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Disruption of Interfascicular Fiber Differentiation in an Arabidopsis Mutant

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Arabidopsis develops interfascicular fibers in stems for needed support of shoots. To study the molecular mechanisms controlling fiber differentiation, we isolated an interfascicular fiber mutant (*ifl1*) by screening ethyl methanesulfonate–mutagenized Arabidopsis populations. This mutant lacks normal interfascicular fibers in stems. Interestingly, some interfascicular cells were sclerified in the upper parts but not in the basal parts of the *ifl1* stems. These sclerified cells were differentiated at a position different from that of interfascicular fibers in the wild type. Lack of interfascicular fibers correlated with a dramatic change of stem strength. Stems of the mutant could not stand erect and were easily broken by bending. Quantitative measurement showed that it took approximately six times less force to break basal stems of the mutant than of the wild type. In addition, noticeable morphological changes were asssociated with the mutant, including long stems, dark green leaves with delayed senescence, and reduced numbers of cauline leaves and branches. Genetic analysis showed that the *ifl1* mutation was monogenic and recessive. The *ifl1* locus was mapped to a region between the 17C2 and 7H9L markers on chromosome 5. Isolation of the *ifl1* mutant provides a novel means to study the genetic control of fiber differentiation.

INTRODUCTION

Fibers are found in various parts of the plant. A variety of cell types can differentiate into fibers. For example, in the primary body, the procambium produces cells that can differentiate into primary xylem fibers or primary phloem fibers. In the secondary body, the vascular cambium produces secondary xylem fibers and secondary phloem fibers. Fibers can also originate from ground tissues of the cortex and the mesophyll of leaves. According to their locations, fibers are classified as being either xylary or extraxylary fibers (Esau, 1977; Mauseth, 1988).

The mechanisms of fiber formation evolved in early vascular plants. For example, multilayers of fibers are present in the stem cortex of the early vascular plant *Lycopodium complanatum*. Because the mechanisms for secondary wall deposition and lignification evolved when vascular plants first emerged on land, it has been assumed that plants evolved to use these mechanisms to produce extraxylary fibers. In the case of xylary fibers, it has been proposed that they evolved from tracheids. The ability to make fibers obviously has strong selective advantages. Along with the deposition of secondary walls and lignin, fibers provide the strength to support the heavy plant body and serve as a physical barrier against herbivore attack (Mauseth, 1988). In addition to reinforcing tissues, fibers render the plant body elastic so that its original size and shape can be resumed when the deforming force is removed. They also allow the plant body to deform without breaking (Mauseth, 1988).

The anatomies of fibers vary considerably. Fibers can be short if their initials are differentiated from cells of nonelongating tissues, such as the vascular cambium or the mesophyll of many dicot leaves. They can be very long if fiber initials are produced in elongating tissues, such as the procambium, the cortex of young internodes, or the mesophyll of monocot leaves. For example, although fibers 1 to 10 mm in length are not unusual (such as leaf fibers from sisal and bowstring hemp), fibers from ramie can be as long as 55 cm (Esau, 1977; Mauseth, 1988; Fahn, 1990).

One common feature of fibers is that they all have thickened secondary walls. Fibers from most plants are also heavily lignified, especially in monocots. There are a few exceptions. For example, little or no lignin is found in extraxylary fibers from plants such as flax and hemp. Because of the agronomical importance of fibers, polysaccharides in flax fibers have been analyzed in detail. In flax stems, fiber bundles originate in the outer part of the primary phloem, and at maturity, the walls of fiber cells are so heavily thickened that their lumens are nearly filled. Mature fibers contain 60 to 70% cellulose (Gorshkova et al., 1996). In addition to cellulose, fibers are rich in pectin and other noncellulosic polysaccharides, which have been well characterized structurally (Davis

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et al., 1990; McDougall, 1993; Gorshkova et al., 1996). It has been observed that flax stems are easily broken at the point at which the fibers start to differentiate, which is consistent with the elastic property of fibers. Because it is easy to get clean fibers by peeling, flax fibers are considered to be convenient materials for the study of wall polymer biosynthesis and identification of fiber-specific genes (Gorshkova et al., 1996).

Early studies by Aloni (1976a, 1976b, and 1978) and Sachs (1972) have shown that signals for fiber differentiation originate in the leaves. The signals flow in a polar direction from the leaves to the root and induce fiber differentiation along their path. Using stems of *Coleus blumei*, Aloni (1979) further showed that the plant hormones auxin and gibberellin (GA) could fully replace the role of leaves in the differentiation of primary phloem fiber. Different ratios of these two hormones resulted in fibers with different shapes. Although high concentrations of auxin stimulated rapid differentiation of short fibers with thick secondary walls, high levels of GA resulted in long fibers with thin walls. In addition, both in vivo and in vitro experiments have demonstrated that cytokinin transported from root apices is also required for fiber differentiation in stems (Saks et al., 1984). For example, the number of newly differentiated fibers decreased significantly by removal of root apices or by lowering the transpiration rate, and this decrease could be reversed by the application of cytokinin. Similarly, in cultured hypocotyl segments of *Helianthus annuus*, no fiber differentiation was observed in the absence of cytokinin (Aloni, 1982). Thus, it seems clear that fiber differentiation is controlled by the interactions of at least three hormonal signals: auxin, GA, and cytokinin.

Although fiber differentiation has been studied by anatomical, structural, and physiological approaches, a number of areas remain to be explored. It is not clear how the hormonal signals are perceived and transduced to direct fiber differentiation. The three hormones required for fiber differentiation are also involved in a number of other developmental processes. It seems likely that different downstream pathways of these hormone signals evolved to direct different processes. For example, although GA is required for both stem elongation and fiber differentiation, a GA-insensitive wheat mutant affected stem height but not fiber differentiation (Lev-Yadun et al., 1996), indicating that downstream of GA there are two different pathways regulating stem elongation and the determination of cell fate of fibers. We also do not know how plants control the spatial differentiation of fibers, specifically, how plants control the differentiation of xylary fibers versus extraxylary fibers.

