ULTRACURVATA1, a SHAGGY-like Arabidopsis gene required for cell elongation

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ABSTRACT

To better understand the genetic mechanisms underlying plant leaf development, we have performed a large-scale screening for Arabidopsis thaliana mutants to identify those displaying abnormally shaped or sized leaves. One of the stronger mutant phenotypes found was that of the ultracurvata (ucu) mutants, whose vegetative and cauline leaves are spirally rolled downwards and show a reduced expansion along the proximodistal axis. We have identified one recessive and two semidominant ucu alleles, the most extreme of which cause severe dwarfism and a constitutive photomorphogenic response. Following a map-based strategy, we have cloned the UCU1 gene, which was found to encode an intracellular kinase closely related to SHAGGY, one of the components of the Wingless/Wnt animal signalling pathway. The responses of ucu mutants to exogenous plant hormones and the genetic analyses of double mutants involving ucu alleles indicate that UCU1 is a key component in several signalling pathways controlling cell expansion and overall plant growth, including those of auxins and brassinosteroids.

Although the leaf is the main photosynthetic plant organ, the question of how plant leaves develop is far from being answered at the genetic level (recently reviewed in Byrne et al., 2001). In order to better understand leaf ontogeny, we performed a large-scale screen for EMS induced mutants displaying abnormally shaped leaves in the model plant Arabidopsis thaliana (Berná et al., 1999). One of the most extreme leaf phenotypes that we found is that of the ultracurvata (ucu) mutants, whose vegetative and cauline leaves are spirally rolled downwards. Here we present the genetic and molecular analysis of three alleles of the ULTRACURVATA1 (UCU1) gene, the strongest of which cause brassinosteroid insensitivity and dwarfism, due to a severe reduction in cell expansion along the proximodistal axis.

Homozygous ucu individuals and the hybrid F1 progeny of their intercrosses and crosses to the wild type (UCU1/UCU1) can be ordered in a descending series of mutant phenotypic strength as follows, the phenotypic effects of ucu-1 and ucu-2 being indistinguishable: ucu1-1/ucu1-1 = ucu1-1/ucu1-2 > ucu1-1/ucu1-3 > ucu1-1/UCU1 > ucu1-3/UCU1 > ucu1-3/ucu1-3 > ucu1-3/UCU1 = UCU1/UCU1.

These results indicate that the mutant alleles have an additive effect and this can be explained assuming that the semidominant ucu1-1 and ucu1-2 alleles are antimorphic and the recessive ucu1-3, hypomorphic. An alternative explanation would be that the UCU1 gene is haploinsufficient, the semidominant alleles being null or extremely hypomorphic. Tetraploid plants with a Col-1 (Columbia-1) genetic background were crossed to either ucu1-1/ucu1-1 or ucu1-2/ucu1-2 mutants with a Ler (Landsberg erecta) background, and the phenotype of the F1 triploid individuals was shown to be wild type.

The Ucu1 mutant phenotype is pleiotropic, ucu1-1/ucu1-1 and ucu1-2/ucu1-2 individuals being dwarf with hypocotyls, leaf petioles, short roots, compact dark-green rosettes and reduced inflorescence length with low fertility, resembling brassinosteroid-deficient mutants (Fig. 1 B,C). Vegetative and cauline leaves of ucu1 mutants are spirally rolled downwards and show a reduced expansion along the proximodistal axis, although they are similar in width to those of the wild type. A reduction in length is suffered by both the lamina and the petiole in ucu1-1/ucu1-1 and ucu1-2/ucu1-2 individuals, but mostly by the lamina in UCU1/ucu1-1 and UCU1/ucu1-2. Only the apical portion of fully expanded leaves is curled in ucu1-3/ucu1-3 plants, whose petioles are apparently normal.

Cell morphology was studied in ucu1/ucu1 individuals, focusing on those organs displaying a reduction in length along the proximodistal axis: the root, hypocotyl, petioles and siliques. No significant differences were found in cell number compared with the wild type, whereas cell length was remarkably diminished. Thus, the organ length reduction displayed by the ucu1 mutants is due to a reduction in cell length, and not correlated with variations in cell number.

In order to characterize some physiological responses of the ucu1 mutants, their growth in the presence of different plant hormones was tested. The mutant phenotype was not rescued by an exogenous hormone in all cases. Sensitivity of the ucu1 mutants to cytokinin (6-benzylaminopurine, BA), gibberellin (GA3), the auxine IAA and abscisic acid (ABA) was similar to that shown by the wild type, but abnormal responses were observed in the presence of the auxin 2,4-D and BR (24-epibrassinolide). Severe root growth inhibition and undifferentiate growth were observed in the mutants when grown at low concentrations of 2,4-D, which does not affect wild-type roots. These root elongation assays indicate that ucu1 mutants are hypersensitive to 2,4-D. In addition, primary root elongation assays revealed that ucu1-1/ucu1-1 and ucu1-2/ucu1-2 plants are extremely insensitive to 24-epibrassinolide and ucu1-3/ucu1-3 plants partially insensitive. Constitutive photomorphogenic response was displayed by ucu1 mutants when grown in the dark, with ucu1-1/ucu1-1 and ucu1-2/ucu1-2 homozygous individuals displaying an extreme de-etiolated phenotype, developing true leaves when grown for 21 days in the dark.

Double mutant combinations were obtained in order to detect interactions between ucu1 alleles and mutant alleles of genes of...
different hormonal signal transduction pathways. Homozygous ucu1-1 plants were crossed to homozygous axr2-1 (auxin resistant2; Wilson et al., 1990) individuals, which have altered perception of auxins, and det2-1 (de-etiolated2; Li et al., 1996), dim1-1 (diminuto; Takahashi et al., 1995) or bri1-1 (brassinosteroid insensitive1; Clouse et al., 1996) mutants, which are defective for brassinosteroid biosynthesis or perception. Phenotypes were shown to be additive in all the double mutants obtained, the only exceptions being combinations involving ucu1 and axr2-1. Whereas UCU1/ucu1-1;AXR2/axr2-1 double heterozygotes are sterile, UCU1/ucu1-3;AXR2/axr2-1 individuals, carrying the weak ucu1-3 allele, are viable and display a synergistic phenotype. The latter was an unexpected result, given that axr2-1 is a completely recessive allele of the AXR2 gene, and ucu1-3 behaves as a completely recessive allele of the UCU1 gene. Both the lethality of UCU1/ucu1-1;AXR2/axr2-1 individuals and the phenotype of UCU1/ucu1-3;AXR2/axr2-1 double heterozygotes clearly indicate a functional relationship between the UCU1 and AXR2 genes.

We mapped the UCU1 gene to the chromosome 4 of Arabidopsis thaliana, near the cleaved amplified polymorphic sequence (CAPS) marker AG. New simple sequence length polymorphisms (SSLP) markers were used as markers to limit the length of the candidate region to 15 kb. Whole sequencing of this region in the three mutant alleles allowed us to obtain single nucleotide polymorphisms (SNP) that rendered new single nucleotide polymorphisms that allowed us to locate the UCU1 gene within a 15 kb interval.

Materials and Methods

Arabidopsis thaliana (L.) Heyhn. Landsberg erecta and Columbia-0 wild-type strains were supplied by the Nottingham Arabidopsis Stock Centre. The tetraploid line CS3151 and the mutants DIM1/ dim1-1 (CS8100), det2-1/det2-1 (CS6159) 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