

Sectors of *liguleless-1* Tissue Interrupt an Inductive Signal during Maize Leaf Development

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The ligule and auricles separate the blade and sheath of normal maize leaves and are absent in *liguleless-1* (*lg1*) mutant leaves. We induced chromosome breakage using X-rays to create plants genetically mosaic for *lg1*. In genetically mosaic leaves, when an *lg1* mutant sector interrupts the normal ligule, the ligule is often displaced basipetally on the marginal side of the sector. Therefore, *lg1* mutant sectors not only fail to induce ligule and auricle, but are also disrupting some form of intercellular communication that is necessary for the normally coordinated development of the ligular region. Our data are consistent with a model in which an inductive signal originates near the midvein, cannot traverse the *lg1* mutant sector, and reinitiates in the wild-type tissue across the sector toward the leaf margin. The *lg1* gene product, therefore, appears to be required for the transmission of this signal and could be involved with reception.

INTRODUCTION

Cellular interactions are important in the development of multicellular organisms. Long-range induction of developmental processes has been studied extensively in plants, especially in regard to hormonal responses (Wareing and Phillips, 1981). For studying short-range interactions, two techniques are generally used. The first is surgical manipulation. Cells or pieces of tissue are removed or grafted to new positions, and if the developmental fate of these cells or their neighbors is altered, then cell-cell interactions are involved. In general, plants are less amenable to such manipulations than animals; however, Siegel and Verbeke (1989) have used grafting to elucidate the presence of a diffusible factor that mediates epidermal redifferentiation in the carpels of *Catharanthus roseus*.

The other major technique for studying cellular interactions is through the use of genetic mosaics. In maize, internal cell layers induce epidermal growth in plants genetically mosaic for *Knotted 1* (Hake and Freeling, 1986; Sinha and Hake, 1990) and in plants genetically mosaic for *D8*, a dominant dwarfing mutation (Harberd and Freeling, 1989). Periclinal chimeras have revealed the influence of subepidermal tissues on a variety of epidermal characteristics. In tomato pedicels, the formation of an abscission zone in the epidermis can be induced by internal cells (Szymkowiak and Sussex, 1989). In contrast, interspecific periclinal chimeras of camellia (Stewart et al., 1972) and tobacco (Marcotrigiano, 1986) show an interaction be-

tween epidermis and mesophyll. Structures such as flowers and leaves have phenotypes intermediate between the donor species or sometimes possess unique phenotypes. In none of these cases has an inducing factor been identified.

Other examples of induction and cell-cell communication in plants include auxin induction of vascular elements as the provascular strands extend into undifferentiated tissues (Sachs, 1975) and pollen recognition involving the glycoprotein products of the *S* loci (Haring et al., 1990).

The ligular region of maize leaves occurs at the junction of the blade and sheath and represents a boundary region between these two developmentally distinct components of the leaf. As shown in Figure 1A, it consists of a fringe of tissue, called the ligule, and two wedges of pale green tissue, called auricles. Development of this region requires precise coordination of pattern-forming events in different tissues and across the breadth of the leaf (Becraft et al., 1990), necessitating cell-cell communication.

The many mutants affecting the ligular region of maize leaves make the development of this region amenable to genetic analysis (Freeling et al., 1988). The *liguleless-1* (*lg1*) gene is of particular interest because recessive *lg1* mutants lack ligules and auricles (Emerson, 1912), as seen in Figure 1B. In addition, the blade-sheath boundary is less distinct in mutant than in wild-type leaves. The wild-type *Lg1* allele is thus involved in ligule and auricle development and in defining the blade-sheath boundary. Genetic mosaic

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analysis has shown that the *Lg1* allele is required in mesophyll tissue for growth of auricles and in epidermis for normal ligule development and differentiation of normal auricle epidermis (Becraft et al., 1990).

While examining genetic mosaics, a unique pattern of ligule formation was often observed: when a ligule was interrupted by a sector of *lg1* segmentally monosomic tissue, the ligule on the wild-type part of the leaf on the marginal side of the sector was displaced in a basal direction. An example is shown in Figure 2. This indicated that the mutant sectors were interfering with the intercellular communication needed to organize the ligular region, and suggested that the *lg1* gene is involved in this communication.

