetative maturation (Telfer et al., 1997). Of the early flowering mutations that we examined (*tfl, elf1, elf3, clf, hy1, hy2, phyB* and *spy*), only the latter four have a significant effect on abaxial trichome production. As noted above, the phenotype of these four can probably be attributed to an increase in either the amount or sensitivity to GA, or to the constitutive activation of a GA response pathway. The phenotype of these mutations is therefore consistent with evidence indicating that GA acts to promote vegetative and reproductive shoot maturation in *Arabidopsis* (Zeevaart, 1983; Wilson et al., 1992; Telfer et al., 1997).

To investigate the role of *HST* in floral induction, we examined the phenotypes of *hst-1* plants that constitutively over-express the flower-meristem-identity genes *LEAFY* (*LFY*) and *APETALA1* (*AP1*). Loss-of-function mutations in *LFY* and *AP1* convert floral meristems into vegetative meristems (Irish and Sussex, 1990; Weigel et al., 1992; Bowman et al., 1993). By contrast, the constitutive expression of *LFY* (Weigel and Nilsson, 1995) or *AP1* (Mandel and Yanofsky, 1995) driven by the cauliflower mosaic virus 35S promoter accelerates floral induction and precociously converts the shoot meristem into a floral meristem. It is significant, however, that neither of these genes completely suppresses vegetative development. Weigel and Nilsson (1995) showed that *LFY* over-expression reduces the number of adult leaves, but has no effect on the number of juvenile leaves. We found that the *35S::AP1* transgene greatly reduces the number of adult leaves and also reduces the number of juvenile leaves, but to a much lesser extent (Fig. 4). These observations can be interpreted to mean that the shoot meristem is incompetent to respond to these floral promoters during the juvenile phase of vegetative development.

To test this hypothesis, we generated plants that were homozygous for *hst-1* and carried the *35S::LFY* or *35S::AP1* transgenes. If *HST* regulates flowering time by affecting the competence of the shoot to respond to *LFY* or *AP1* (rather than up-regulating their expression), we predicted that *hst-1* would enhance the effect of *35::LFY* and *35S::AP1* by the same amount that it accelerates the transition to the adult phase of development. The phenotype of *hst-1 35S::LFY* and *hst-1 35S::AP1* plants supports this prediction (Figs 4, 5). *hst-1 35S::LFY* plants flower with 4 fewer leaves than *35S::LFY* plants; they have the same number of adult leaves as *35S::LFY* plants, and the same number of juvenile leaves as *hst-1* plants. In the same way, the accelerated flowering of *hst-1 35S::AP1* plants is attributable primarily to a reduction in the length of the juvenile phase.

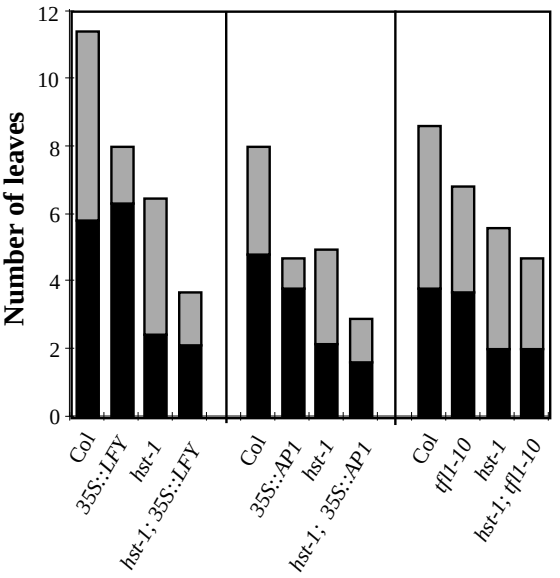

Fig. 4. The effect of *hst-1* and *35S::LFY, 35S::AP1* and *tfl-1* on the number of juvenile and adult leaves in single and double mutant plants. The interaction between *hst-1* and *35S::LFY* (left panel); The interaction between *hst-1* and *35S::AP1* (center panel); The interaction between *hst-1* and *tfl-1* (right panel). All genotypes are in Col. Juvenile leaves (■); adult leaves (□).

