

Interactions between *INCURVATA* genes in *Arabidopsis thaliana*

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ABSTRACT One of the most frequently represented phenotypic classes among the leaf morphological mutants available in *Arabidopsis thaliana* is that named *Incurvata* (*lcu*), whose members display curled, involute leaves. Here we describe ethyl methanesulfonate-induced alleles of the *INCURVATA9* (*ICU9*) gene, which cause several leaf abnormalities, such as organ radialization and the presence of abaxial trichomes in juvenile leaves. These morphological aberrations can be interpreted as a consequence of a partial transformation of ventral into dorsal leaf identity, which makes the *ICU9* gene a candidate for being involved in the specification and/or maintenance of leaf dorsoventrality. In order to identify genetic operations at work during leaf morphogenesis, we have obtained double mutants involving alleles of ten *ICU* loci, finding that *ICU9* is functionally related to other five *ICU* genes. All these six genes seem to be essential for the leaf to properly develop.

Plant leaves are laminar and polar structures patterned along the three axes that are established in the earliest stages of leaf organogenesis: the mediolateral, the proximodistal, and the dorsoventral, the last defining a dorsal (adaxial), and a ventral (abaxial) domain (Byrne *et al.*, 2001). Most leaves are characteristically flattened, displaying bilateral symmetry and dorsoventrality, while other plant organs have radial symmetry. A coordination mechanism has to be invoked to account for the matching areas of the adaxial (dorsal) and abaxial (ventral) leaf sides, despite the fact they display dorsoventral asymmetry, being dissimilar in the number, size, spatial arrangement and differentiation state of their cells. Under the above hypothesis, mutants displaying deviations from the planarity that characterizes wild-type leaves can be regarded as being defective in the putative mechanisms that coordinate the growth of the dorsal and ventral tissues of the leaf. For the purpose of testing such a hypothesis, we decided to study *Arabidopsis thaliana* mutants displaying leaf curling, which were considered to probably suffer uncoordinated growth of the adaxial and abaxial sides of the leaf. Since the *incurvata* (*lcu*) mutants display curled leaves, they may be useful for the identification of the genes required for leaf dorsoventrality.

We have isolated several *lcu* lines, most of which carry recessive alleles, exceptions being *lcu4*, *lcu5* and *lcu6*, mutations that are, respectively, semidominant, completely dominant and recessive lethal with effect on the heterozygote. The complementation and linkage analyses of *lcu* mutations (Serrano-Cartagena *et al.*, 2000) indicate that they belong to 14 genes, two of which correspond to already described genes: *CURLY LEAF* (*CLF*; Goodrich *et al.*, 1997) and *HASTY* (*HST*; Telfer and Poethig, 1998). Two

recessive *incurvata* mutations, *lcu9-1* and *lcu9-2*, were induced by ethyl methanesulfonate (Berná *et al.*, 1999) and display a pleiotropic phenotype with complete penetrance and variable expressivity. The juvenile leaves of *lcu9* mutants show abaxial trichomes, a trait that is never found in their wild-type ancestor *Landsberg erecta* (*Ler*). The first two leaves of some *lcu9* individuals are almost completely cylindrical, apparently as a result of partial dorsalization, a transformation of abaxial (ventral) into adaxial (dorsal) cell fates (Fig. 1 A-C). This observation suggests a role for the *ICU9* gene in the specification and/or maintenance of leaf dorsoventrality. Leaf radialization in *lcu9* loss-of-function mutants seems to arise from dorsalization, an observation that does not fit with current models for leaf dorsoventrality, based on the phenotypes of dorsalized gain-of-function or ventralized loss-of-function mutants (Waites and Hudson, 1995; McConnell and Barton, 1997; Waites *et al.*, 1998).

The observation of phenotypic synergism between non allelic mutations, as opposed to additivity, might be used as a criterion for identifying the genetic operations at work in a developmental process (Díaz-Benjumea *et al.*, 1989; Díaz-Benjumea and García-Bellido, 1990; Serrano-Cartagena *et al.*, 2000). A genetic analysis of 13 *lcu* mutants, including *clf*, *hst*, *lcu2*, *lcu4* and *lcu5* mutations, allowed us to define two genetic operations required for a proper leaf development, one involving *CLF* and *ICU2*, and the other involving *HST* and *ICU4*. In order to analyze the genetic interactions between additional *ICU* genes, we have extended this study to double mutants involving alleles of the above mentioned genes, as well as *lcu6*, *lcu7*, *lcu8*, *lcu9* and *lcu15*.