To address these issues, we have initiated a genetic approach to study fiber differentiation. Using the model plant Arabidopsis, we have isolated one mutant that lacks normal interfascicular fibers in inflorescence stems. Interestingly, although the lower parts of the mutant stems were devoid of extraxylary fibers, sclerified cells were formed between vascular bundles in the upper parts. Consequently, the lower parts of the mutant stems were easily broken as well as incapable of supporting the shoot. These results directly demonstrate the essential role of fibers in plant structure and form.

RESULTS

Interfascicular Fiber Development in Arabidopsis Inflorescence Stems

Using the model plant Arabidopsis to study fiber differentiation, we first examined the fiber formation in the wild-type Arabidopsis plants. A lignin staining reagent phloroglucinol–HCl was used to show fiber distribution in inflorescence stems of 5-week-old plants. Stems were divided into four equal segments to represent different developmental stages. In the top part of the stem, discrete xylem bundles were seen, but no lignified extraxylary fibers were evident (Figure 1A). Toluidine blue staining of these sections also did not show recognizable extraxylary fiber bundles (data not shown), indicating that no fibers were differentiated at the top part of the stem. In the second segment, lignified extraxylary fibers located between vascular bundles were seen (Figure 1C). Lignin staining in fibers was much less intense than that seen in xylem cells. These two to three layers of fibers were located at the same level as phloem and thus formed an arch-shaped pattern between xylem bundles. In the third segment, approximately four layers of interfascicular fibers were lignified as intensely as xylem bundles (Figure 1E). At the basal part, four to five layers of interfascicular fibers together with xylem bundles formed a thick ring of lignified tissues (Figure 1G). These indicate that interfascicular fiber formation is well correlated with the timing of stem maturation and of needed strength for support of shoots.

Isolation of the *ifl1* Mutant

We screened ethyl methanesulfonate–mutagenized populations of Arabidopsis for mutants with altered fiber formation. Several mutants showing varying degrees of changes in fiber formation were isolated. Dramatic effects on both interfascicular fiber formation and stem strength were seen with one mutant; therefore, it was chosen for further analysis. In this mutant, normal lignified interfascicular fibers were absent in inflorescence stems of 5-week-old plants (Figure 1). In the top part of the stem, the overall staining pattern, such as small xylem bundles, was similar to that seen in the wild type (Figure 1B). However, from the second segment to the basal segment, dramatic differences were observed (Figures 1D, 1F, and 1H). Although interfascicular fibers were formed in wild-type stems, the mutant did not show any lignified interfascicular fibers. Interestingly, a few interfascicular cells next to xylem were lignified in the second and third segments (Figures 1D and 1F). In addition, vascular bundles in

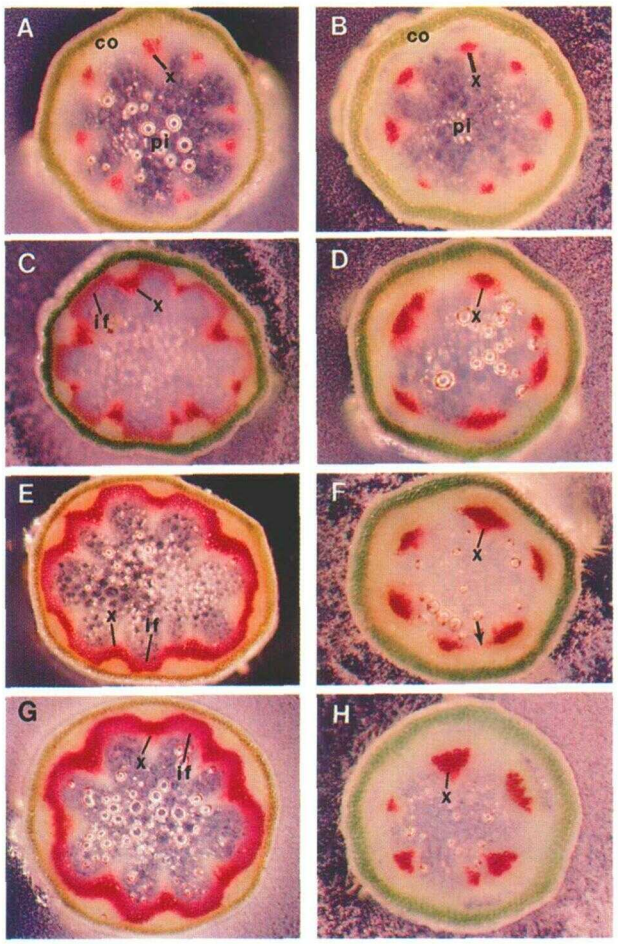

Figure 1. Interfascicular Fiber Formation in Stems of the Mutant and Wild-Type Arabidopsis.

Stems from 5-week-old plants were divided into four equal segments. Sections from the middle part of each segment were stained for lignin with phloroglucinol–HCl. Lignin in fibers and xylem bundles stained red. **(A)**, **(C)**, **(E)**, and **(G)** are from the wild type. **(B)**, **(D)**, **(F)**, and **(H)** are from the mutant.

(A) and **(B)** Sections from the top segments of the wild type **(A)** and the mutant **(B)**. Only xylem walls were lignified.

(C) and **(D)** Sections from the second segments showing that although the wild type **(C)** developed three layers of interfascicular fibers, the mutant **(D)** did not.

(E) and **(F)** Sections from the third segments showing that fibers in the wild type **(E)** were heavily lignified, whereas the mutant **(F)** had only a few lignified interfascicular cells (arrow).

(G) and **(H)** Sections from the basal segments showing that the wild type **(G)** developed four to five layers of lignified fibers, whereas the mutant **(H)** did not.

co, cortex; if, interfascicular fiber; pi, pith; x, xylem.

the mutant seemed to be wider than in the wild type. In the basal stem section where fibers were most heavily lignified in the wild type (Figure 1G), the mutant completely lacked lignified interfascicular fibers (Figure 1H).

To investigate whether the mutation completely blocks interfascicular fiber formation or only disrupts lignin deposition in interfascicular fibers, thin stem sections from both the mutant and wild-type plants were examined at a higher magnification. In the wild type, interfascicular fibers (small cells with thick walls) were clearly seen to form an arch-shaped pattern between vascular bundles (Figure 2A). However, no fiberlike cells were evident in this region of the mutant (arrow in Figure 2C). In addition, it was obvious that the mutant had wider vascular bundles and more vessels (Figure 2D) than did the wild type (Figure 2B). These results indicate that the absence of lignin staining in interfascicular regions of the mutant is due to the lack of fibers. Thus, we named the mutant locus the *ifl1* (interfascicular fiberless) locus.