Loss-of-function mutations of the *TERMINAL FLOWER* (*TFL*) gene are strikingly similar to *35S::LFY* plants in that they are early flowering and produce terminal flowers instead of an inflorescence. Indeed, genetic analysis (Shannon and Meeks-Wagner, 1993), and the expression pattern of *LFY* in *tfl* mutant plants (Weigel et al., 1992; Bradley et al., 1997) indicate that the phenotype of such mutants is due, at least in part, to *LFY* mis-expression. We found that *hst-1* interacts with *terminal flower1-10* (*tfl1-10*) in the same way that it interacts with *35S::LFY* and *35S::AP1* (Fig. 4). *hst-1 tfl1-10* double mutants had about the same number of juvenile leaves as *hst-1* plants, and the same number of adult leaves as *tfl1-10* plants, and therefore flowered with fewer leaves than *tfl1-10* single mutants. These results support the conclusion that *HST* regulates the competence of the shoot to respond to *LFY* and *AP1*, and to whatever other floral inducers may be up-regulated in *tfl* mutant plants.

Additional support for the hypothesis that *HST* affects flowering time by regulating the competence to respond to *LFY* and *AP1*, rather than up-regulating the expression of these genes, is provided by the phenotype of *hst-1*; *lfy-2* and *hst-1;ap1-10* double mutants. We found that neither *lfy-2* or *ap1-10* suppress the effect of *hst-1* on vegetative phase change or on leaf number (Fig. 6). This result suggests that these aspects of the hst phenotype do not require the expression of *LFY* or *AP1*, although the evidence for this conclusion is not as strong in the case of *LFY* because *lfy-2* is a relatively weak allele.

As a final test of the hypothesis that *hst-1* does not up-regulate *LFY* transcription, we examined the effect of this mutation on the expression a *LFY::GUS* reporter gene in transgenic plants. We did not observe any increase in the distribution or the amount of GUS staining in *hst-1 LFY::GUS*

Fig. 5. The phenotype of *35S::LFY* (left), *hst-1* (middle) and*35S::LFY*; *hst-1* double mutants (right). All genotypes are in Col.

3- and 5-day-old seedlings in comparison to seedlings carrying only the *LFY::GUS* construct (Fig. 7); indeed, *hst-1 LFY::GUS* seedlings consistently stained less intensely than *LFY::GUS* plants, although no attempt was made to quantitate this difference. As previously reported (Blázquez et al., 1997; Hempel et al., 1997) we found that the *LFY::GUS* construct is expressed in very young leaf primordia, but not in older leaf primordia or in the shoot meristem.

HASTY does not act through GA

Gibberellins are known to promote flowering and vegetative phase change in many plants, including *Arabidopsis* (Zeevart, 1983; Zimmerman et al., 1985; Wilson et al., 1992; Evans and Poethig, 1995; Chien and Sussex, 1996; Telfer et al., 1997). To investigate the possibility that *HST* regulates these processes by affecting GA production or response, we constructed double

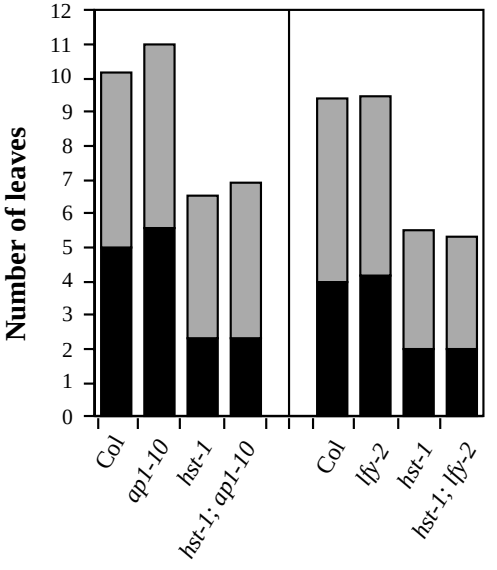

Fig. 6. The effect of *hst-1* and *lfy-2*(left) and *hst-1* and *ap1-10* (right) on the number of juvenile and adult leaves. All genotypes are in Col. Juvenile leaves (■); adult leaves (□)

