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Short Report

Genetic architecture of leaf morphogenesis in *Arabidopsis thaliana*

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ABSTRACT In an attempt to identify genes involved in leaf morphogenesis in *Arabidopsis thaliana*, we screened for new mutants showing abnormal leaves and conducted genetic analyses of already obtained mutants. Our large-scale mutant search, which got close to but did not reach saturation of the genome, showed that the lines obtained fell into 94 complementation groups. Many of these genes were mapped using a high-throughput linkage analysis method, based on the simultaneous PCR coamplification of 21 polymorphic microsatellites and the fluorescent semiautomated detection of their products. In addition, in an attempt to ascertain wether intraspecific variability might be a source of information on the genetic controls underlying plant leaf morphogenesis, we analyzed variations in the architecture of vegetative leaves in a large sample of *Arabidopsis thaliana* natural races, concluding that such morphological traits are unlikely to develop under monogenic controls. Hence, a mapping population of the recombinant inbred lines of Lister and Dean was analyzed to identify quantitative trait loci (QTL) harboring naturally occurring alleles that contribute to natural variations in leaf architecture and to eventually correlate their intervals with the map positions of genes identified by mutation.

The primary pathway for carbon and energy uptake by plants is the leaf, an organ of utmost importance in agriculture. Although the biotechnological manipulation of plant leaves offers great potential, little is known about the genetic controls underlying leaf development. Though most plant leaves are simple structures, many developmental processes are involved in leaf ontogeny. They include, among others, the positioning and initiation of leaf primordia at the flanks of the shoot meristem, the specification of leaf identity as opposed to that of other organs which are assumed to be modified leaves, the establishment of dorsal and ventral identities within the organ, the definition of domains such as ligule, sheath and blade in some monocotyledonous plants, as well as petiole and lamina in dicots, the control of cell division and expansion, the formation of patterns such as those of venation, trichomes or stomata, the mechanisms responsible for the diversity of compound and simple leaves and those that specify heteroblastic differences among different leaves within a plant. A large body of detailed information on what actually happens at a morphological level is available for most, if not all, such processes but only a few studies have focused on the nature, action and interactions of the genes driving the sequence of developmental events that contribute to the making of a leaf (recently reviewed in Byrne *et al.*, 2001).

With a view to identifying genes involved in leaf morphogenesis, we followed three different experimental approaches in *Arabidopsis thaliana*. On the one hand, we conducted phenotypic and genetic analyses of 115 leaf mutants isolated by previous authors, establishing that they fall into 47 complementation groups (Serrano-Cartagena *et al.*, 1999; 2000). In addition, we screened for new mutants with abnormally shaped leaves, induced by EMS treatment (Berná *et al.*, 1999; Fig. 1A) or fast neutron bombardment (Robles and Micol, submitted), whose genetic analysis showed that they belong to 94 and 8 genes, respectively. We have determined the map positions of 76 of these genes (Robles and Micol, 2001; Fig. 1B), taking advantage of the availability of a high-throughput mapping procedure previously developed with this purpose in our laboratory, based on the simultaneous PCR coamplification of 21 polymorphic microsatellites and the fluorescent semiautomated detection and sizing of the products (Ponce *et al.*, 1999). The molecular markers used are spaced over the entire genome at intervals of 6.7 to 57.7 cM.

In an attempt to ascertain if intraspecific variability might be a source of information on the genetic controls underlying plant leaf morphogenesis, we have analyzed variations in the architecture of vegetative leaves in a large sample of *Arabidopsis thaliana* natural races. A total of 188 ecotypes from the *Arabidopsis* Information Service collection were grown and classified into 14 phenotypic classes, which were defined according to petiole length, marginal configuration and overall lamina shape. Ecotypes displaying extreme and opposite variations in the above-mentioned leaf architectural features were crossed and their F_2 progeny found not classifiable into discrete phenotypic classes. Furthermore, leaf trait-based classification of the ecotypes being crossed was found not to be correlated with estimations of their genetic distances, calculated after determining variations in repeat number at 22 microsatellite loci. Since these results suggested that intraspecific variability in leaf morphology arises in *Arabidopsis thaliana* from mutations at quantitative trait loci (QTL), we then studied a mapping population of the recombinant inbred lines of Lister and Dean. A total of 100 lines were grown and the third and seventh leaves of 10 individuals from each line collected when fully expanded, and morphometrically analyzed. QTL harboring naturally occurring alleles that contribute to natural variations in leaf architecture will be identified and their intervals correlated with the map positions of the genes identified by mutation.