Because a few interfascicular cells next to xylem were lignified in the middle part of stems of 5-week-old mutant plants (Figures 1D and 1F), we investigated whether more cells were sclerified in interfascicular regions of older mutant plants. Stems of 8-week-old *ifl1* and wild-type plants were divided into four segments of equal length, and sections from the middle of each segment were stained for lignin. Stems of the 8-week-old wild type showed the same pattern of interfascicular fiber distribution as did stems of the 5-week-old wild type, with the exception of a few more layers of fibers (data not shown). In the *ifl1* mutant, the features observed in 5-week-old mutants were still obvious in the lower parts of 8-week-old stems, including lack of normal interfascicular fibers and wider vascular bundles (Figure 3). However, it was clear that xylem bundles were gradually joined by lignified interfascicular cells in the upper part of stems. The walls of these lignified interfascicular cells were birefringent when viewed through polarization filters, indicating that they are sclerified cells (data not shown).

As clearly shown in Figure 3, there were a few significant differences between the formation of interfascicular fibers in the wild type and that of sclerified cells in the mutant. First, in contrast to the formation of interfascicular fibers in lower parts of the wild-type stem (Figure 1), sclerified cells were formed in upper parts (Figures 3A and 3B) but not in the basal part of the mutant stem (Figure 3D). Second, in contrast to the simultaneous formation of three layers of interfascicular fiber cells in the wild type, sclerified cells first appeared next to xylem bundles and then gradually extended out from vascular bundles in the mutant (Figure 3C). In addition, although one layer of sclerified cells was initially formed in lower parts of the mutant stem (Figure 3C), two to three layers of sclerified cells were differentiated in upper parts of the mutant stem (Figure 3A). A few lignified phloem fibers opposite to vascular bundles were seen in the top part of the mutant stem (Figure 3A). Phloem fibers were also seen in the wild type, although they were present only in the basal parts of stems (data not shown).

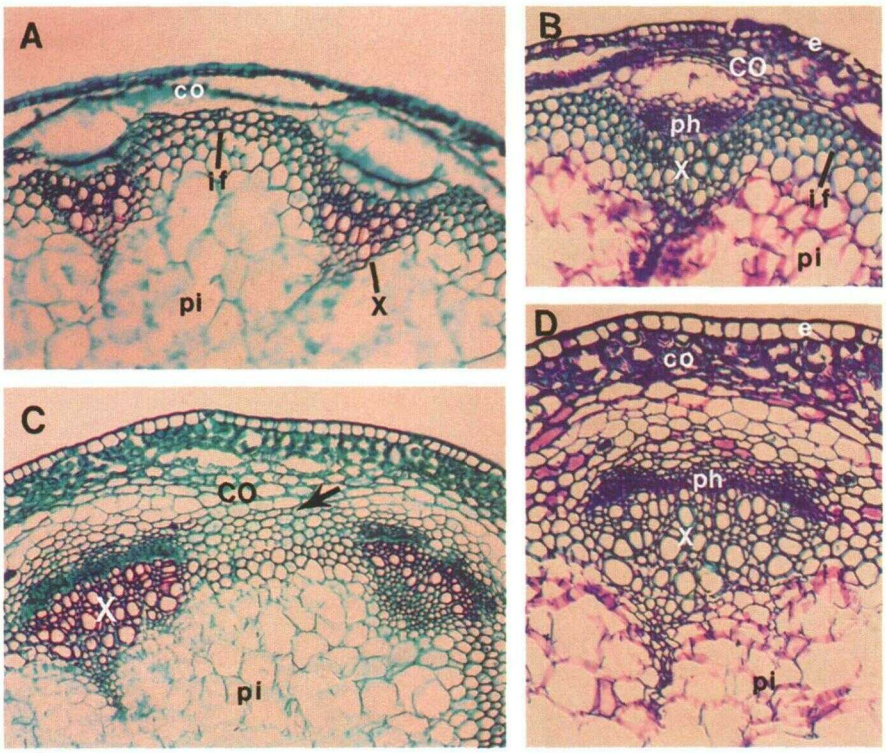

Figure 2. The *ifl1* Mutant Lacks Normal Interfascicular Fibers in Stems.

Thin stem sections (15 μm) were prepared from 6-week-old wild-type and *ifl1* plants and stained with either safranin O and fast green or toluidine blue. Lignified walls stained light red by safranin O (**[A]** and **[C]**) or blue by toluidine blue (**[B]** and **[D]**).

(A) A section from the wild type showing interfascicular fibers forming an arch-shaped pattern.

(B) A close-up of a vascular bundle of the wild type.

(C) A section from the mutant stem showing the absence of interfascicular fibers (arrow).

(D) A close-up of a vascular bundle of the mutant.

co, cortex; e, epidermis; if, interfascicular fibers; ph, phloem; pi, pith; x, xylem.

Another important feature was the positional difference between the sclerified cells in the mutant and the interfascicular fibers in the wild type. Although the interfascicular fibers in the wild type were positioned at the same level as phloem and thus formed arch-shaped patterns between bundles, the sclerified cells in the mutant were located at the same level as xylem and thus were not seen as arch-shaped patterns (Figures 4A and 4B). This positional difference can be clearly seen in longitudinal sections of the interfascicular regions of the mutant and the wild type (Figures 4C and 4D). Both the mutant and the wild-type plants had four layers of cortical cells. Although the interfascicular fibers were formed next to the cortical cells in the wild type (Figure 4C), the sclerified cells and cortical cells were separated by six to eight layers of interfascicular parenchyma cells in the mutant (Figure 4D and Table 1). In addition, the interfascicular fiber cells in the wild type were much longer than were the sclerified cells in the mutant.

Phenotype of the *ifl1* Mutant

A visible feature associated with the *ifl1* mutation was long stems. During the first week after bolting, the length of stems was similar between the mutant and wild-type plants. From the second week after bolting, the mutant stems elongated much faster than did wild-type stems. Although wild-type stems only could grow to ~38 cm in length during their lifetime, the mutant stems continued to grow to as long as 59 cm (Figure 5). Because the mutant and the wild-type plants had internodes with similar length and flowered at the same time (data not shown), the long stems in the mutant resulted from new growth of apical meristems rather than internodal elongation.