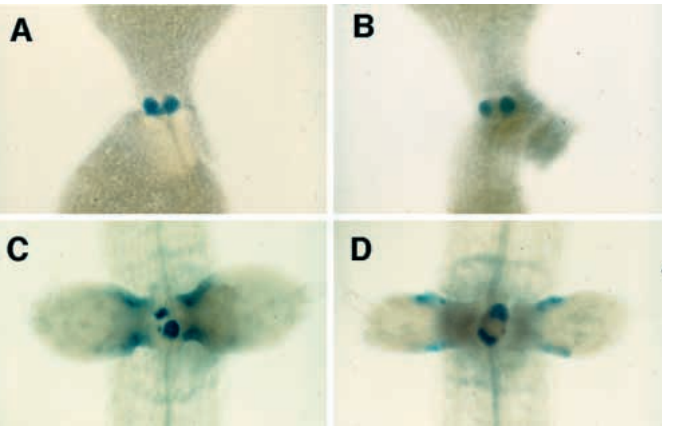

Fig. 7. *LFY::GUS* expression in a *hst-1* (A,C) and a wild type (B,D) background at 3 days (A,B) and 5 days (C,D) days after planting. At 3 days after planting, GUS activity is visible in the primordia of leaves one and two. GUS activity is confined to the basal margin of the first two leaves in 5-day-old *hst-1* and wild-type seedlings, but is expressed uniformly throughout the primordia of leaves 3 and 4. *hst-1* seedlings exhibit slightly lower levels of GUS activity than wild-type seedlings, although the distribution of GUS activity is identical in both genotypes.

mutants between *hst-1* and *ga1-3*, a biosynthetic mutant that has been reported to have little or no GA (Zeevaart and Talon, 1992), and *gai*, a semidominant GA-insensitive mutation that is proposed to affect GA perception or signal transduction (Koornneef et al., 1985).

Under long day conditions and in the absence of exogenous GA, *ga1-3* plants develop as dark green, severely dwarfed plants which fail to produce abaxial trichomes (Chien and Sussex, 1996; Telfer et al., 1997) and flower, but do not bolt (Sun et al., 1992; Wilson et al., 1992) (Fig. 8A). *ga1-3 hst-1* double mutants have an intermediate phenotype (Fig. 8C). They are similar to *ga-3*, in that they are dwarf and dark green and fail to bolt. They are like *hst-1* in that they flower early and produce abaxial trichomes, albeit at a later leaf position than *hst-1* (leaf 5 vs. leaf 3). The double mutant also loses adaxial trichomes in the same pattern as *hst-1* (*i.e.*, starting at the apex of the leaf). A similar result was obtained with *gai*. In the progeny of self-pollinated *hst/+ gai/gai* plants, *hst-1 gai* double mutants were found to produce abaxial trichomes significantly earlier than their *HST gai* siblings (leaf 2.9±0.2 vs. leaf 4.5±0.4, $P<0.01$). The interaction between *hst-1* and these two mutations demonstrates that neither GA nor the GA response pathway is required for the effect of *hst-1* on vegetative phase change.