Although until recently map-based cloning has been considered time-consuming and expensive in *Arabidopsis thaliana*, techniques developed in recent years and the information provided by the

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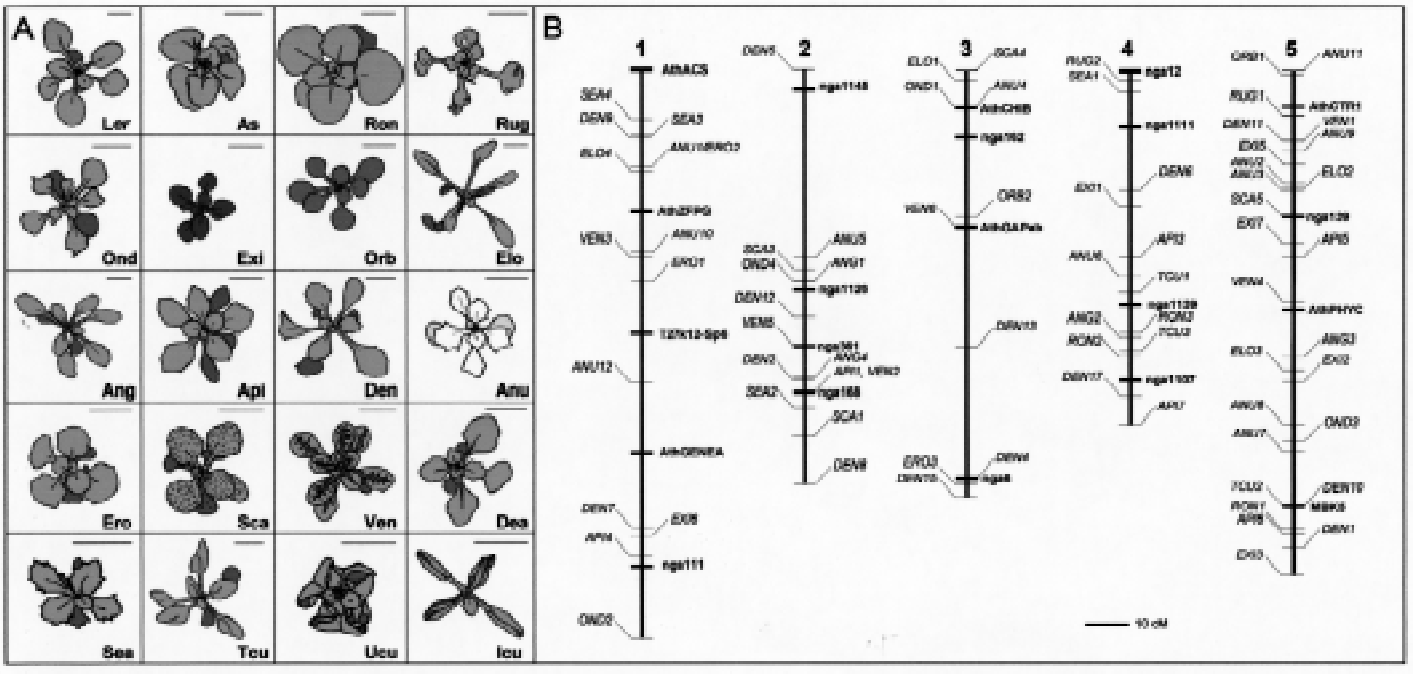