RESULTS

To investigate further the displacement phenomenon exemplified in Figure 2, we created more *lg1* genetic mosaics using chromosome breakage induced by X-rays. The strategy is diagrammed in Figure 3. The recessive *lg1* mutant allele was marked with *albescens* (*al*), a recessive mutation conditioning a chlorophyll deficiency, and *b*, which lacks anthocyanin pigmentation in the plant body. Seedlings heterozygous for all three loci, in coupling linkage, were X-irradiated. Such plants are purple-green pigmented and have normal ligular regions. X-ray-induced breakage and loss of the chromosome arm carrying the dominant, wild-type alleles uncovered the recessive alleles, allowing expression of the recessive phenotypes. Clones derived from such an event appeared as sectors of white, liguleless tissue.

We also conducted control experiments with plants that were heterozygous *al* and homozygous *Lg1* to confirm that hypoploidy for chromosome 2S or hemizyosity for *al* was not causing differential growth of leaf tissue.

Table 1 summarizes the sectors obtained in this experiment. Approximately 40% of the *lg1* sectors that included all tissue layers showed ligule displacement. Ligule displacement was only observed when sectors included all tissue layers of the leaf. In the control experiment, sectors that occupied the internal cell layers or all cell layers were visible. In 185 cases, the ligular region appeared absolutely normal and showed no displacement. The two control sectors that showed ligule displacement appeared phenotypically different from any other sectors observed in the control population. They showed perturbations in the blade and appeared yellow and necrotic, whereas *al* sectors are normally white and healthy. Therefore, it is likely that these sectors resulted from an unrelated event such as segmental nullisomy caused by breakage and loss of the same chromosome arm from both homologs. Mitochondrial aberrations can also cause similar phenotypes (Newton et al., 1990). Abnormal sectors such as these

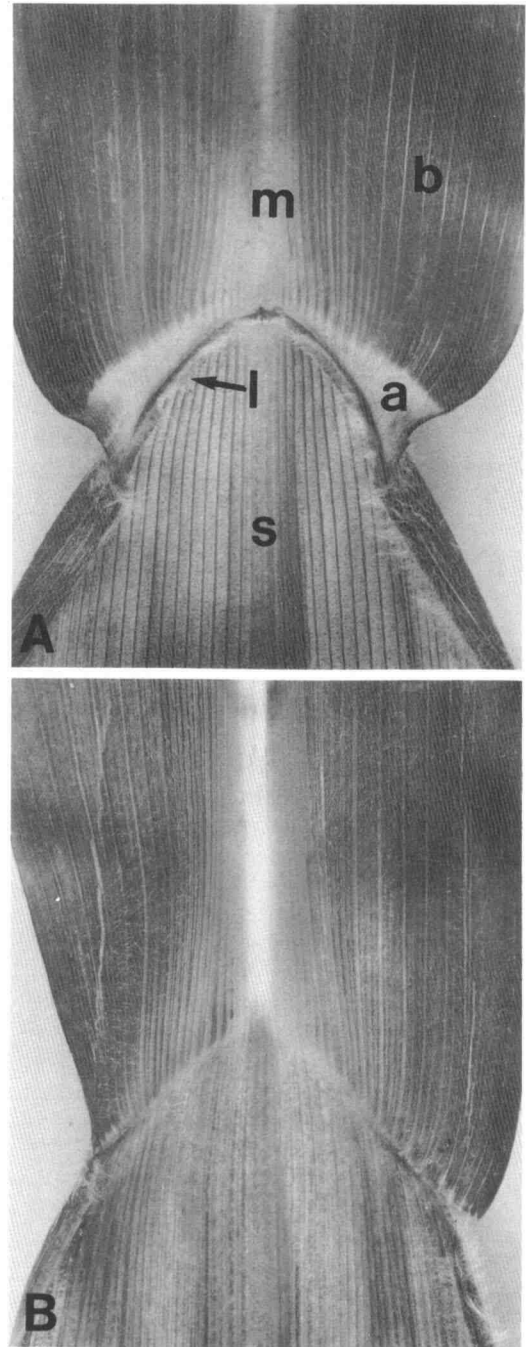


Figure 1. The Ligular Region of Normal and *lg1* Maize Leaves.

(A) A normal leaf. b, blade; s, sheath; a, auricle; l, ligule; m, midrib.
(B) An *lg1* mutant leaf.