The vegetative phenotype of *hst* does not require *AGAMOUS*

Transgenic plants in which *AGAMOUS* (*AG*) is constitutively expressed under the control of the CaMV 35S promoter are phenotypically similar to *hst* mutants in that they are early flowering, have leaves that are curled upwards, and produce abaxial trichomes slightly earlier than normal (Mizukami and Ma, 1992, 1997). To determine if these aspects of the *hst* phenotype are due to the mis-expression of *AG* during the vegetative phase of development, we generated double mutants

Fig. 8. The phenotype of *ga1-3* (A), *hst-1* (B) and *ga1-3; hst-1* (C) double mutant plants. All genotypes are in Ler.

between *hst-1* and *ag-1*. This strong *ag* allele did not significantly affect any aspect of the *hst-1* phenotype, other than floral morphology (Fig. 9). Double mutant plants were indistinguishable from *hst-1* with respect to abaxial trichome production, leaf morphology, and total leaf production; *hst-1 ag-1* plants produced abaxial trichomes starting with leaf 1.5±0.4, and had a total of 2.9±0. 6 leaves, whereas *hst-1* plants had abaxial trichomes on leaf 1.6±0.2 and had a total of 2.7±0.2 leaves. We conclude, therefore, that the effect of *hst* on vegetative morphology or flowering time is not a consequence of the inappropriate expression of *AG*.

DISCUSSION

We undertook an analysis of shoot maturation in *Arabidopsis thaliana* by screening for mutations that affect the timing of abaxial trichome production, a trait that distinguishes leaves produced early in shoot development (juvenile leaves) from leaves produced later (adult leaves). Some of the mutations recovered in this screen affected the rate of shoot development but did not have a major effect on the timing of the juvenile-to-adult transition (Telfer et al., 1997), whereas others primarily affected the timing of this transition. Mutations of the *HST* gene are in the latter class, suggesting that *HST* may be part of a 'developmental clock' that regulates the timing of shoot maturation Our analysis of *hst-1* – a strong allele of this locus – indicates that *HST* functions early in development to delay the transition from a juvenile, reproductively incompetent phase, to an adult, reproductively competent phase of shoot development.

Fig. 9. The phenotype of *hst-1* (left) and *hst-1; ag-1* (right) double mutant plants. All genotypes are in Ler.

The relationship between vegetative and reproductive maturation

The observation that *hst* affects both vegetative phase change and flowering time raises a long-standing question about the relationship between these two aspects of shoot development. It has long been known that plants acquire reproductive competence during the adult phase of vegetative development, and that flowering time can be modified by conditions that delay or accelerate vegetative maturation (Hackett, 1985; Zimmerman et al., 1985). There is very little information about the relationship between the vegetative phase of the shoot and reproductive competence in herbaceous species, but in many woody plants floral induction does not occur in response to inductive photoperiods until the shoot begins to express vegetative traits characteristic of the adult phase of development. However, certain woody plants can be induced to flower in the juvenile phase, even though they do not normally do so (Zimmerman et al., 1985). This latter observation is inconsistent with the idea that vegetation maturation is a prerequisite for reproductive competence, and raises the possibility that these phenomena are actually independent processes whose expression is coordinated by common regulatory factors.

hst mutations truncate the juvenile phase of shoot development, but do not usually reduce the number of adult leaves produced by the shoot. This aspect of its phenotype suggests that *HST* is not directly involved in the process of floral induction, but may regulate the way the shoot responds to the factors that induce flower development. We addressed this problem by asking if *hst-1* accelerates flowering in plants that constitutively express *LFY* or *AP1*. These genes are required for the transformation of a vegetative meristem into a floral meristem (Irish and Sussex, 1990; Weigel et al., 1992; Bowman et al., 1993), and it is believed that the factors that regulate floral induction do so by affecting the level of *LFY* and *AP1* expression and the responsiveness of the shoot to these factors (Blázquez et al., 1997; Hempel et al., 1997). Evidence that the shoot undergoes a change in its competence to respond to these factors during development is provided by the observation that constitutive expression of either *LFY* or *AP1* causes plants to flower precociously, but only after they have

begun to produce leaves with abaxial trichomes (Weigel and Nilsson, 1995) (Fig. 4). It has also been shown that *LFY* is normally expressed at low levels early in shoot development, and that the level of its expression increases during shoot development and in response to factors (GA or long days) that induce flowering (Blázquez et al., 1997; Hempel et al., 1997).