Fig. 1. Phenotypes and map positions of mutations studied in this work. *(A) Diagram showing representative individuals of the phenotypic classes defined for the complementation analysis of EMS-induced mutants with abnormal leaves mapped in this work. Drawings were made from pictures taken 21 days after sowing. Scale bars indicate 5 mm. Abbreviations:* Ler *(Landsberg* erecta*), As (Asymmetric leaves; rounded lamina, with some degree of bilateral asymmetry and margins slightly revolute), Ron (Rotunda; broad and rounded lamina), Rug (Rugosa; wrinkled lamina), Ond (Ondulata; undulated lamina), Exi (Exigua; small and dark leaves), Orb (Orbiculata; small, rounded and yellowish leaves), Elo (Elongata; narrow and elongated lamina and long petiole), Ang (Angusta; narrow lamina), Api (Apiculata; pointed lamina, with slightly incised margins), Den (Denticulata; pointed lamina, with dentate margins), Anu (Angulata; yellowish leaves with dentate margins), Ero (Erosa; rounded lamina, with dentate margins), Sca (Scabra; rounded and protruded lamina), Ven (Venosa; conspicuous venation. Some lines displaying incise margins), Dea (Dentata; serrated margins), Sea (Serrata; small leaves with strongly serrated margins), Tcu (Transcurvata; margin obliquely revolute), Ucu (Ultracurvata; lamina spirally rolled downwards) and Icu (Incurvata; involute margins).* **(B)** *Genetic map of* Arabidopsis thaliana*, showing positions of genes mapped in this work. The microsatellite markers used appear in boldface.*

Arabidopsis Genome Initiative now make it possible to complete positional projects in a short period of time (Lukowitz *et al.*, 2000). The mapping data, as well as the F_2 mapping populations obtained in this work, lay the foundations for positional attempts to clone the mutated genes, which will be identified by narrowing down the genetic intervals presented here. In fact, efforts to clone some of the genes mapped in this work are in progress in collaboration with other groups. Moreover, mendelization of the QTL intervals found, which will end with the molecular definition of QTL alleles, will provide additional information on the genes that contribute to the making of plant leaves.

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). Linkage analysis was performed as described in Ponce *et al.*, 1999.

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References

BERNA, G., ROBLES, P. and MICOL, J.L. (1999). A mutational analysis of leaf morphogenesis in *Arabidopsis thaliana. Genetics* 152: 729-742.

BYRNE, M., TIMMERMANS, M., KIDNER, C. and MARTIENSSEN, R. (2001). Development of leaf shape. *Curr. Opin. Plant Biol.* 4: 38-43.

LUKOWITZ, W., GILLMOR, C.S. and SCHEIBLE, W.-R. (2000). Positional cloning in *Arabidopsis*. Why it feels good to have a genome initiative working for you. *Plant Physiol.* 123: 795-805.

PONCE, M.R., QUESADA, V. and MICOL, J.L. (1998). Rapid discrimination of sequences flanking and within T-DNA insertions in the *Arabidopsis* genome. *Plant J.* 14: 497-501.

PONCE, M.R., ROBLES, P. and MICOL, J.L. (1999). High-throughput genetic mapping in *Arabidopsis thaliana. Mol. Gen. Genet.* 261: 408-415.

ROBLES, P. and MICOL, J.L. (2001). Genome-wide linkage analysis of *Arabidopsis* genes required for leaf development. *Mol. Gen. Genomics*, in press.

SERRANO-CARTAGENA, J., ROBLES, P., PONCE, M.R. and MICOL, J.L. (1999). Genetic analysis of leaf form mutants from the *Arabidopsis* Information Service collection. *Mol. Gen. Genet.* 261: 725-739.

SERRANO-CARTAGENA, J., CANDELA, H., ROBLES, P., PONCE, M.R., PEREZ-PEREZ, J.M., PIQUERAS, P. and MICOL, J.L. (2000). Genetic analysis of *incurvata* mutants reveals three independent genetic operations at work in *Arabidopsis* leaf morphogenesis. *Genetics* 156: 1363-1377.