Another conspicuous feature of the *ifl1* mutant was that the stems could not stand as erect as the wild-type stems. As shown in Figure 6A, the stems of the wild-type plant generally stood erect. However, the stems of the *ifl1* mutant nat-

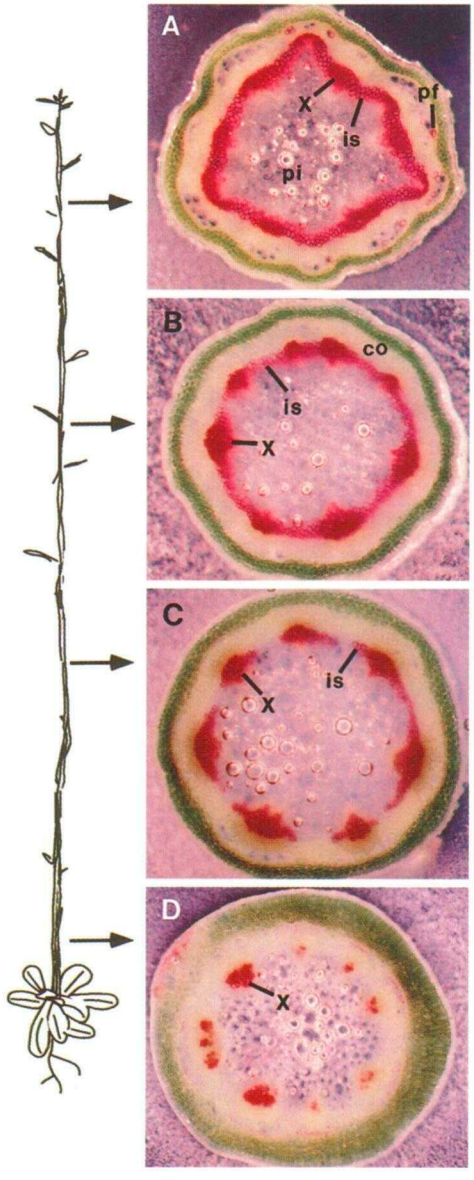

Figure 3. Sclerification of Interfascicular Cells in the *ifl1* Mutant Stem.

Stems from 8-week-old *ifl1* plants were divided into four equal segments, as shown in the diagram at left, and free-hand sections from the middle portion of each segment were stained for lignin with phloroglucinol–HCl. Lignin stained red in xylem walls and fibers.

(A) A section from the top segment showing the presence of two to three layers of heavily lignified interfascicular sclerenchyma.

(B) A section from the second segment showing one to two layers of lignified interfascicular sclerenchyma.

(C) A section from the third segment showing interfascicular sclerenchyma starting to extend out from each xylem bundle.

(D) A section from the basal segment showing the absence of lignified interfascicular sclerenchyma.

co, cortex; is, interfascicular sclerenchyma; pf, phloem fiber; pi, pith; x, xylem.

urally lay on the ground (data not shown). For a better view of the morphology of the stems, 6-week-old *ifl1* plants were placed on the edge of a table and photographed (Figure 6B). It is obvious that the mutant stems were pendent. Eight-week-old *ifl1* plants still exhibited the same phenotype (data not shown) probably because of the lack of sclerified interfascicular cells in the basal parts of the mutant stems (Figure 3). This is consistent with the function of fibers providing strength for support.

During our routine care of the *ifl1* plants, we noticed that the stems were easily broken by bending. The basal parts of the stems were most susceptible to breakage. However, wild-type stems generally could not be broken by bending. To analyze quantitatively the strength of stems, we measured the force needed to pull stems apart. Stems from 6-week-old plants were divided into five equal segments, and the force required to break each segment was measured. As seen in Figures 7A and 7B, in the top segments of stems, the breaking forces were similar between the wild type and the *ifl1* mutant. However, dramatic differences were seen in the lower segments of stems. Whereas increasing force was required to break older stems of the wild type, much less force was required for older stems of the *ifl1* mutant. To break the basal stem segment of the mutant, a force of only ~0.27 kg was needed. This was approximately six times less than that required for the wild type (Figure 7). These results indicate that lack of fibers in the *ifl1* mutant renders the stems plastic, consistent with the notion that fibers provide elasticity that enables organs to maintain their proper shapes and positions.

In addition to the long and brittle stems, there were a few other significant phenotypic changes in the *ifl1* plants. First, both leaves and stems of the mutant were dark green compared with those of the wild type. No major difference in leaf color was noticed before bolting. However, after bolting, *ifl1* leaves gradually became dark green. Second, rosette leaves of the mutant showed delayed senescence. They stayed dark green after 2 months, whereas most wild-type leaves under the same conditions started to senesce at this stage (data not shown). Third, there were fewer rosette leaves and rosette buds in the mutant than in the wild type (Table 1). In the wild type, new rosette buds were initiated after the first bolting stem was removed. However, in the mutant, no new rosette buds were formed after the removal of the first few stems. Fourth, the *ifl1* mutant had fewer cauline branches. Wild-type stems generally had approximately five leaves and developed axillary buds. However, in the mutant, there were fewer cauline leaves, and generally they were devoid of axillary buds (Table 1). Removal of apical buds of the mutant stems did not induce formation of axillary buds.