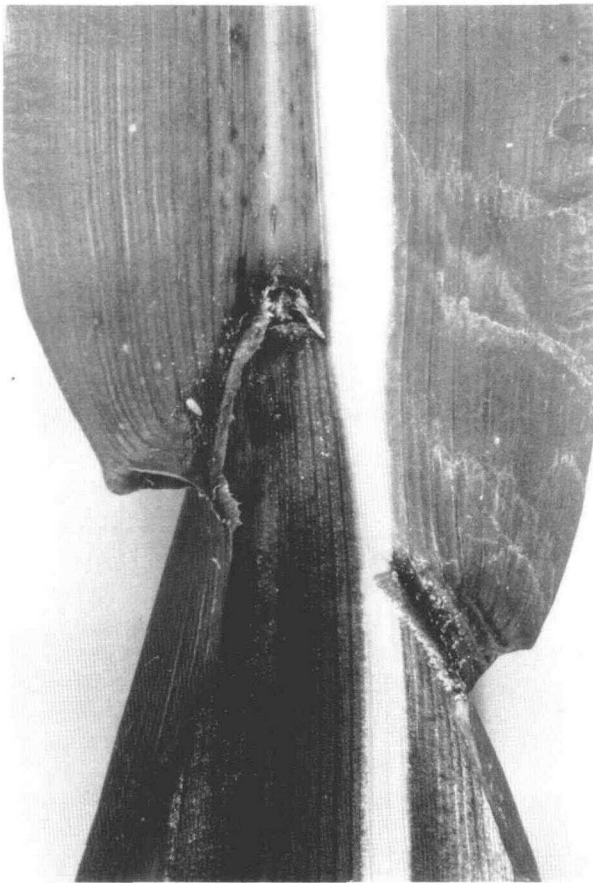


Figure 2. The Ligular Region of a Genetically Mosaic Leaf.

The white, liguleless sector has all the attributes of *lg1* mutant leaves. The ligule formed by the normal tissue on the right, marginal side of the sector is displaced basipetally relative to the normal ligule on the rest of the leaf, suggesting that the *lg1* tissue interrupts a signal that normally coordinates development of the ligular region.

were disregarded in the experimental population. We conclude that ligule displacement is a direct result of losing the *Lg1* allele, and that segmental monosomy for 2S or hemizyosity for *al* does not cause ligule displacement.

Displacement ranged from 0 to 2.3 cm and sector width ranged from less than 1 mm to 1.2 cm. The amount of ligule displacement showed no relationship to any of the other parameters measured. Figure 4 shows examples of a narrow sector with a large displacement and a wider sector with little displacement. Regression analysis showed no statistical correlation between the amount of ligule displacement and either sector width ($r^2 = 0.02$, $P = 0.18$), distance from the midrib ($r^2 = 0.03$, $P = 0.16$), or position on the leaf ($r^2 = 0.005$, $P = 0.59$).

DISCUSSION

In *lg1* genetically mosaic plants, sectors of mutant tissue have all the phenotypic attributes of *lg1* mutant leaves (Becraft et al., 1990). When such mutant sectors pass through the ligular region of an otherwise normal leaf, the ligule and auricles often do not align from one side of the sector to the other; the ligule on the side toward the margin is displaced in the basipetal direction. This clearly indicates that these sectors of mutant tissue are interfering with some form of intercellular communication that normally results in a continuous ligule across the leaf. Furthermore, the blade tissue between the ligule's normal position and its displaced position normally would have developed as

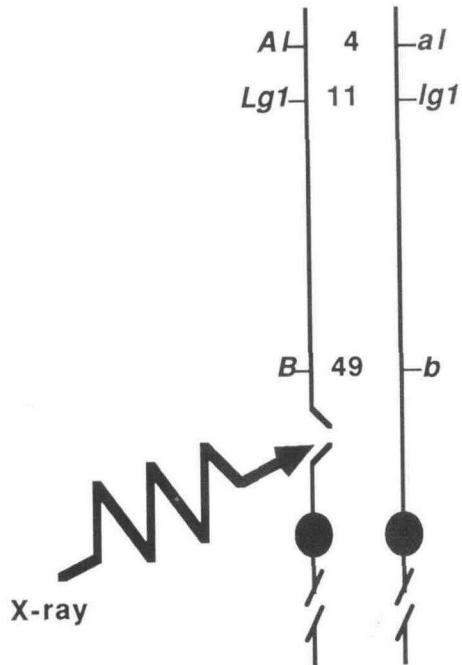


Figure 3. Diagrammatic Representation of the Genetic Constitution of Chromosome 2S in Plants Used for Genetic Mosaic Analysis.

