Development 125, 3843-3851 (1998)
Printed in Great Britain © The Company of Biologists Limited 1998
DEV0190

CLAVATA2, a regulator of meristem and organ development in *Arabidopsis*

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Accepted 23 July; published on WWW 7 September 1998

SUMMARY

Mutations at the *CLAVATA2* (*CLV2*) locus of *Arabidopsis* result in enlarged shoot and flower meristems, as well as alterations in the development of the gynoecia, flower pedicels, and stamens. The shoot and flower meristem phenotypes of *clv2* mutants are similar to weak *clv1* and *clv3* mutants. We present genetic analysis that *CLV2* may function in the same pathway as *CLV1* and *CLV3* in the regulation of meristem development, but function separately in the regulation of organ development. We also present evidence that *clv2* phenotypes are altered when the mutants are grown under short-day light conditions. These alterations include flower-to-shoot transformations, as well as a nearly complete suppression of the flower phenotypes, indicating that the requirement for *CLV2* changes in response to different physiological conditions. The *stm-1* mutation dominantly suppresses *clv2*, and *clv2* mutations suppress the strong *stm-1* allele, but not the weak *stm-2* allele.

Key words: Organogenesis, Cell differentiation, Cell division, Pedicel, Gynoecium, *CLAVATA2*, *Arabidopsis*

INTRODUCTION

All above-ground organs are derived from the shoot meristem. The shoot meristem accomplishes continuous organ formation by maintaining a pool of undifferentiated cells at the center of the meristem and directing appropriately positioned descendant cells toward organ formation and eventual differentiation (Clark, 1996). Several genes have been described that appear to specifically regulate shoot meristem development in *Arabidopsis*. Mutations at the *CLV1* and *CLV3* loci accumulate a massive population of undifferentiated cells at the shoot meristem, while mutations at the *WUS* and *STM* loci result in shoot meristems that fail to maintain organogenesis (Barton and Poethig, 1993; Clark et al., 1993, 1995; Laux et al., 1996). The flower meristem has long been thought to be a modified shoot meristem, and *clv1*, *clv3*, *stm* and *wus* mutations exert similar effects on flower meristem development as they do on shoot development (Weigel and Clark, 1996). Genetic interactions between *clv1*, *clv3*, *stm* and *wus* mutants suggest that these genes may all function in a related pathway (Clark, 1997). *CLV1* and *CLV3* appear to function in the same pathway (Clark et al., 1995), while *wus* mutants are epistatic to both *clv1* and *clv3* mutants (Laux et al., 1996). *STM* appears to function in a balanced, competitive manner with *CLV1* and *CLV3* (Clark et al., 1996), raising the possibility that these genes act on a common downstream target, perhaps the *WUS* gene.

Two models have been proposed for the action of the *CLV1* and *CLV3* loci. One model predicts that they regulate the rate of division of the central undifferentiated cells. For example, the *clv1* mutant phenotype could be explained by postulating that the undifferentiated cells divide more rapidly in the mutant, implying that the role of *CLV1* is to repress cell division. Another hypothesis is that these genes regulate the undifferentiated/differentiated state of the shoot meristem cells. Under this hypothesis, *CLV1* would act to promote the transition of cells from an undifferentiated state toward organ formation. Thus, in a *clv1* mutant, cells on the flanks of the meristem that would normally contribute to organ primordia would often remain undifferentiated, enlarging the shoot meristem.

CLV1 codes for a putative receptor-kinase and is specifically expressed at the shoot and flower meristem (Clark et al., 1997). Thus, CLV1 likely relays positional information to regulate the development of cells at the shoot meristem. *STM* codes for a homeodomain-containing protein and likely acts as a transcription factor (Long et al., 1996). *STM* is also expressed in a central region of the shoot meristem.

Understanding the control of meristem development requires detailed analysis of critical regulators. *clv2* mutants have been superficially described (McKelvie, 1962; Koornneef et al., 1983; Griffin, 1994), indicating a possible role for *CLV2* in regulating meristem development. We have carried out a detailed phenotypic and genetic analysis to determine the role that *CLV2* plays in meristem development, and whether *CLV2* works on the same pathway as *CLV1* and *CLV3*.

MATERIALS AND METHODS

Plant material

The *clv2-1* and *clv2-4* alleles have been previously described (Koornneef et al., 1983; Pogany et al., 1998). The *clv2-3* allele was identified in a screen of fast neutron mutagenized wild-type Columbia

seeds purchased from the Lehle Seed company. The *clv2-2* and *clv2-5* alleles were identified in screens of T-DNA transformed wild-type Wassilewskija (Ws) (Forsthoefel et al., 1992). The *clv2-5* allele was kindly provided by Robert Williams.

Seeds were sown on a 1:1:1 mix of top soil:perlite:vermiculite and imbibed for 7 days at 4°C. Plants were grown at 22°C under approximately 800 foot-candles of constant cool white fluorescent light. Plants were fertilized once a week. Plants in short-day conditions were grown at 22°C under approximately 800 foot-candles of constant cool white fluorescent light for 8 hours each 24 hour period.

Tissue and image processing

Scanning electron microscopy (SEM) was carried out as described (Bowman et al., 1989), except the Hitachi S3200N SEM allowed images to be collected digitally. In situ mRNA hybridization was carried out as described (Clark et al., 1997), except that light-field and dark-field images were taken separately and combined in Adobe Photoshop 4.0 after digitization. Slides were scanned and digitized using a Polaroid SprintScan35. Brightness, contrast and color balance were adjusted using Photoshop.

RESULTS

clv2 alleles

We have examined the phenotypes of five *clv2* alleles (Table 1; Materials and methods). *clv2-1* and *clv2-4* were isolated from the Landsberg *erecta* (L*er*) background (Koornneef et al., 1983; Pogany et al., 1998). *clv2-2*, which was isolated from the Ws background, and *clv2-3*, which was isolated from the Columbia background, were backcrossed into L*er* before analysis. All alleles were recessive. Overall, *clv2-1*, *clv2-3* and *clv2-4* exhibit very similar phenotypes, while *clv2-2* exhibited a weaker phenotype. *clv2-5*, which was isolated from T-DNA mutagenesis in the Ws ecotype, was not backcrossed into L*er* and thus could not be directly compared to the other alleles. Linkage of *clv2* alleles to *CLV1*, *STM* and *AP1* were consistent with the previously published map position (Koornneef et al., 1983).