Genetic Analysis

To investigate whether the *ifl1* mutation was dominant or recessive, the mutant was backcrossed with the wild-type

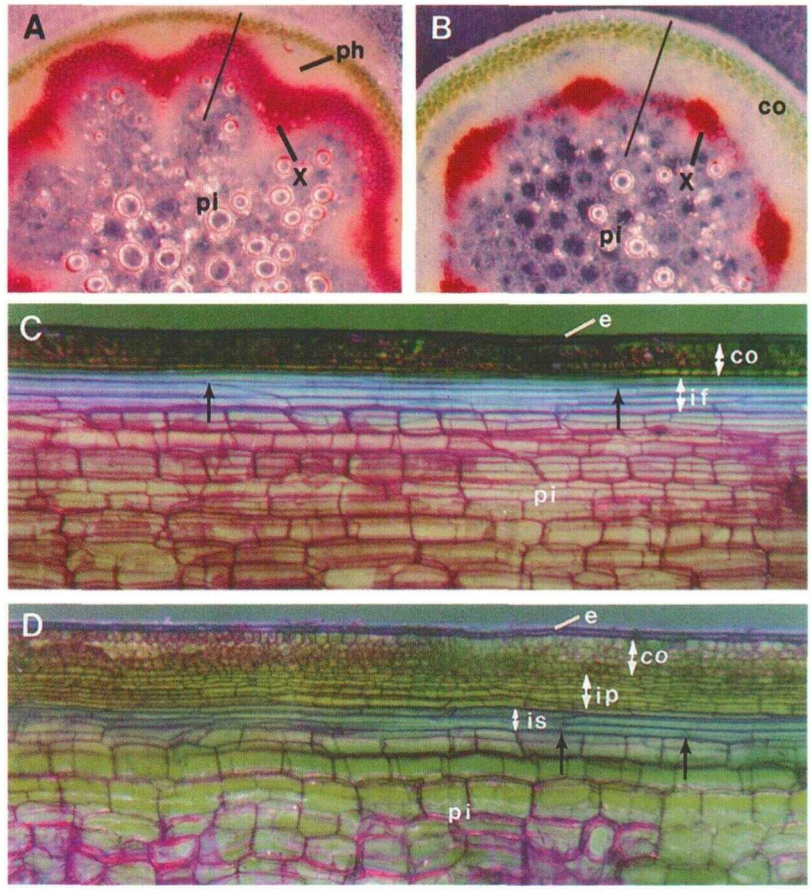

Figure 4. Interfascicular Sclerenchyma Cells in the *ifl1* Mutant Form at a Position Different from That of Interfascicular Fibers in the Wild Type.

Freehand sections were stained with either phloroglucinol–HCl (**[A]** and **[B]**) or toluidine blue (**[C]** and **[D]**) to show the anatomy.

(A) A cross-section of the wild-type stem showing interfascicular fibers positioned at the same level as phloem.

(B) A cross-section of the mutant stem showing interfascicular sclerenchyma positioned at the same level as xylem.

(C) A longitudinal section of the the wild-type stem through an interfascicular region (shown as a black line in **[A]**) showing long interfascicular fiber cells next to cortex. The two ends of one fiber cell are indicated by single-headed arrows.

(D) A longitudinal section of the mutant stem through an interfascicular region (shown as a black line in **[B]**) showing short interfascicular sclerenchyma cells separated from cortex by interfascicular parenchyma. The two ends of one sclerenchyma cell are indicated by single-headed arrows.

co, cortex; e, epidermis; if, interfascicular fiber; ip, interfascicular parenchyma; is, interfascicular sclerenchyma; ph, phloem; pi, pith; x, xylem.

Columbia. All of the F_1 progeny showed the wild-type phenotype, indicating that the *ifl1* mutation is recessive. For segregation analysis, the F_1 progeny was selfed, and F_2 plants were scored for the segregation of the *ifl1* mutation. Of 368 plants analyzed, 91 showed the interfascicular fiberless phenotype. This gave a 3:1 segregation ratio of the wild type to *ifl1*, indicating that the defect resulted from a single gene mutation. All other phenotypes of the *ifl1* mutant, including long stems, dark green leaves with delayed senescence, and reduced numbers of cauline leaves and branches, completely cosegregated with the interfascicular fiberless phenotype.

The chromosomal location of the *ifl1* locus was determined by using codominant cleaved amplified polymorphic sequences (CAPS) markers (Konieczny and Ausubel, 1993). No linkage of the mutation was found on chromosomes 1 to 4. However, a close linkage was found when the CAPS markers DFR and LFY3 on chromosome 5 were used for mapping (Figure 8). Of 338 F_2 mapping plants that segregated for the *ifl1* mutation, five recombinants were detected between the marker LFY3 and the *ifl1* locus. This placed the *ifl1* locus 0.7 centimorgans (cM) away from LFY3. Of 314 F_2 mapping plants analyzed, there were 144 crossovers between the CAPS marker DFR and the *ifl1* locus. Further mapping defined the *ifl1* locus to a genetic distance 0.3 cM away from 7H9L, which is located on the left side of the locus, and 0.3 cM away from 17C2, which is located on the right side of the locus (Figure 8).

Table 1. Comparison of the Number of Cell Layers, Stems, and Leaves between the Wild Type and the *ifl1* Mutant[a]

Phenotype	Wild Type	*ifl1*
Cell layers between epidermis and interfascicular fibers or sclerenchyma	4	8 ± 2
Rosette leaves	16 ± 4	8 ± 2
Rosette buds	8 ± 3	3 ± 2
Axillary leaves	5 ± 2	2 ± 1
Axillary buds	5 ± 2	1 ± 1

[a] Data are the mean values ±SE from 20 plants.

DISCUSSION

Arabidopsis inflorescence stems develop extraxylary fibers in the interfascicular regions. The formation of interfascicular fibers is coincident with the timing of needed strength for shoot support. Because these fibers are easily identified by simple histological staining, Arabidopsis seems to be an ideal system in which to study fiber differentiation. In particular, mutational analysis may be applied for this purpose because fibers are not essential for plant survival under greenhouse conditions. In contrast, disruption of xylem formation may be lethal to plants because the xylem is essential for water and solute transport. Considering the possibility that fibers evolved by activating the same mechanisms for secondary wall formation and lignification as used for xylem formation, the knowledge gained from the study of fiber differentiation may also be useful in understanding xylogenesis. Isolation of the *ifl1* mutant, which lacks interfascicular fibers, marked the first step in this direction.

Normal Interfascicular Fiber Formation Is Disrupted in the *ifl1* Mutant

In wild-type stems, interfascicular fibers are positioned at the same level as the phloem. The *ifl1* mutation apparently blocks the initiation of normal interfascicular fiber differentiation in stems. This was clearly shown in stems of 5-week-old mutants (Figure 1). The lack of normal interfascicular fibers is not due to the delay of fiber differentiation because no fibers were formed in the basal stems of 8-week-old plants. However, the mutation seems to have no effect on the formation of xylem fibers and phloem fibers. Both xylem and phloem fibers are present in mutant stems. Interestingly, phloem fibers are formed in upper parts rather than lower parts of the mutant stems, as was observed in the wild type. These results suggest that the mutation mainly affects normal extraxylary fiber initiation. They also indicate that fiber formation in different tissues is regulated by different controlling pathways.