The study of the genetic interactions between *lcu9* and other *lcu* mutations has allowed us to classify the corresponding genes according to the type of interaction found in the double mutants. We found synergistic interactions between *lcu9* mutations and *hst*, *lcu4*, *lcu8* and *lcu15*, suggesting that all of them participate in a single genetic operation. The phenotype of the *lcu4 lcu9* double mutant is interesting, since it shows cylindrical protuberances covered with trichomes arising from the abaxial leaf surface (Fig. 1E). This suggests that *lcu4 lcu9* leaves acquire or retain meristematic activity, which is never observed in the wild type. On the other hand, the *lcu9 hst* and *lcu9 lcu15* double mutants are dwarf, their leaves being reduced to succulent projections of vegetative tissue that arise from abnormally large apical meristems (Fig. 1 D,F, respectively). These leaves show extremely reduced lateral and proximodistal expansion.

The phenotypes of the *lcu8 lcu15*, *lcu4 lcu8* and *lcu15 hst* double mutants are also synergistic since they are extreme and

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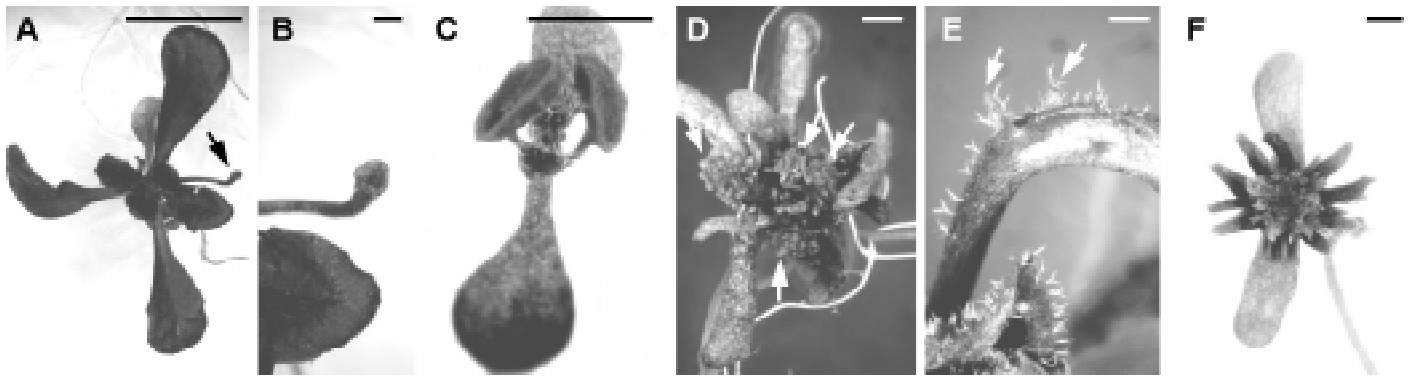


Fig. 1. Some phenotypic traits of *icu9* mutants and double mutants involving *icu9* alleles. (A) *icu9-1* individual displaying a completely radialized first leaf, which is magnified in (B). (C) *icu9-2* plant, whose first pair of leaves are trumpet-shaped, showing a more severe radialization in their basal regions. (D) *icu9 hst* double mutant, showing several extremely large apical meristems. (E) Leaf abaxial protuberances in a leaf of an *icu9 icu4* double mutant. (F) *icu9 icu15* double mutant, displaying an abnormally enlarged meristem. Photographs shown in (A,B,C,D,E and F) were taken 25, 14, 58, 37 and 28 days, respectively, after sowing. Scale bars indicate 5 mm in (A) and (C) and 1 mm in the remaining cases.

share some features with other double mutants involving *icu9* alleles. Taken together, our results suggest that *HST*, *ICU4*, *ICU8*, *ICU9* and *ICU15* act in the same genetic operation to specify and/or maintain leaf dorsoventrality in *Arabidopsis thaliana*.

Materials and Methods

Plants were grown as previously described (Ponce *et al.*, 1998), at 20±1°C and 60-70% relative humidity under continuous fluorescent light (7,000 lx).

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