Table 1. *clv2* alleles

Allele	Mutagen	Ecotype	Source
clv2-1	EMS	L*er*	Koornneef, 1983
clv2-2	T-DNA	Ws	this article
clv2-3	fast neutron	Col	this article
clv2-4	EMS	L*er*	Pogany et al., 1998
clv2-5	T-DNA	Ws	this article

clv2 mutants affect the shoot and flower meristem

All *clv2* alleles exhibited an enlarged inflorescence shoot meristem when compared to wild type (Fig. 1A-I). While not remarkably larger in circumference, the *clv2* shoot meristems were much taller than those observed at the apices of wild-type plants. *clv2* shoot meristems are enlarged during vegetative development as well (Griffin, 1994; data not shown). Under normal growth conditions (Materials and methods) fasciation of the meristem was not observed in *clv2* mutants (but see short-day growth conditions below). This phenotype is similar to that observed in weak *clv1* (*clv1-6*, *clv1-5*) and *clv3* (*clv3-3*) alleles. As with *clv1* and *clv3* mutations, the size of the *clv2* shoot meristems varied, with the shoot meristems of the weak *clv2-2* allele often observed similar to wild type (data not shown), and the shoot meristems of the other *clv2* alleles occasionally enlarging significantly in circumference (Fig. 1I).

clv2 mutations similarly affected the flower meristem. The number of organs generated by the flower meristem was increased (Fig. 2). Under standard growth conditions, the number of carpels generated in each *clv2* flower was almost twice that of wild type, while the number of stamens was also

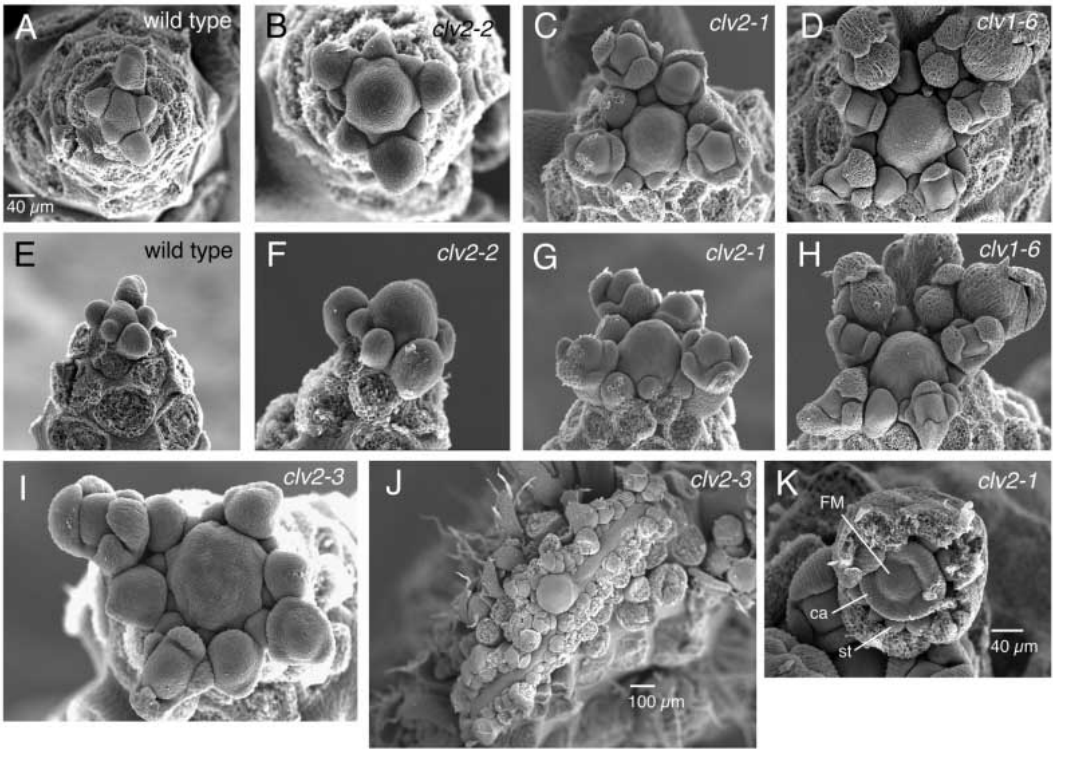

Fig. 1. *clv2* meristem defects. The shoot apical meristems of wild-type (A,E), *clv2-2* (B,F), *clv2-1* (C,G) and *clv1-6* (D,H) plants are shown from a top (A-D) and side (E-H) view revealing the larger *clv2* and *clv1* shoot meristems. Occasionally, the *clv2* shoot meristems develop a larger, irregular appearance (I). (J) A *clv2-3* fasciated shoot apical meristem from a plant grown under short-day light conditions (see Text). (K) A stage 6 *clv2-1* flower meristem, revealing a flower meristem (FM) interior to the whorl 4 carpels (ca). st, stamen. A-I are shown at the same magnification.

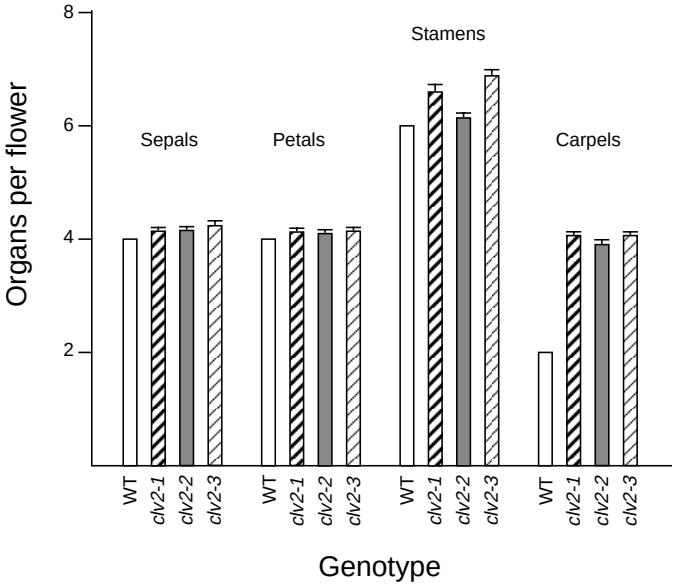

Fig. 2. The number of organs in wild-type and *clv2* mutant flowers. Bars represent the mean number of indicated organs for flowers of wild-type Landsberg (WT), *clv2-1*, *clv2-2* and *clv2-3* plants. At least 150 flowers were counted for each mean. Only the first ten flowers on any given plant were analyzed. Standard error is indicated. Filamentous organs were included in the counts (see text).