Genes that regulate floral induction by affecting the transcription of *LFY* and *AP1* should have little or no effect on the flowering time of plants carrying *35S::LFY* or *35S::AP1* transgenes; in contrast, genes that either directly or indirectly affect the activity of *LFY* or *AP1* are expected to modify the effect of these transgenes on flowering time. We found that *hst-1* enhances the effect of both *35S::LFY* and *35S::AP1* on flowering to the same extent that it accelerates vegetative phase change. That is, plants expressing either of these transgenes in combination with *hst-1* had an additive phenotype: they produced essentially the same number of juvenile leaves as *hst-1* plants and the same number of adult leaves as plants that contained only the transgene. This result confirms the conclusion that *35S::LFY* and *35S::AP1* are unable to induce flowering during the juvenile phase of vegetative development, and supports the hypothesis that *HST* regulates flowering time by affecting the competence of the meristem to respond to *LFY* and *AP1* (i.e. via its effect on vegetative phase change) rather than by directly increasing the activity of these genes. The observation that *hst-1* mutants have approximately the same number of adult leaves as wild-type plants provides additional evidence for the conclusion that *hst-1* does not increase the activity of *LFY* or *AP1* because constitutive transcription of these genes in plants harboring a *35S::LFY* and *35S::AP1* transgene leads to a truncation of the adult phase.

In light of the results obtained from the double mutant analysis described above, the effect of *hst-1* on the *LFY::GUS* reporter gene was somewhat surprising. We found that this transgene is expressed at a slightly reduced level in a *hst-1* mutant background. This result is consistent with the observation that *hst* alleles occasionally increase the number of bracts in the inflorescence, and implies that *HST* may function as a positive regulator of *LFY* transcription. Thus, *HST* may have several developmental functions. One of these functions is to promote a juvenile, reproductively incompetent phase of development. A second function may be to promote floral morphogenesis once the shoot is in a reproductive phase of development. Given the pleiotropic nature of the *hst* mutant phenotype, it would not be surprising if *HST* functioned in other aspects of plant development as well.

Juvenile development consists of several discrete phases

In a previous study (Telfer et al., 1997) we found that, in contrast to other juvenile leaves, the first two leaves of the rosette are incompetent to produce abaxial trichomes. This observation, and the fact that leaves one and two are morphologically distinct from other rosette leaves (Telfer and Poethig 1994; Poethig, 1997), suggests that the juvenile phase of development may actually consist of two developmentally distinct phases. Additional support for this conclusion is provided by the phenotype of *hst* mutants. Although *hst* mutations affect the morphology of leaves one and two, they usually do not cause these leaves to produce abaxial trichomes. Furthermore, *hst-1 35S::LFY* and *hst-1 35S::AP1* plants always have at least two rosette leaves. These results imply that although *HST* is expressed during the production of leaves one and two, it may not be the only gene regulating the vegetative identity and the reproductive competence of the shoot meristem during this early period of shoot development.

HST and GA

As noted above, previous studies have shown that GA promotes vegetative phase change and flowering in woody plants, maize and *Arabidopsis*. We therefore investigated the possibility that *HST* regulates either the synthesis or response to GA. The introduction of *hst-1* into the severely GA-deficient *ga1-3* mutant background does not correct the dwarf, dark green phenotype of *ga1-3*. However, *hst-1* does restore the production of abaxial trichomes – albeit at a slightly later leaf position than in the *hst* single mutant. *hst-1* also partially suppresses the delay in flowering time conditioned by *ga1-3*. *hst-1* interacts in a similar fashion with the GA-insensitive mutation, *gai*. These results suggest that *HST* does not act by regulating the level of GA (because *ga1-3* is incapable of producing GA) or by activating a GA response pathway. An alternative possibility is that *HST* acts downstream of the GA signal transduction pathway, affecting only a subset of the processes influenced by GA. This interpretation would imply that GA promotes vegetative phase change by repressing the expression of *HST*. In this case, the intermediate phenotype of the double mutant would have to be attributed to residual HST function in *hst-1*, which is somehow increased by *ga1-3* and *gai*. The difficulty with this explanation is that *hst-1* is one of the strongest alleles in our collection and is therefore expected to have little or no wild-type function. We favor the conclusion that *HST* and GA function in different regulatory pathways that interact to regulate vegetative and reproductive maturation.