The mutant *lg1* allele was marked with *al*, a recessive mutation causing a chlorophyll deficiency, and *b*, which lacks anthocyanin in leaves. *AI*, *Lg1*, and *B* are the dominant wild-type alleles that confer normal chlorophyll, ligule and auricle development, and anthocyanin production in the leaves and plant, respectively. The numbers refer to the genetic positions of these loci on the chromosome. Seedlings heterozygous for all three loci, in coupling linkage, were X-irradiated. X-ray-induced breakage and loss of the chromosome arm carrying the dominant wild-type alleles of these genes allows expression of the recessive alleles. Clones derived from such an event appear as sectors of white, liguleless tissue, in otherwise purple-green, normal leaves.

Table 1. Summary of *lg1* and Control Sectors

Sector Type ^a	Number Observed	Number Displaced
<i>lg1</i> sectors		
Epidermal	11	0
Subepidermal	3	0
All layers	62	25
Control sectors	187	2 ^b

The *lg1* sectors were obtained by X-irradiating plants that were heterozygous *al lg1 b/Al Lg1 B*. Control sectors were generated by X-irradiating plants of the genotype *al Lg1 b/Al Lg1 b*.

^a The sector types refer to the leaf cell layers that are included in the mutant sector.

^b The two sectors in the control population that showed ligule displacements also had growth aberrations that were not confined to the ligular region of the leaf.

sheath. Therefore, this experimental manipulation has led to the transformation of sheath tissue into blade, suggesting that this communication is involved in specifying cell fate.

Alternative explanations for ligule displacement based on differential growth of the mutant tissue within sectors relative to the normal tissue outside the sectors are unlikely. There is no expectation or evidence of differential growth for several reasons. First, a detailed comparison of growth in wild-type and *lg1* mutant leaves revealed that the patterns of growth were the same, except for the local specialized divisions that give rise to the ligular structures (Sylvester et al., 1990). Second, control sectors demonstrated that segmental monosomy (which may be expected to affect growth) did not cause ligule displacement. Finally, a growth differential would be evident as some form of morphological disturbance in the region of the sector, and this was not observed. Furthermore, it is not clear how differential growth within a sector would result in the displacement of structures found outside the sector. Data from previous experiments support these arguments. Sectors of wild-type tissue were created in dwarf plants mutant for the autonomously acting *D8* mutation (Harberd and Freeling, 1989). These sectors would be expected to overgrow the surrounding dwarf tissue. Surprisingly, they only rarely overgrew the surrounding tissue and when they did, it was clearly evident and never affected the position of structures outside the sectors. An interruption of cellular communication during development is the best explanation of these results, and this is what we based our model on.

Several facts of maize leaf development allowed us to derive a model to explain ligule displacement in these genetic mosaics: (1) Differentiation and maturation occur first at the tip of the leaf and progress basipetally (Sylvester et al., 1990). (2) Ligule initiation begins near the midrib and proceeds toward the margins (Hake et al., 1985; Sylvester