slightly increased. The sepals and petals were only rarely affected. This is similar to the number of organs observed in weak *clv1* mutants (Clark et al., 1993). In addition, *clv2* flowers developed an additional whorl of organs in the center of the flower meristem. At the time of carpel initiation (stage 6; all stages according to Smyth et al., 1990), the carpel primordia in *clv2* mutant flowers initiated in a ring around the center of the flower meristem, instead of terminating the flower meristem as in wild type (Fig. 1K; see also Smyth et al., 1990). This results in the development of a gynoecium interior to the gynoecium formed by the whorl 4 carpels (Fig. 3J). All of these features are also similar to observations of weak and intermediate *clv1* and *clv3* mutants (Clark et al., 1993).

clv2 mutants affect organ development

Mutations at *clv2* also affected the development of several organ types. The flower pedicels in *clv2* mutants were about 50% longer than wild type (Fig. 4). While wild-type pedicels were approximately 5 mm in length, pedicels in *clv2* plants averaged approximately 7.5 mm for the stronger alleles. These measurements were all performed in the Landsberg *erecta* background. *erecta* mutations have been demonstrated to reduce pedicel length (Torii et al., 1996), indicating that *clv2* suppressed this *erecta* phenotype.

The morphology of the gynoecia were also altered in *clv2* mutant backgrounds. Strikingly, the portion of the gynoecium covered by valves with developing ovules can be greatly reduced in *clv2* flowers. In wild-type gynoecia, the valves extend from shortly below the stigmatic tissue to the base of the gynoecium, with only a very short segment between the bottom of the valves and the attachment site of sepals, petals and stamens (Clark and Meyerowitz, 1994; Fig. 3A). In the most severely affected *clv2* flowers, the gynoecium completely lacked valves (Fig. 3B). Some *clv2* flowers lacked valves only on the basal portion of the gynoecium (Fig. 3D), while other *clv2* flowers developed multiple valves that extended to the base of the gynoecium, with a valveless region extending between these valves from the base to the apex of the gynoecium (Fig. 3C,E). The morphology of the cells in the valveless region is similar to the morphology of cells between the valves in wild-type gynoecia (Fig. 3F-H). The cells typical of the style region, which is normally found between the top of the valves and the stigmatic tissue in wild-type gynoecia (Bowman, 1994), are only found in the region immediately adjacent to the stigmatic tissue in the mutants as well (Fig. 3H,I). The extent of the valveless phenotype was quite variable in *clv2* flowers, with many flowers not exhibiting the

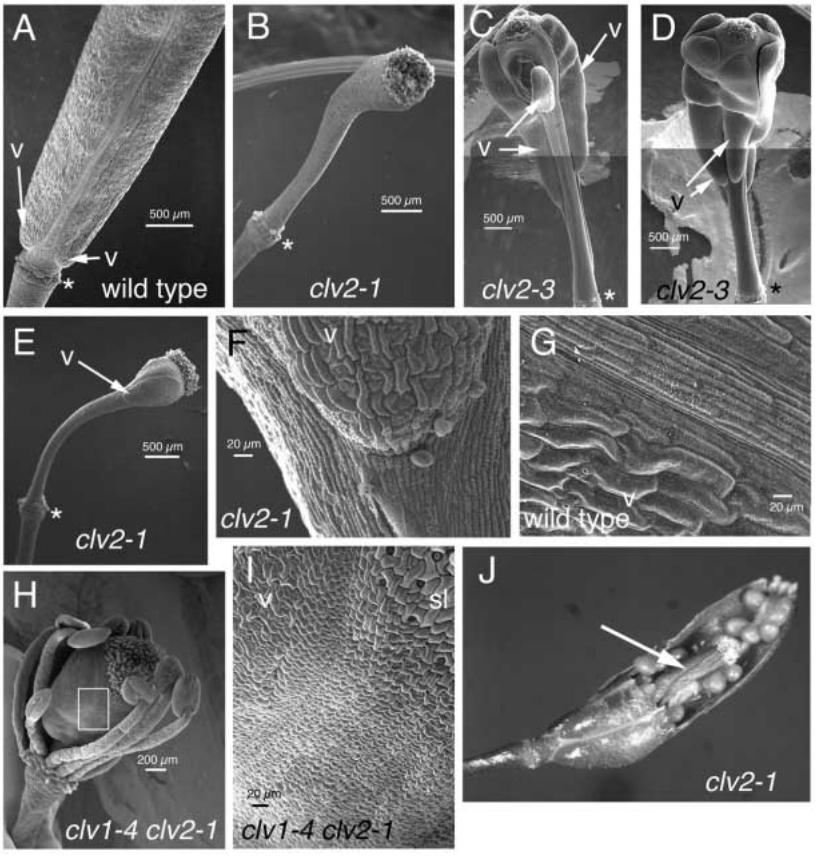

Fig. 3. *clv2* gynoecium defects. (A) A wild-type gynoecium with valve (v) tissue extending adjacent to the attachment site for the sepals, petals and stamens (*). *clv2* gynoecia often lack valve tissue on the entirety or a portion of the gynoecium (B-E). (F,G) Higher magnification views of E and A, respectively, showing the edge of the valves, and revealing that the cells of the valveless region in *clv2* are similar to cells between the valves of wild type. A *clv1-4 clv2-1* gynoecium in H and higher magnification in I shows the cells characteristic of the style (sl) are only found at the apex of the gynoecium. The box in H indicates the region shown in I. (J) *clv2* flowers develop carpels interior to the whorl 4 gynoecium (arrow).

