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Short Report

Mutations in the *ULTRACURVATA2* gene of *Arabidopsis thaliana*, which encodes a FKBP-like protein, cause dwarfism, leaf epinasty and helical rotation of several organs

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ABSTRACT Contrary to wild-type *Arabidopsis thaliana* vegetative leaves, which are flattened organs, those of *ultracurvata* (*ucu*) mutants are spirally rolled downwards and show reduced expansion along the proximodistal axis. We have identified six *ucu* lines, whose genetic analysis indicates that they fall into two complementation groups, *UCU1* and *UCU2*. Here we describe three recessive *ucu2* alleles, whose homozygotes are dwarf with a compact rosette, and display some organs that are helically rotated along the apical-basal axis, a trait that is more pronounced in roots, pistils and mature siliques. Following a map-based strategy, we have identified the *UCU2* gene, which was found to encode a peptidyl-prolyl cis-trans isomerase of the FKBP (FK506-binding protein) family of proteins.

With the purpose of contributing to the dissection of the mechanisms underlying plant leaf ontogeny, we have followed three complementary genetic approaches in the model system *Arabidopsis thaliana*: the study of natural variations in leaf architecture among wild-type races (Candela *et al.*, 1999; J. M. Pérez-Pérez, J. Serrano-Cartagena and J. L. Micol, submitted), the analysis of leaf mutants obtained by other authors (Serrano-Cartagena *et al.*, 1999; 2000), and the isolation of new leaf mutants (Berná *et al.*, 1999; P. Robles and J. L. Micol, submitted). One of the most extreme leaf phenotypes that we found is that of the *ultracurvata* mutants (Figure 1B). Unlike wild-type *Arabidopsis thaliana* vegetative leaves, which are flattened organs (Figure 1A), those of *ultracurvata* (*ucu*) mutants are spirally rolled downwards and show a reduced expansion along the longitudinal axis. Genetic analysis indicates that they fall into two complementation groups, *UCU1* and *UCU2*.

We have identified three recessive alleles of the *ULTRACURVATA2* (*UCU2*) gene, two of which were isolated in our laboratory: *ucu2-1* and *ucu2-3*, respectively induced by fast neutron bombardment (Robles and Micol, unpublished) and T-DNA insertional mutagenesis (this work). Another line, CS3397, was obtained from the ABRC and later found to be a double mutant carrying the *ucu2-2* and *gi-2* (*gigantea*) mutations, both putatively induced by X rays. Homozygous *ucu2* individuals are dwarf and poorly fertile, with a distorted and short inflorescence. In some of their terminal flowers, stamens show carpelloid tissues and a few sepals carry aborted ovules. Roots, hypocotyl, stems and carpels are helically rotated along their apical-basal axis in the three homozygous *ucu2* lines, a trait that is more pronounced in roots, pistils and mature siliques (Figure 1D).

In an attempt to clone the *UCU2* gene, we first studied the segregation of the kanamycin resistance marker associated with the T-DNA in the T-DNA induced *ucu2-3* allele, which was found to be untagged. Then, using a mapping population of 660 F_2 individuals from a cross between *ucu2-1* (in a L*er* genetic background) and Columbia-0, the *UCU2* gene was mapped on chromosome 3, 8.8±1.8 cM away from the nga162 SSLP marker, and 2.26±0.91 cM from the AtDMC1 CAPS marker. We ruled out allelism between *UCU2* and either *AXR2* (*AUXIN RESISTANT2*; Wilson *et al.*, 1990) or *DIM1* (*DIMINUTO*; Takahashi *et al.*, 1995), genes already known to map to the above mentioned region, after crossing *ucu2-1* plants to *axr2-1* and *dim1-1* individuals. New SSLP markers were obtained, allowing us to isolate 23 recombinants that limited the candidate region to an interval encompassed by four BACs. Sequencing of several candidate genes contained within this region in the *ucu2-3* mutant revealed a 40 bp deletion in a putative gene that codes for a peptidyl-prolyl cis-trans isomerase with significant sequence similarity with the immunophilin familiy of FKBPs (FK506-binding proteins), whose animal homologues are involved in protein folding and steroid receptor activation (Galat, 2000). This *FKBP-like* gene is composed of eight exons and is transcribed to a 1360 nt mRNA that codes for a 41 kDa protein of 356 amino acids. The deletion carried by the *ucu2-3* allele causes a frameshift that eliminates the entire COOH-terminal domain of the protein. Molecular analyses of *ucu2-1* and *ucu2-2* are in progress.