Physiological studies have shown that the hormones auxin, cytokinin, and GA control fiber differentiation both qualitatively and quantitatively (Aloni, 1987). Although the nature of the *ifl1* mutation is not yet known, it appears that the *ifl1* mutation does not cause hormonal deficiency because no growth retardation was observed in the mutant compared with the wild type. In addition, exogenous application of these hormones did not rescue the *ifl1* mutant phenotype (data not shown). Thus, it seems likely that the mutation affects a specific step controlling initiation of fiber formation by these hormones. This is apparently different from those found in the known GA-, auxin- or cytokinin-responsive mutants because these hormone-responsive mutants exhibit phenotypes distinct from the *ifl1* mutant (Estelle and Klee, 1994; Finkelstein and Zeevaart, 1994).

It has been shown that positional pattern of fiber differentiation is controlled by polar auxin flow along the stem (Aloni, 1987). Because interfascicular fibers form at precise positions in wild-type Arabidopsis stems, it is possible that in the *ifl1* mutant, the polar transport of controlling factors, such as auxin, is disrupted so that no normal interfascicular fibers are induced. If this is the case, the disruption must have occurred only along the sites where normal interfascicular fibers are differentiated but not along the vascular bundles because the normal vascular pattern is maintained in the *ifl1* mutant. A number of auxin transport mutants, such as *pin-formed* (*pin1*) (Okada et al., 1991), *root curl in naphthylphthalamic acid* (*rcn1*) (Garbers et al., 1996), and *lopped* (*lop1*) (Carland and McHale, 1996), have been isolated. These mutations resulted in distinct phenotypes that are completely different from the *ifl1* mutant, indicating that *pin1*, *rcn1*, and *lop1* are specific for a subset of responses. It will be interesting to test whether there is any alteration of auxin transport in the *ifl1* mutant stems.

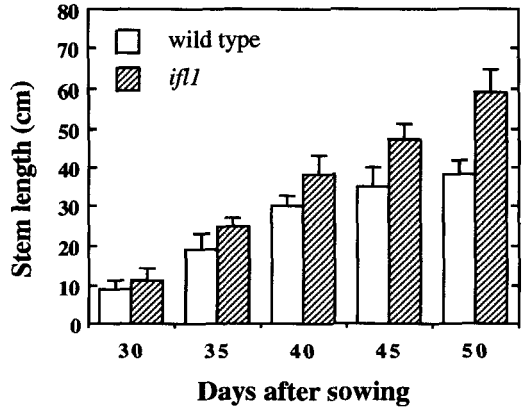

Figure 5. The *ifl1* Mutant Has Long Stems.

The main stems from 20 individual wild-type or *ifl1* plants were measured for stem lengths consecutively at 5-day intervals. Data are the mean values ±SE of 20 plants.

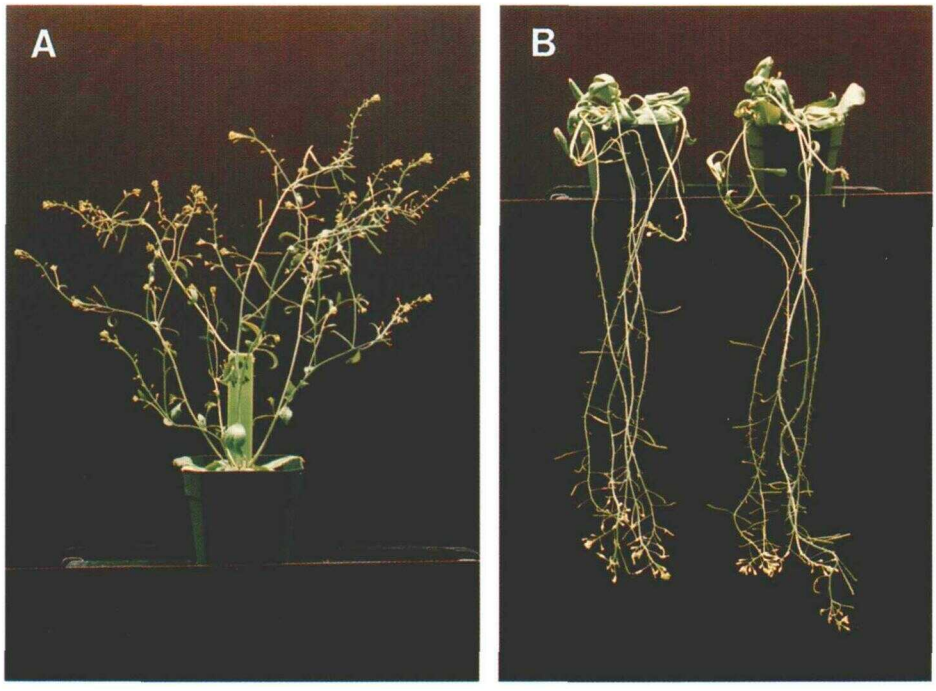

Figure 6. The *ifl1* Mutant Stems Cannot Stand Erect.

The wild-type and the *ifl1* plants were kept on the edge of a counter.
(A) Wild-type stems stand erect.
(B) *ifl1* stems are pendent.

Interfascicular Cells Are Sclerified in the Upper Parts of the *ifl1* Stems

Sclerification of interfascicular cells in the *ifl1* mutant is intriguing. It is possible that in Arabidopsis there exists a default pathway to induce sclerification of interfascicular cells. In wild-type plants, differentiation of normal interfascicular fibers might suppress this pathway. The mutant plant appears to trigger this default pathway only at late stages of plant development because interfascicular fibers are not formed at basal stems. Based on the polar auxin flow theory (Aloni, 1987), sclerification of interfascicular cells in the *ifl1* mutant might be induced by the lateral diffusion of auxin and other controlling factors from vascular bundles. This is consistent with the fact that in the mutant, the sclerified cells gradually extended out from xylem bundles (Figure 3). In contrast, multilayers of interfascicular fibers form simultaneously between vascular bundles in the wild type.

ifl1 Stems Are Easily Broken

One striking phenotype of the *ifl1* mutant is its inability to keep stems erect. This is consistent with the function of fibers in providing strength for support. Fibers also provide elasticity that enables organs to maintain their proper shapes and positions. By contrast, lack of fibers in the *ifl1* mutant renders stems plastic. Compared with wild-type stems, the stems of the mutant were much more easily broken by bending or pulling. This is a direct demonstration of the roles of extraxylary fibers in the maintenance of plant form.