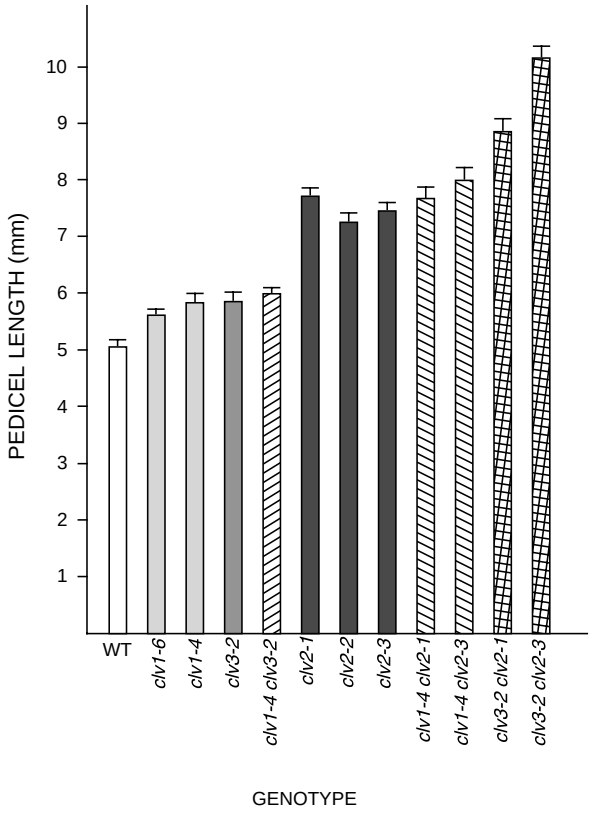

Fig. 4. *CLV* loci regulate pedicel length. The pedicel length for mature siliques was measured. At least 50 flowers were examined for each mean. Only the first ten flowers on any given plant were analyzed. The mean and standard error are indicated.

phenotype. The percentage of flowers with significant valveless phenotypes are indicated in Table 2.

The valveless phenotypes was only rarely observed in weak *clv1* or *clv3* mutants (Table 2, and data not shown). However, a significant number of gynoecia of strong *clv1* and *clv3* alleles did lack valves (Table 2). This is discussed in more detail under Genetic Interactions, below. Mutations at the *ETTIN* locus also result reduced coverage of the gynoecium with valve tissue (Sessions and Zambryski, 1995).

Table 2. Frequency of valveless phenotype

Genotype	Valveless	Normal	Percent valveless
clv2-1	71	245	22.5
clv2-2	49	405	10.8
clv2-3	80	422	15.9
clv2-4	179	214	45.5
clv1-1	12	341	3.4
clv1-4	109	256	29.9
clv3-2	66	200	24.8
clv1-4 clv3-2	157	190	45.2
clv1-4 clv2-1	168	60	73.7
clv2-1 clv3-2	251	97	72.1

The number of gynoecia analyzed in which the distribution of valves were normal is indicated under *Normal*. Gynoecia in which a valveless region extended at least 50% of the length of the gynoecium, or gynoecia in which the basal 25% completely lacked valves were counted as *Valveless*.

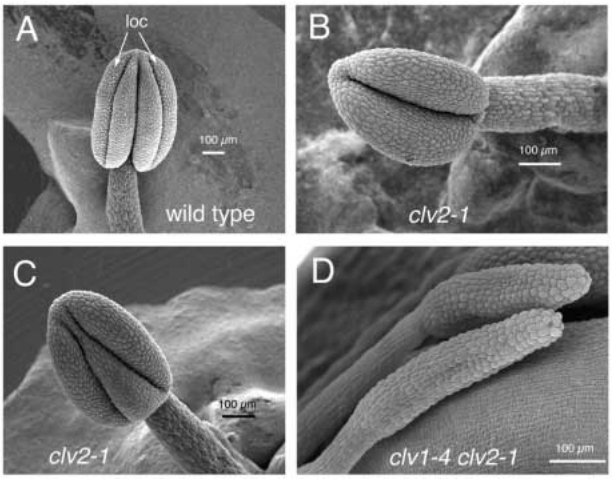

Fig. 5. *clv2* stamen defects. Wild-type stamens (A) develop two locules (loc). In *clv2* mutants, the stamens occasionally develop only a single locule (B), partially fused locules (C), or no locules (D).

In many *clv2* mutant flowers, stamens developed abnormally. Wild-type stamens develop an anther with two locules on the end of a stalk or filament (Clark and Meyerowitz, 1994; Fig. 5A). *clv2* mutant flowers developed anthers in which the locules were partially fused (Fig. 5C), or only contained a single locule (Fig. 5B). *clv2* mutants also developed stamens that lacked anthers. 11% of *clv2-1* stamens, 12% of *clv2-2* stamens and 10% of *clv2-3* stamens were antherless (over 250 stamens analyzed per genotype). This compared with previously published analysis of *clv1-1*, *clv1-5* and *clv1-4* flowers in which 4%, 5% and 6% of stamens, respectively, developed without anthers (Clark et al., 1993). The antherless stamens often contained anther-like cells at their apex whether they were found in *clv1*, *clv2*, *clv3* single mutants or various *clv* double mutants (Fig. 5D), suggesting that perhaps these are extremely reduced anthers.

Short-day phenotypes

A number of novel features of the development of *clv2* plants was revealed when the mutants were grown under short-day light conditions (SD; 8 hours of light per 24 hour period). Wild-type L*er* plants and most *clv2* alleles flower very late under SD conditions when compared to growth under continuous light (Martinez-Zapater et al., 1994; Table 3). The weak *clv2-2* allele, however, flowered significantly earlier than wild type. However, *clv2-2* was originally isolated in the Ws genetic

Table 3. Days to flowering under short-day conditions

Genotype	Mean days to flowering
L*er*	68.4±1
clv2-1	67.5±0.9
clv2-2	54.3±2.2
clv2-3	69.8±0.8
clv2-4	66.2±1.3

Plants were grown under 8 hours light per 24 hour period. The mean number of days between transfer from 4°C and visible flowers for approximately 30 plants is indicated for each genotype. Standard error is given.