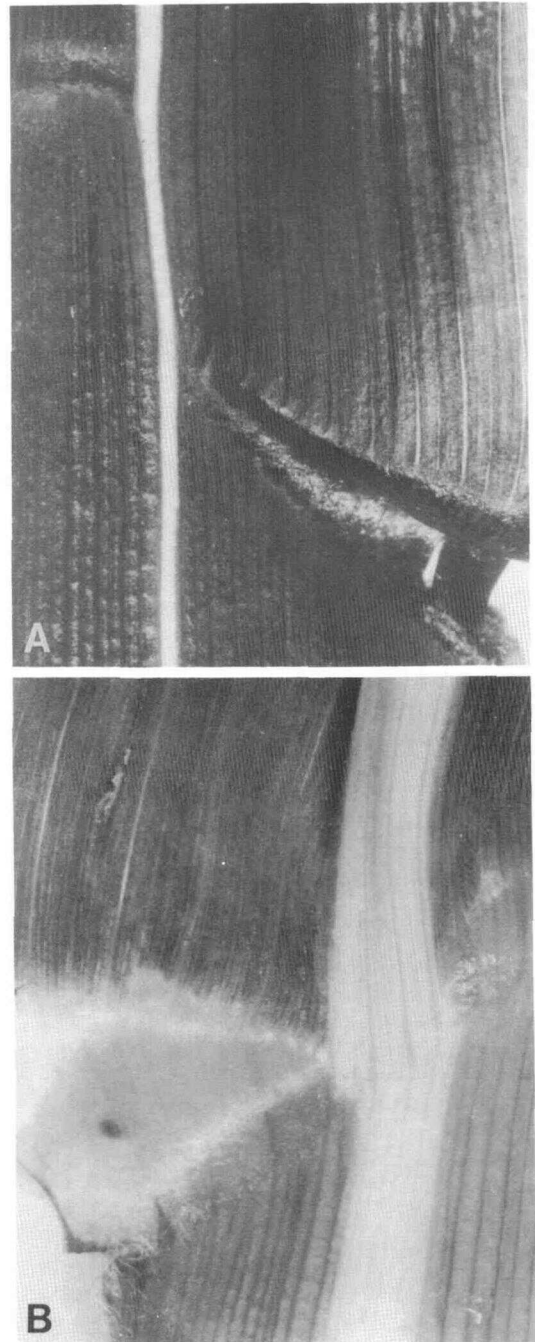


Figure 4. Two Examples of Ligule Displacement Caused by *lg1* Sectors.

(A) A narrow sector that caused a large displacement.

(B) A wider sector that caused little displacement, but where it is clear that the auricle appears to begin anew on the marginal side of the sector.

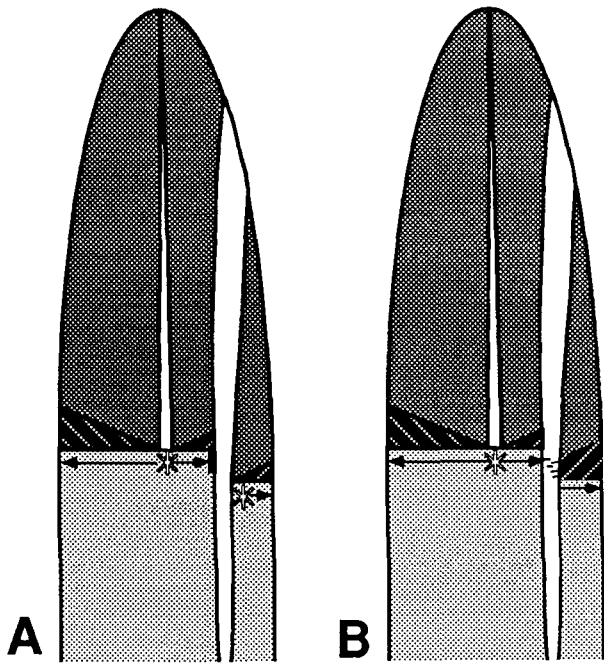


Figure 5. Two Alternative Models for How *lg1* Sectors Can Interrupt a Developmental Signal.

(A) The reinitiation model. The original signal emanates from near the midvein and is blocked by the *lg1* sector. When the tissue on the marginal side of the sector fails to receive the signal, a new, delayed signal is initiated. "*" denotes initiation of the signal, and the arrows indicate signal transmission. The area without stippling represents the *lg1* mutant sector.

(B) The diffusion model. The signal is actively propagated autocatalytically in normal tissue but diffuses across the mutant sector. During the time it takes to diffuse across the sector, the normal cells directly across the sector lose their competence to respond, and only the less mature, more basal cells can respond by forming a ligule and propagating the signal to the margin. This model also depicts an expanded auricle on the marginal side of the sector, which is the expected result, were this model correct.

et al., 1990). (3) Homozygotes for the *lg1* mutation are blocked at the earliest recognizable stage of differentiation of the ligular region, at about plastochron 2 (Becraft et al., 1990; Sylvester et al., 1990). The basic tenets of our model are as follows: A signal that organizes development of the ligular region emanates from near the midrib and is transmitted toward the margin. The signal is probably autocatalytic; cells that receive the signal then produce and transmit the signal to the next cells. Sectors of *lg1* mutant tissue interrupt the transmission of the signal. This results in delayed ligule initiation in the marginal tissue. Because leaf development proceeds basipetally, the delay in ligule initiation results in basipetal displacement on the marginal side of the sector. Accordingly, the *lg1* gene is

most likely involved in reception and/or transmission of this signal.

The way in which *lg1* sectors interrupt this signal can be envisaged in the two ways depicted in Figure 5. The first is that the signal cannot traverse the *lg1* mutant tissue. After some time, when the wild-type tissue marginal to the sector fails to receive the signal, that tissue, by some default mechanism, reinitiates the signal and transmits it to the margin. The second possibility is that the signal can diffuse (or otherwise travel) across the mutant sector but at a slower rate than normal signal transmission. During this time, cells directly across the sector from the ligule lose their competence, and only the less mature, more basal cells can respond by initiating a ligule and propagating the signal.