Interestingly, interfascicular cells were sclerified at late stages of stem development in the *ifl1* mutant. However, this sclerification did not occur in the basal part of the stem. As a result, a snap point is located in the lower part of the *ifl1* stem instead of in the top part, as observed in flax stems (Gorshkova et al., 1996). As soon as interfascicular cells were sclerified, the *ifl1* stems could not be broken by bending. Thus, the brittle phenotype of the *ifl1* stems is due to the lack of fibers, which seems to be different from the previous identified culm brittleness mutants of grasses (reviewed in Reiter, 1994) or cell wall mutants of Arabidopsis (Reiter et al., 1993; Turner and Somerville, 1997). Culm brittleness in the grass mutants was considered to be caused by the change of secondary wall structure and/or composition. In the case of the barley mutants, it was demonstrated that the decrease of cellulose content resulted in the brittleness phenotype (Kokubo et al., 1989, 1991). In Arabidopsis, two cell wall mutants with de-

creased stem strength were caused by deficiency either in cellulose deposition in secondary wall (Turner and Somerville, 1997) or in fucose synthesis (Reiter et al., 1993). These mutants indicate that the alteration of stem strength can result from a variety of changes of cell types or wall properties.

The *ifl1* Mutant Has Pleiotropic Phenotypes

In addition to the lack of normal interfascicular fibers, the *ifl1* mutant exhibits some other noticeable phenotypes, including long stems and reduced numbers of lateral branches. It is known that stem elongation and apical dominance are controlled by hormones. For example, deficiency in either GA or brassinosteroid results in a dwarf phenotype with no stem elongation (Finkelstein and Zeevaart, 1994; Clouse, 1996). Constitutive perception of the GA signal or exogenous spraying of GA stimulates stem elongation (Finkelstein and Zeevaart, 1994). However, the *ifl1* phenotype is distinct from that of known GA mutants because the long-stem phenotype of GA-responsive mutants, such as *spindly*, is due mainly to the increase of internode length (Jacobsen and Olszewski, 1993), which is not the case for *ifl1*.

The continuous growth of apical meristem and reduced numbers of lateral branches in *ifl1* resemble apical dominance, a process controlled by both auxin and cytokinin (Estelle and Klee, 1994). However, removal of apex did not induce lateral branch formation, indicating that the *ifl1* phenotype may be somewhat different from apical dominance. Although we do not know whether the auxin level is altered in the *ifl1* mutant, it seems unlikely that the *ifl1* phenotype results from overproduction of auxin. The *ifl1* mutant does not exhibit any phenotypes similar to those observed in auxin overproduction mutants, such as extreme proliferation of roots and inhibition of stem growth (Boerjan et al., 1995; King et al., 1995).

Pleiotropic phenotypes resulting from single gene mutations have been observed in a number of Arabidopsis mutants. For example, the *lop1* mutant of Arabidopsis displayed various abnormal growth patterns, such as narrow and deformed leaves, male-sterile flowers, and sporadic occurrence of bladeless regions along the midrib (Carland and McHale, 1996). In the cases of the Arabidopsis *deetiolated 2* (*det2*) and *constitutive photomorphogenesis and dwarfism* (*cpd*) mutants, which are defective in the synthesis of brassinolide, pleiotropic phenotypes were shown throughout plant development (Chory et al., 1991; Li et al., 1996; Szekeres et al., 1996). It is not yet known whether the pleiotropic effects observed in the *ifl1* mutant have any correlation with fiber differentiation. Because the mutation is monogenically recessive, it is possible that the *IFL1* gene is involved in an early step controlling these multiple processes. All of these processes, including fiber differentiation, are controlled by hormones such as auxin, cytokinin, and GA. Thus, it is possible that the *ifl1* mutation affects a step involved in transduction of these hormone signals. Isolation and characterization of the *IFL1* gene will be essential for understanding the molecular bases of the pleiotropic phenotypes shown in the *ifl1*

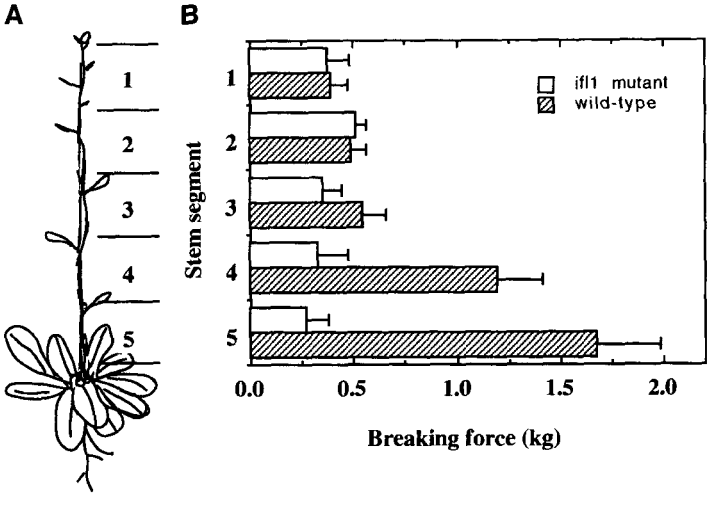

Figure 7. The *ifl1* Mutant Stems Are Easily Broken.

(A) A diagram of an Arabidopsis plant showing the stem divided into five equal segments. Each segment was used to measure the breaking force.

(B) Measurement of breaking force as the function of each stem segment. Stems of 6-week-old plants were used for the experiments. Data are the mean values ±SE of 10 samples.