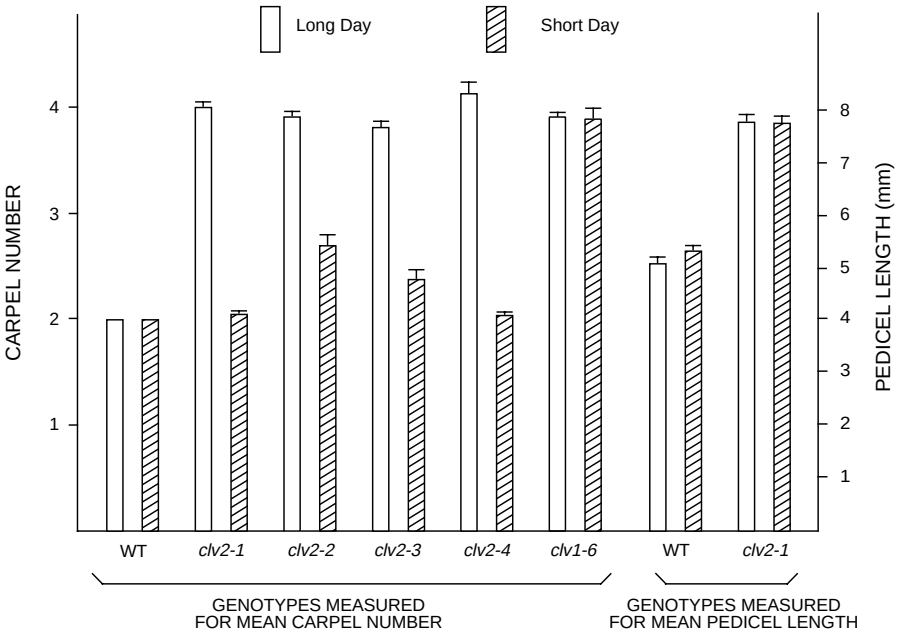

Fig. 6. *clv2* flower phenotypes suppressed by short-day conditions. Wild-type plants, *clv2* mutants and the *clv1-6* mutant were grown under continuous light (Long Days) or 8 hours light/24 hour period (Short Days). At least 50 flowers were examined for the number of carpels per flower and pedicel length. Only the first ten flowers on any given plant were analyzed. The mean and standard error are indicated.

background and the difference in flowering time may be the result of Ws-specific genetic factors that were not lost during the backcrosses of *clv2-2* into the L*er* background.

One clear effect of growth under SD conditions was the suppression of the *clv2* flower phenotype (Fig. 6). The number of organs in the flowers of most *clv2* alleles grown under SD were almost identical to wild type. No additional organs were formed interior to the whorl 4 gynoecium, and the valveless phenotype was not observed (data not shown). The weak *clv2-2* allele was not as strongly suppressed, with many flowers developing additional carpels (Fig. 6). However, not all *clv2* phenotypes were suppressed under SD conditions. The shoot meristem remained enlarged compared to wild type. In fact, the shoot meristems of *clv2-1* and *clv2-3* plants occasionally fasciated under SD conditions (Fig. 1J). In addition, the length of pedicels in *clv2* plants grown under SD conditions were identical to those of *clv2* plants grown under continuous light (Fig. 6).

An equally dramatic phenotype was observed in *clv2-2* and *clv2-5* mutants grown under SD conditions. In these plants, flowers along the inflorescence were occasionally converted into shoot meristems. In approximately half of the *clv2-2* plants examined, at least one flower was converted into a shoot meristem. In plants that were affected, usually multiple flowers were converted to shoot meristems (Fig. 7A). These numbers do not include plants in which the first several flowers produced after the cauline leaves were transformed into shoots, as we have observed that this phenomenon occurs, albeit rarely, even in wild-type plants grown under continuous light. Instead, we observed that *clv2* flowers at any length along the inflorescence could become transformed into shoots, even after the production of ten or twenty normal flowers. Flowers on secondary and tertiary inflorescences could also be transformed into shoots. The flowers that did develop as shoots were not subtended by a leaf; however, they did produce several cauline leaves before initiating flowers. The *clv2-5* allele, which is interestingly also from the Ws background, is the only other *clv2* allele that exhibited this phenotype, but it did so at a lower frequency. Only 3 of 36 *clv2-5* plants examined exhibited flowers transformed into shoots. This phenotype was never observed in wild-type plants.

The other *clv2* alleles (*clv2-1*, *clv2-3* and *clv2-4*) did not exhibit the dramatic transformation seen occasionally in *clv2-2* and *clv2-5* plants. They did, however, display a more subtle transformation of flowers into shoots when grown under SD conditions. The flowers that were produced shortly after the transition to flowering exhibited phenotypes similar to weak *leafy* mutants (Weigel et al., 1992). This consisted of the failure of organs to initiate in clear whorls, especially for the sepals and petals, and additional sepals were formed at the expense of petals (Table 4). This was only apparent on the earliest flowers formed after the transition to flowering, and the affect was reduced acropetally. *leafy* and *apetala1* mutants also exhibit a similar acropetal reduction in shoot-to-flower transformation (Weigel et al., 1992; Bowman et al., 1993). Interestingly, *clv2-1* enhances the partial flower-to-shoot transformation observed in the weak *leafy-5* allele grown under continuous light (data not shown).

clv1-1 and *clv1-6* mutants, which exhibit defects in shoot

Table 4. Flower-to-shoot transformations of *clv2* flowers under short-day conditions

	Mean organ number/flower			
Genotype	Sepals	Petals	Mosaic Sepal/Petal	Whorl 1 + 2 organs
wild-type	4.3±0.1	4.1±0.1	<0.1	8.4
clv2-3	5.2±0.1	3.0±0.1	0.2±0.1	8.4

The number of sepals, petals and mosiac sepal/petal organs were determined for the first ten flowers initiated by the primary inflorescence on each of five wild-type and *clv2-3* plants grown under short-daylight conditions (see Materials and methods). The mean and standard error are indicated. The combined number of whorl 1 and 2 organs was determined by adding the mean number of sepals, petals and mosaic organs.