Because of the similarity between the phenotypes of *ucu2/ucu2* and *ucu1/ucu1* plants (see Table 1), we obtained double mutants involving the recessive *ucu2* allele and either the semidominant *ucu1-1* or the recessive *ucu1-3* alleles. The *ucu1-1/ucu1-1*;*ucu2-1/ucu2-1* (Figure 1C) and *ucu1-3/ucu1-3*;*ucu2-1/ucu2-1* double mutants were sterile and phenotypically indistinguishable,

TABLE 1

BODY PARAMETERS OF *UCU* MUTANTS

Genotype	Root length	Fresh weight	Dry weight	Lamina length	Lamina width	Petiole length
L*er*	64±9	24.4±4.0	1.9±0.4	5.85±0.79	5.30±0.70	3.23±0.62
ucu1-1/ucu1-1	29±13	10.7±3.9	1.0±0.5	2.94±0.27	4.91±0.57	0.87±0.17
ucu1-3/ucu1-3	41±11	15.2±3.5	1.4±0.5	5.04±0.63	4.89±0.33	2.23±0.36
ucu2-1/ucu2-1	32±10	9.4±3.4	0.8±0.3	2.74±0.36	4.57±0.54	1.56±0.35

Values are means of at least 15 measurements. Lengths are indicated in mm, weights in mg. Root length was determined 11 days after sowing in seedlings grown on vertically oriented agar plates. Weight was determined 21 days after sowing. Lamina and petiole parameters refer to first leaves collected 21 days after sowing.

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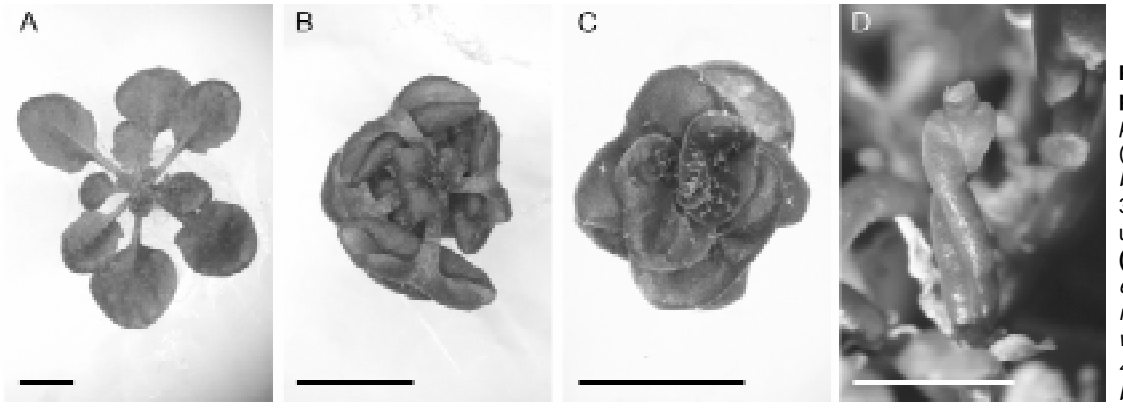

Fig. 1. The Ucu2 mutant phenotype. *(A-C) Rosette phenotypes of* ultracurvata2 (ucu2) *mutants.* **(A)** *The Columbia-0 (Col-0) ecotype,* **(B)** ucu2-3/ucu2-3, **(C)** ucu2-1/ucu2-1; ucu1-1/ucu1-1 *double mutant.* **(D)** *Detail of a* ucu2-1/ucu2-1 *elongated pistil displaying helical rotation of the carpels. Pictures were taken 21 days (A-C) and 45 days (D) after sowing. Scale bars: A-C, 4 mm; D, 2 mm.*

ressembling brassinosteroid-deficient mutants. The phenotypes of the double mutants involving alleles of the *UCU2* and *UCU1* genes can be interpreted as merely additive, as would be expected if their gene products act in an independent manner. The *UCU1* gene encodes an intracellular kinase closely related to SHAGGY, one of the components of the Wingless/Wnt animal signalling pathway. As regards to *UCU2*, although several genes for members of the FKBP family of immunophilins have been found in the genome of *Arabidopsis thaliana*, mutations have been described only at one of them, *PASTICCINO1 (PAS1)*, encoding a FK506-binding protein. Given that *pas1* mutant alleles affect both embryonic and vegetative development, it has been proposed that *PAS1* is involved in the control of cell division (Vittorioso *et al.*, 1998). Future studies should provide insight into the functions of *UCU* genes and their role in leaf morphogenesis and overall plant growth.

Materials and Methods

Arabidopsis thaliana (L.) Heyhn. Landsberg *erecta* and Columbia-0 wild-type strains were supplied by the Nottingham *Arabidopsis* Stock Centre (ABRC). The mutants CS3397, *DIM1/dim1-1* (CS8100) and *axr2-1/axr2-1* (CS3077) were supplied by the *Arabidopsis* Biological Resource Centre. 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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