Mutations in the ULTRACURVATA2 gene of Arabidopsis thaliana, which encodes a FKBP-like protein, cause dwarfsim, 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 peptidylyl-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 (Bernà 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were isolated in our laboratory: ucu2-1 and ucu2-3, respectively induced by fast neutron bombardment (Robles and Micol, unpublished) and T-DNA insertional mutagenesis (this work).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 carpeloid tissues and a few sepals carry aborted ovules. Roots, hypocotyl, stems and carpels are helically rotated along their apical-basal axis in the three homzygous 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 F2 individuals from a cross between ucu2-1 (in a Ler 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 peptidylyl-prolyl cis-trans isomerase with significant sequence similarity with the immunophilin family of FKBP receptors (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/ucu2-3 double mutants were sterile and phenotypically indistinguishable.

**TABLE 1**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Root length</th>
<th>Fresh weight</th>
<th>Dry weight</th>
<th>Lamina length</th>
<th>Lamina width</th>
<th>Petiole length</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ler</td>
<td>64±9</td>
<td>24.4±0.4</td>
<td>19.0±0.4</td>
<td>5.8±0.7</td>
<td>3.5±0.7</td>
<td>1.2±0.7</td>
</tr>
<tr>
<td>ucu1-1/ucu1-1</td>
<td>29±13</td>
<td>10.7±3.9</td>
<td>1.0±0.5</td>
<td>5.9±0.27</td>
<td>4.9±1.0</td>
<td>0.7±0.17</td>
</tr>
<tr>
<td>ucu3-1/ucu3-3</td>
<td>41±11</td>
<td>15.2±3.5</td>
<td>1.4±0.5</td>
<td>4.0±0.65</td>
<td>5.8±1.0</td>
<td>0.9±0.33</td>
</tr>
<tr>
<td>ucu2-1/ucu2-1</td>
<td>32±10</td>
<td>9.4±3.4</td>
<td>8.0±1.3</td>
<td>2.7±0.36</td>
<td>4.5±0.54</td>
<td>1.5±0.35</td>
</tr>
</tbody>
</table>

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.
resembling 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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References