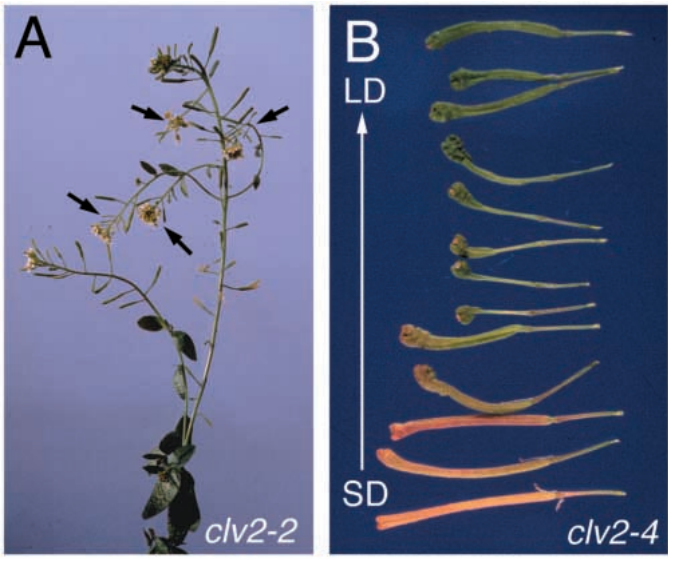

Fig. 7. *clv2* short-day defects. *clv2-2* plants grown under short-day (SD) light conditions (A) can exhibit shoots in the place of flowers (arrows) along the inflorescence. (B) Consecutive flowers along an inflorescence of a *clv2-4* plants that was transferred from SD to continuous light conditions reveal an abrupt transition from suppressed to mutant phenotype.

meristem size and flower organ number similar to that observed in *clv2* alleles, were also grown under SD conditions. These *clv1* alleles exhibited none of the changes in phenotype that *clv2* mutants underwent. The number of organs initiated by the flower was unchanged (Fig. 6, and data not shown), and no flower-to-shoot transformations were observed. *clv3-1* and *clv3-2* mutants also did not exhibit the suppression of flower organ number or flower-to-shoot transformations when grown under SD conditions(data not shown).

To determine the developmental period during which *clv2* flowers are sensitive to SD conditions, *clv2-3* and *clv2-4* plants were shifted from SD to continuous light after the transition to flowering. Thus, the earliest flowers formed on these plants developed entirely under SD conditions, while much later arising flowers developed entirely under continuous light. We observed a very marked transition from the suppressed phenotype to the *clv2* phenotype, indicating that the developmental period during which *clv2* flowers are sensitive to the light conditions is brief (Fig. 7B). This presumably corresponds to stages 2 to 5, when the flowers are initiating organs. Interestingly, the flowers formed during the transition appeared to be very strongly affected and displayed strong valveless phenotypes.

clv2 mutants result in expanded *CLV1* expression

CLV1 expression in wild-type plants is restricted to a central region of the shoot meristem and stage 2 through stage 5 flower meristems (Clark et al., 1997). Because *clv2* mutants result in phenotypes similar to *clv1* mutants, we considered the possibility that *clv2* mutant phenotypes were the result of reductions in the pattern or level of *CLV1* expression. In situ RNA hybridization experiments were performed on sections of both wild-type and *clv2-1* inflorescence tissue. The results as shown in Fig. 8 revealed an expanded pattern on *CLV1* expression in the enlarged *clv2* shoot meristem. As the signal in these images was achieved under identical conditions, it would appear that the level of *CLV1* mRNA accumulation is not reduced in the *clv2* mutant plants.

clv2 displays complex interactions with *clv1* and *clv3* mutants

An important question is whether *CLV2* acts in the same pathway as *CLV1* and *CLV3*. *clv2* mutants affect the development of the shoot and flower meristem in a manner similar to that of weak *clv1* and *clv3* mutants, yet *clv2* mutants also display a number of phenotypes not previously observed in *clv1* or *clv3* mutants. To test whether this resulted from *CLV2* functioning in a separate pathway from *CLV1* and *CLV3*, we generated doubles mutants of *clv1* and *clv2* as well of *clv2* and *clv3*.

We generated double mutants of the strong *clv1-4* and *clv3-2* mutations with various *clv2* alleles. While meristem size in these strong alleles is variable, we did not detect any difference in meristem size between the double mutants and the strong single mutants (Fig. 9). However, mutating *clv2* in a weak or intermediate *clv1* or *clv3* mutant background (*clv1-1*, *clv1-6*, *clv1-5*, *clv1-7*, *clv3-1*) did lead to an enlargement of the shoot meristem (Fig. 9, and data not shown). *clv1-7* is the weakest *clv1* allele and develops a shoot meristem even smaller than that of *clv1-6* (data not shown). The *clv1-7 clv2-2* double mutant lead to the development of massively enlarged shoot meristems comparable to strong *clv1* or *clv3* single mutants.

The phenotypes of *clv1 clv2* and *clv2 clv3* flowers revealed

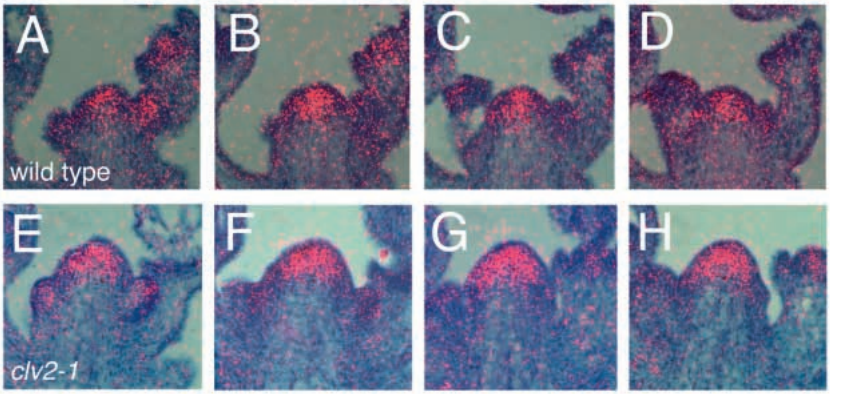

Fig. 8. *CLV1* mRNA expression is expanded in *clv2* mutants. In situ RNA hybridization with an antisense *CLV1* probe reveals transcript accumulation in serial sections of the shoot meristem of wild-type plants (A-D). In serial sections of an enlarged *clv2-1* shoot meristem (E-F), *CLV1* transcripts are detected in a larger area. Red grains indicate hybridization to the *CLV1* probe (see Materials and methods). All images shown at the same magnification.