Does HST negatively regulate floral homeotic genes?

The early flowering phenotype, adaxially curled leaves and small, semi-sterile flowers of *hst* plants resemble the phenotype of mutations of *CURLY LEAF (CLF)* (Goodrich et al., 1997) The phenotype of these mutations does not overlap completely, but this similarity suggest that *HST* and *CLF* may have related functions. *CLF* encodes a polycomb-like protein that represses the expression of the floral homeotic gene, *AGAMOUS (AG)*, during vegetative and reproductive development (Goodrich et al., 1997). Many aspects of the vegetative and floral phenotypes of *clf* are suppressed by a strong, loss-of-function mutation in *AG* (*ag-1*), implying that the inappropriate expression of *AG* is largely responsible for the *clf* mutant phenotype. By contrast, we found that *ag-1* does not suppress the vegetative phenotype of *hst* or its early flowering phenotype. We conclude, therefore, that these traits do not result from the mis-expression of AG during vegetative development. Loss-of-function mutations in *LFY* and *AP1* also had no effect on the vegetative phenotype of *hst-1*. Although we did not examine the interaction between *hst-1* and other genes involved in floral morphogenesis, it is significant that constitutive expression *PI* and *AP3* (Krizek and Meyerowitz, 1996) and *UFO* (Lee et al., 1997) does not reproduce the effects of *hst* on vegetative morphology. We think it is unlikely, therefore, that *HST* acts as a negative regulator of these floral homeotic genes. The possibility that

HST negatively regulates other genes involved in floral morphogenesis (e.g. *APETALA2*) remains an open question.

How does HST compare to other mutations that affect phase change?

Several genes involved in shoot maturation have been identified in maize and *Pisum*. In maize, such genes are defined by dominant, gain-of-function mutations (*Teopod1*, *Teopod1* and *Teopod3/Corngrass*) that prolong the expression of juvenile traits (Poethig, 1988; Lawson and Poethig, 1995). In contrast to *hst*, these mutations have relatively little effect on the onset of the adult phase or on reproductive maturation, suggesting that they act specifically to promote the expression of a juvenile program of vegetative differentiation rather than to regulate the switch from juvenile to adult development. Among the many genes that affect flowering time in *Pisum*, alleles of the *Lf* locus most closely resemble *hst* (Wiltshire et al., 1994). In comparison to the dominant Lf^d allele, the lf^a and *Lf* alleles accelerate both changes in leaf shape and flowering time. A comparison of the effect of *Lf* alleles on flowering time in many different genetic backgrounds indicates that this gene plays an important role in determining the minimum flowering node, that is, the point at which the shoot meristem first becomes competent to flower (Murfet 1975).

Several late flowering mutations delay abaxial trichome production (Telfer et al., 1997), demonstrating that they function in both the vegetative and reproductive maturation of the shoot. Similarly, some mutations that affect phytochrome or GA metabolism (Wilson et al., 1992; Jacobson and Olszewski, 1993; Reed et al., 1996) also affect both of these processes. However, most of these mutations have much less of an effect on vegetative maturation than they do on flowering time, so it is unlikely that their primary function is to regulate vegetative maturation. *hst* mutations are unusual in that they appear to affect flowering time primarily by accelerating the transition to a reproductively competent development stage; *hst* alleles sometimes also accelerate flowering once the plant is in an adult phase (i.e. reduce the number of adult leaves), but this effect is minor compared to their effect on the duration of the juvenile phase. How changes in the vegetative phase of the shoot are linked to changes in its reproductive competence may be revealed by further studies of *HST* and other genes that affect both of these important developmental transitions.

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