The experimental data support the reinitiation model, shown in Figure 5A, over the diffusion model in Figure 5B. The expectation was that if the delayed ligule initiation were the result of slow diffusion of the signal across the sector, then wider sectors should show more displacement. On the other hand, if the ligule initiation signal could not traverse the mutant tissue, then sector width would be unrelated to the amount of displacement. There was no correlation between the amount of displacement and either sector width, position on the leaf, or distance from the midrib. A second observation also supports the reinitiation model: auricles on the marginal side of the sector begin from a point, as though they had reinitiated. This is visible on the leaf in Figure 4B. By the diffusion model, the auricle would be expected to be as expanded, or possibly more expanded, than normal. Therefore, it appears most likely that the signal cannot traverse *lg1* mutant tissue, and that the *lg1* gene is involved in the intercellular propagation of this developmental signal.

Ligule displacement was only observed in *lg1* sectors that occupied all leaf cell layers. This suggests that wild-type cells in either epidermal or internal tissues permit proper organization of the ligular region. Because only three subepidermal sectors were obtained and displacement only occurred 40% of the time, we cannot rule out the possibility that the epidermis serves no function in ligular organization. However, in our original experiment (Becraft et al., 1990), we obtained 15 subepidermal sectors, of which none showed displacement (data not presented). This supports the suggestion that wild-type epidermis has the ability to organize the ligular region.

The question arises of why all *lg1* sectors do not cause displacement. The *lg1* phenotype shows variable expressivity, and it has been suggested that the *lg1* mutation may be leaky or that ligule can sometimes form in the absence of *lg1* function (Becraft et al., 1990). One possibility is that this leakiness sometimes allows enough function to permit signal transmission, even though no ligule forms. Another possibility is that the region of the leaf competent to receive the signal may be narrow and may not always allow enough leeway for displacement to occur.

We can definitively rule out the possibility of genetic differences between sectors (e.g., breakpoints of different proximity, interstitial breaks, and so forth) accounting for this variability. We obtained nine sectors that persisted for more than one node on the plant. Within each of these sectors, some leaves showed ligule displacement, whereas others did not. Because each sector represents a somatic clone derived from a single cell (Poethig, 1987), this within-sector variability is not due to genetic variability.

Interestingly, a similar ligule displacement phenomenon is observed in several other mutants. The *Lg4* mutation causes projections of sheath tissue into the blade. Where the sheath tissue interrupts the ligule, basipetal ligule displacement often occurs on the marginal side (J. Fowler, this laboratory, unpublished observations). Leaves on *twisted* mutant plants often form two midribs, with the second midrib causing basipetal ligule displacement where it interrupts the ligule (K. Dawe and M. Freeling, unpublished observations). This suggests that the signal cannot transmit through sheath or midrib tissue and that the signal is initiated at the second midrib independently of the first.

Specification of the ligular region appears to be a complex process involving at least a dozen genetic loci (Freeling, et al., 1988) and cell-cell communication. Because the *Ig1* gene appears to be directly involved in this intercellular communication, further analysis of *Ig1* will provide answers as to the nature of such short-range developmental signals in plants. It will also prove interesting to discover how the other genes act to determine the fate of cells in developing leaves.

METHODS

Generation of Genetic Mosaics

Sectors of *Ig1* tissue were induced in normal leaves as previously described (Becraft et al., 1990). The general strategy is diagrammed in Figure 3. Briefly, germinating seedlings heterozygous for *al*, *Ig1*, and *b*, in coupling linkage, were X-irradiated. When the chromosome arm carrying the dominant alleles of these loci is broken and lost, the recessive alleles are uncovered, allowing expression of the recessive phenotypes. Somatic clones derived from a cell in which such an event occurred appeared as sectors of white, liguleless tissue in otherwise purple-green, normal plants. Control sectors were generated in seedlings that were heterozygous *al* but homozygous for the wild-type *Lg1* allele.

Sector Analysis

Sectors were examined and measured at the ligule. The phenotype conferred by *al* (lack of chlorophyll) was assayed by examining chlorophyll autofluorescence in fresh tissue sections with fluorescence microscopy. The phenotype conferred by *b* (lack of anthocyanin) was examined with bright-field microscopy of fresh sections. Measurements included sector width, distance from the midrib to the sector, and position of the sector as a fraction of the

distance from the midrib to the margin. Ligule displacement was measured as the vertical distance between the positions of the ligules at the inner and outer borders of the sector. Statistical correlation between ligule displacement and the other measured parameters was analyzed with multiple regression using the MREGRESS program in the MSUSTAT statistical package (Richard Lund, Montana State University, Bozeman, MT).

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