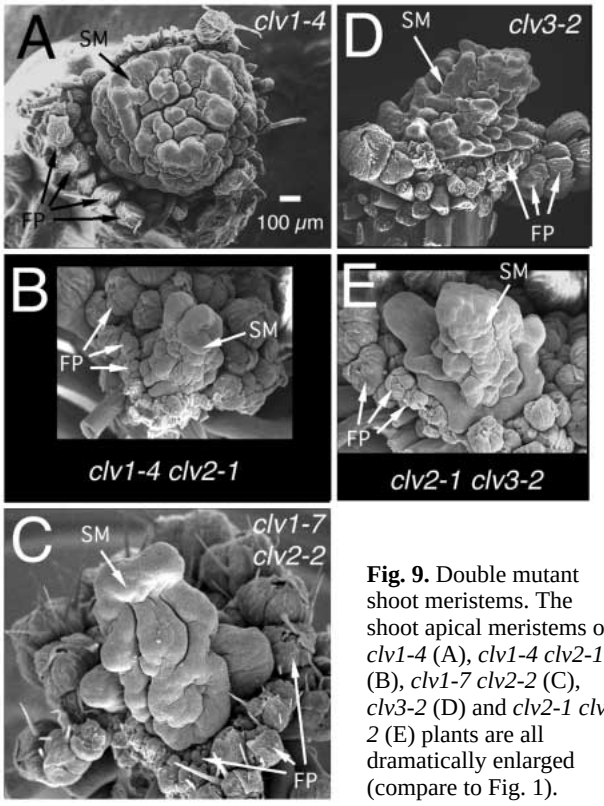

Fig. 9. Double mutant shoot meristems. The shoot apical meristems of *clv1-4* (A), *clv1-4 clv2-1* (B), *clv1-7 clv2-2* (C), *clv3-2* (D) and *clv2-1 clv3-2* (E) plants are all dramatically enlarged (compare to Fig. 1).

a complex interaction in the flower. The number of organs initiated by the double mutant flowers were similar to (or perhaps less than) those initiated by strong *clv1* or *clv3* single mutants (Table 5). In this aspect, *clv1-4* and *clv3-2* were epistatic to *clv2*. However, the effects of *clv2* on organ development was additive with *clv1* and *clv3*. The pedicels of *clv1* and *clv3* plants were found to be slightly longer than those of wild type and this was unchanged in the *clv1 clv3* double mutant. *clv2 clv3* double mutants developed dramatically longer pedicels than in *clv2* or *clv3* alone. Similarly, strong *clv1* and *clv3* mutant alleles also give rise to the valveless phenotype observed in *clv2* mutants. However, the *clv1 clv2* and *clv2 clv3* double mutant plants displayed a higher frequency and more extensive valveless phenotype compared to either single mutant, with nearly every gynoecium exhibiting the phenotype (Table 2). In fact, the slight reduction in mean carpel number in the *clv1 clv2* and *clv2 clv3* flowers compared to *clv1* and *clv3* single mutants, respectively, may be due to the high frequency of missing valves on the double mutants, as we use the number of valves to determine carpel number.

Table 5. Regulation of carpel number

Genotype	Mean number of carpels/flower
L*er*	2.0
clv2-1	4.0±0.06
clv2-2	3.9±0.04
clv2-3	3.8±0.07
clv2-4	4.1±0.08
clv1-6	4.0±0.03
clv1-4	6.3±0.14
clv3-2	6.3±0.13
clv1-4 clv2-1	5.7±0.21
clv1-4 clv2-2	6.3±0.13
clv2-1 clv3-2	5.5±0.18
clv2-2 clv3-2	6.0±0.13

The number of carpels per gynoecium was determined for 50 flowers of each genotype. The first ten flowers of each plant were analyzed. The mean and standard error are indicated.

clv2 displays complex interactions with *stm* mutants

STM and *CLV1*/*CLV3* appear to function in a competitive manner in the regulation of shoot meristem development. This is based on the genetic interaction displayed between *clv1*/*clv3* and *stm* mutations (Clark et al., 1996). *clv1*/*clv3* mutations dominantly suppress the strong *stm-1* allele, while *stm-1* dominantly suppresses *clv1*/*clv3* alleles. The *clv1*/*clv3 stm* double mutants show a loss of meristem homeostasis (i.e., the meristems vary greatly in size, they often become enlarged, but always terminate prematurely). *clv1*/*clv3* mutations also suppress the weak *stm-2* allele: the *clv3-2 stm-2* double mutant, for example, develops dramatically fasciated stems and each inflorescence produces hundreds of organs. The reduction of organs in the *stm-2* flowers is also partially suppressed by *clv1*/*clv3* mutations.

We tested the ability of the *stm-1* allele to dominantly suppress *clv2*, as well as the ability of *clv2* to suppress both the strong *stm-1* and weak *stm-2* allele. To determine whether *stm-1* can partially suppress *clv2-2* in a dominant manner, we scored the progeny of a plant homozygous for *clv2-2* and heterozygous for *stm-1*. As expected, one-quarter of the progeny germinated with the stm phenotype and were presumably homozygous for *stm-1* (however, see below for the adult phenotypes of these double mutants). The remaining progeny were scored for potential suppression and the genotypes of these plants at the *STM* locus (*stm-1* / + or + / +) were determined by testing progeny (Table 6). While the results indicate that *stm-1* dominantly suppresses the *clv2-2* phenotype, the correlation of *stm-1* heterozygosity and *clv2-2* suppression is not complete. This indicates that *stm-1* suppression of *clv2-2* is not as effective as *stm-1* suppression of *clv3-1*, where the phenotype was completely correlated with genotype (Clark et al., 1996).

The *clv2-2 stm-1* and *clv2-3 stm-1* double mutants were also analyzed. These plants frequently developed the ‘rescued’ phenotype characteristic of *clv1 stm-1* and *clv3 stm-1* double mutants (data not shown). Namely, postembryonic growth consisting of rosettes of leaves and inflorescences bearing

Table 6. *stm-1* dominantly suppresses *clv2-2*

Carpel Phenotype	Number of plants	Number of plants segregating *stm-1*	
		Observed	Expected
Normal clv2-2	11	1	7.3
Suppressed clv2-2	18	16	12

The self progeny of *clv2-2*/*clv2-2 stm-1*/+ plants were scored as either having the normal *clv2-2* carpel phenotype or a partially suppressed phenotype. Plants were then scored for *stm-1* segregation in the next generation. For each phenotypic class, the number of plants segregating *stm-1* is compared with the number that would be expected to segregate *stm-1* if *stm-1* had no effect on the carpel phenotype of these plants.

several flowers were observed. These structures were indicative of the development of meristems in the *clv1* and *clv3* double mutants with *stm-1*. Thus, *clv2* partially suppressed the *stm-1* phenotype. However, no suppression was observed in the *clv2-1 stm-2* double mutants. These plants were indistinguishable from *stm-2* single mutants and developed similar numbers of organs per flower (data not shown). Thus, *stm-2* was epistatic to *clv2* mutants.