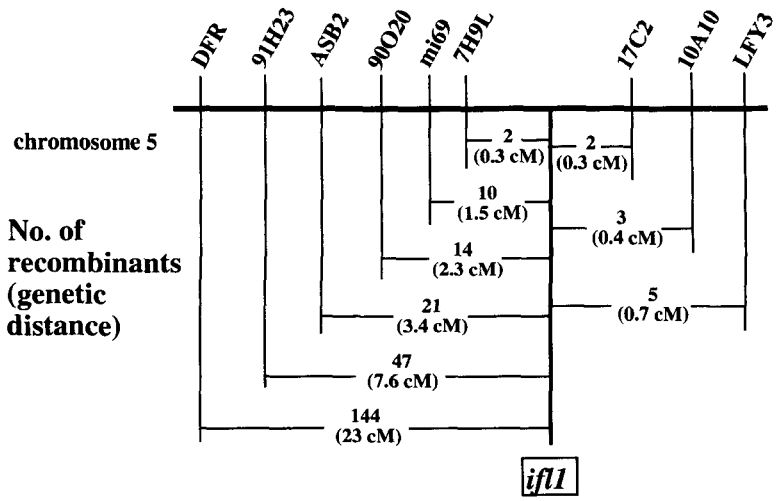

Figure 8. Genetic Mapping of the *ifl1* Locus.

A total of 338 F_2 mapping plants were used for mapping with the markers on the right side of the locus, and a total of 314 F_2 mapping plants were used for mapping with the markers on the left side of the locus. All of the markers used for mapping are CAPS markers except 7H9L, which is a restriction fragment length polymorphism marker. Markers 91H23, 90O20, mi69, and 7H9L were developed during our mapping work.

mutant. Because the *ifl1* locus has been mapped on chromosome 5 at a resolution of 0.3 cM, it is expected that the *IFL1* gene will be cloned soon by using the map-based approach.

METHODS

Mutant Screening

M_2 plants of ethyl methanesulfonate–mutagenized populations of *Arabidopsis thaliana* ecotype Columbia (Lehle Seeds, Round Rock, TX) were grown in the greenhouse. Inflorescence stems from individual plants were free-hand sectioned with a razor blade, and sections were stained for lignin with phloroglucinol–HCl (1% phloroglucinol in 6 N HCl). The sections were observed under a dissection microscope using dark-field illumination. Plants showing altered fiber formation were saved and backcrossed with wild-type Columbia four times to reduce unlinked background mutations.

Histology

Stem segments were fixed overnight in 4% paraformaldehyde at 4°C. After washing in a PBS solution (10 mM phosphate buffer, pH 7.2, 138 mM NaCl, and 3 mM KCl), they were dehydrated through a gradient series of ethanol and xylene and finally embedded in paraffin. Thin sections (15 μm) were prepared from paraffin-embedded tissues by using a microtome and transferred onto poly-L-lysine–coated slides. Sections were then deparaffined in xylene and rehydrated through a gradient series of ethanol. For anatomical observation, sections were stained either with 0.025% toluidine blue or with 1% safranin O (2 min) and 0.5% fast green (30 sec). After staining, sections were dehydrated through a gradient series of ethanol and xylene and mounted in a Polymount medium (Fisher Scientific, Pittsburgh, PA). Sections were observed under a compound microscope using bright-field illumination.

Breaking-Force Measurement

Inflorescence stems were divided into five equal segments, and each segment was used for breaking-force measurement (Reiter et al., 1993). The instrument used for measuring breaking force was a digital force/length tester (model DHT 4-50; Larson Systems Inc., Minneapolis, MN). Two clamps were fixed on both arms. Both ends of the stem segment were clamped, and a force was applied manually until the segment was broken.

Genetic Analysis

The mutant was backcrossed with wild-type Columbia. The resulting F_1 plants were selfed, and the F_2 progeny was used for segregation analysis. For genetic mapping, the mutant was backcrossed with wild-type Landsberg *erecta*. The resulting F_1 plants were selfed, and the F_2 progeny was used for mapping. Leaves from homozygous F_2 mutants were collected and used for genomic DNA isolation (Cocciolone and Cone, 1993). A polymerase chain reaction–based gene mapping procedure using cleaved amplified polymorphic sequences (CAPS) markers was used (Konieczny and Ausubel, 1993). The information on CAPS markers DFR (Konieczny and Ausubel, 1993), ASB2 (Niyogi et al., 1993), 17C2, 10A10, and LFY3 (Konieczny and Ausubel, 1993) was from the Arabidopsis database. The 17C2 and 10A10 markers were developed by J. Bender (Department of Biochemistry, Johns Hopkins University, Baltimore, MD). CAPS markers 91H23, 90O20, and mi69 and restriction fragment length polymorphism marker 7H9L were developed during our mapping work.

The 91H23 and 90O20 primer sequences originated from Arabidopsis expressed sequence tag clones EST91H23 and EST90O20, respectively. The mi69 primer sequences were from the mi69 clone (Liu et al., 1996). The 91H23 primers (5′-GTCGTCAAATGCTGGTGCCA-3′ and 5′-TCGGTACGAAATGAGTGAAGA-3′) amplified a 0.71-kb fragment of the Columbia ecotype and a 0.68-kb fragment of the Landsberg *erecta* ecotype. XhoI digestion of the 91H23-amplified DNA from Columbia produced 400- and 310-bp fragments, with that from Landsberg *erecta* producing 400- and 280-bp fragments. The 90O20 primers (5′-GGATTCGGCTTAGAGAGT-3′ and 5′-TTACAGTGGCAGTGAAAGCGC-3′) amplified a 1.2-kb fragment. The BglII enzyme did not cut the 90O20-amplified DNA from ecotype Columbia but cut that from Landsberg *erecta* into 1- and 0.2-kb fragments. The mi69 primers (5′-AAGGGATTAGTTGCGGTTGA-3′ and 5′-GTTCTCTCATTGTCTCCTCA-3′) amplified a 1.1-kb fragment. RsaI digestion of the mi69-amplified DNA from ecotype Columbia produced 590- and 540-bp fragments, with that from Landsberg *erecta* producing 560- and 540-bp fragments. The 7H9L fragment was amplified from the left end of yeast artificial chromosome clone yUP7H9 with the thermal asymmetric interlaced–polymerase chain reaction procedure (Liu and Whittier, 1995). The 7H9L probe hybridized with 5.5-, 3.1-, 2.1-, and 1.2-kb fragments of EcoRI-digested ecotype Columbia genomic DNA and with 7.0-, 6.2-, 5.0-, and 4.2-kb fragments of EcoRI-digested ecotype Landsberg *erecta* genomic DNA.

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