DISCUSSION

We have examined the phenotypes of *clv2* mutants in order to understand the role that *CLV2* plays in regulating meristem development in *Arabidopsis*. Our observations indicate that *CLV2* plays a role not only in regulating shoot and flower meristem development, but also the development of several organ types.

clv2 phenotypes/*CLV2* functions

The effects of *clv2* mutations on meristem development are apparent in the enlarged shoot meristems, as well as the additional organs and the additional whorl of organs that are initiated in *clv2* flowers. These phenotypes are similar to that observed in weak *clv1* and *clv3* mutants. Thus, like *CLV1* and *CLV3*, *CLV2* is required to prevent the accumulation of undifferentiated cells at the shoot and flower meristem. The same models proposed for *CLV1*/*CLV3* action (inhibition of undifferentiated cell division, and promotion of organ formation) could also be proposed for *CLV2*. *clv2* mutant plants also develop abnormal organs, namely, elongated pedicels, gynoecia lacking valves and reduced anthers. Evidence outlined below suggests some of these organ defects are not indirect consequences of alterations in meristem structure.

The requirement for *CLV2* during flower meristem development is dependent on physiological conditions. During growth in continuous light, *CLV2* is required for normal development of the flower meristem. However, when grown under short-day (SD) light conditions, *clv2* mutant flowers are nearly identical to wild type. This could be because the change in physiological state associated with SD growth conditions results in an altered flower meristem that no longer requires *CLV2* function. An alternative explanation is that a redundant or compensating factor is active, or active at a higher levels, only under SD conditions. The fact the *clv2* mutants develop normal flower meristems with elongated pedicels under SD conditions is direct evidence that *CLV2* regulation of pedicel length is independent of its regulation of flower meristem development. *clv2* flowers under SD conditions, however, do not exhibit valveless phenotypes or reduced stamens, indicating that an alteration of the flower meristem is at least a prerequisite for these developmental abnormalities.

clv2 mutants also exhibit a novel flower-to-shoot transformation specifically under SD growth conditions. For the Ws-derived *clv2-2* and *clv2-5* alleles, this consists of late-arising flowers occasionally converted completely into functional shoot meristems. We are unaware of any other mutation that gives rise to a similar phenotype. While mutations such as *leafy* and *apetala1* covert flowers into shoots, they affect all of the flowers of the inflorescence, and generally exhibit partially conversion. For the other *clv2* alleles, a more subtle flower-to-shoot transformation, reminiscent of weak *leafy* alleles, affect the flowers that are initiated shortly after the transition to flowering. That these flower identity phenotypes are only observed under SD conditions is likely the result of the reduced level of flower induction generally believed to exist under SD conditions.

Genetic interactions

A critical question is how does *CLV2* fit into the hierarchy of genes known to regulate shoot and flower meristem development. *clv2* mutants, overall, are similar to *clv1* and *clv3* mutants, although there are several differences. First, all *clv2* alleles have phenotypes relatively weak compared to *clv1* and *clv3* alleles. One possible explanation is that the *clv2* alleles are all partial-loss-of-function alleles. However, three alleles all have very similar phenotypes (*clv2-1*, *clv2-3* and *clv2-4*), while there is often great variability between partial-loss-of-function alleles (see for example, the various partial-loss-of-function *clv1* alleles; Clark et al., 1993, 1997). In addition, the *clv2-5* allele is linked to a T-DNA insertion (S.-H. Jeong and S. E. C., unpublished) and many, albeit not all, T-DNA insertions result in null alleles. Another difference between *clv2* mutants and *clv1*/*clv3* mutants is that *clv2* alleles exhibit more dramatic organ defects (pedicel elongation, valveless gynoecia, reduced stamens) than comparable *clv1* alleles. Finally, *clv1*/*clv3* mutants are not suppressed by SD growth conditions, nor do they exhibit flower-to-shoot transformations. Thus *CLV2* could function in a separate pathway from *CLV1*/*CLV3* entirely, or could function with *CLV1*/*CLV3* to regulate meristem development and separately to regulate organ development.

To distinguish between these possibilities, *clv1 clv2* and *clv2 clv3* double mutants were generated and analyzed. The data are consistent with *CLV2* functioning with *CLV1*/*CLV3* in the regulation of shoot and flower meristem development. This is based on the observations that the strong *clv1* and *clv3* alleles were epistatic to *clv2* in terms of the size of the shoot meristem and the number of organs initiated by the flower meristem. However, the *clv2 clv3* double mutant was additive in terms of pedicel length and both the *clv1 clv2* and *clv2 clv3* double mutants were additive in terms of valveless gynoecia, consistent with a model in which *CLV2* functions in a separate pathway in the regulation of organ development. If *CLV2* functions in the same pathway as *CLV1*/*CLV3*, then it should exhibit similar interactions with *stm* mutations. *clv1*/*clv3* and *stm* dominantly suppress each other's phenotypes, and the same is true for *clv2* and *stm*. However, *clv2* is unable to suppress a weak *stm* allele. This is perhaps related to the weak *clv2* phenotypes. As CLV1 likely acts as a signaling molecule and STM likely functions as a transcription factor (Long et al., 1996), it will be interesting to determine how the *CLV2* gene product fits into this developmental hierarchy.

This work is supported by grant IBN-9506952 from the National Science Foundation – Developmental Mechanisms Program. The scanning electron microscope used was acquired under grant BSR-83-14092 from the National Science Foundation. We thank David Bay for photographic assistance, and Keiko Torii for critical reading of the manuscript. We thank Sang Ho Jeong for determining the frequency of flower-to-shoot transformations in *clv2-5*.

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