Int. J. Dev. Biol. 45 (S1): S51-S52 (2001)

Short Report

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

JOSÉ M. PÉREZ-PÉREZ, MARÍA R. PONCE and JOSÉ L. MICOL*

División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, Alicante, Spain

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 *ultracurvata1* (*ucu1*) mutants, whose vegetative and cauline leaves are spirally rolled downwards and show a reduced expansion along the longitudinal axis, in contrast to wild type leaves, which are flattened organs. We have identified one recessive and two semidominant *ucu1* 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 *ucu1* mutants to exogenous plant hormones and the genetic analyses of double mutants involving *ucu1* 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 *ucu1* individuals and the hybrid F_1 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 *ucu1-1* and *ucu1-2* being indistinguishable: *ucu1-1/ucu1-1* = *ucu1-1/ucu1-2* > *ucu1-1/ucu1-3* > *ucu1-1/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 L*er* (Landsberg *erecta*) background, and the phenotype of the F_1 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, ressembling 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), giberellin (GA_3), 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* aleles and mutant alleles of genes of

***Addresscorrespondence to:** J. L. Micol. División de Genética and Instituto de Bioingeniería, Universidad Miguel Hernández, Campus de Elche, 03202 Elche, Alicante, Spain. e-mail: jlmicol@umh.es

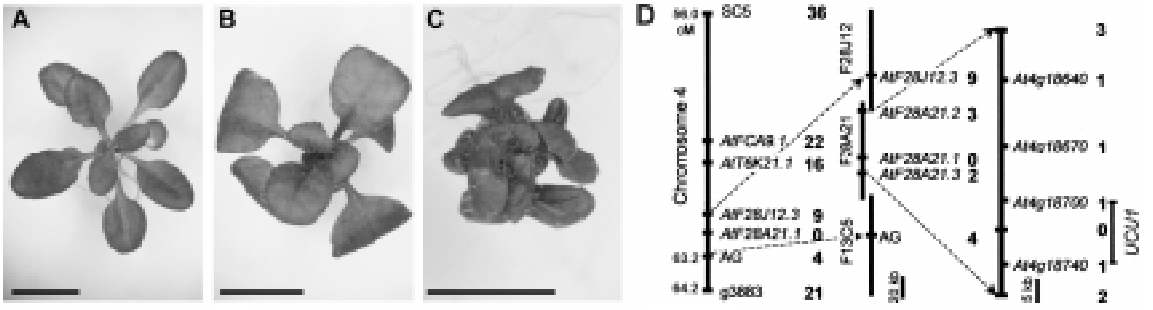

Fig. 1. Mutant phenotypes and positional cloning of the *UCU1* gene. *(A-C) Leaf phenotype of* ucu1 *mutants. Rosettes are shown from* **(A)** *Landsberg* erecta (*L*er), **(B)** ucu1-3/ucu1-3, *and* **(C)** ucu1-2/ucu1-2 *individuals. Photographs were taken 21 days after sowing. Scale bars, 4 mm.* **(D)** *Positional cloning of the* UCU1 *gene. Analysis of 1,620 chromosomes gave 57 recombinants (shown in black) relative to 9 polymorphic markers. Names of the SSLP markers designed and used for the first time in this work are shown in italics. Sequencing of some genes within the candidate region rendered new single nucleotide polymorphisms that allowed us to locate the* UCU1 *gene within a 15 kb interval.*

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 dominant 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 obtained within this region and were used to screen for recombinants (Fig. 1D). These markers localized *UCU1* to an interval of ~80 kb within the F28A21 bacterial artificial chromosome (BAC). Sequencing of some of the genes contained within this BAC allowed us to obtain single nucleotide polymorphisms (SNP) that 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 identify two different mutations in the coding sequence of an already described gene: *SHAGGY-like kinase etha* (Dornelas *et al*., 1998).

SHAGGY/GSK3-like protein kinases act as key components in metazoan pattern formation, their activity being required in the Wingless/Wnt pathway for the correct establishment of dorsoventral axes in vertebrates, anterior-posterior segment polarity in *Drosophila*, and differential cell fates in *Caenorhabditis* and *Dictyostelium*, among others (Kim and Kimmel, 2000). In these systems, SHAGGY-mediated phosphorilation of downstream elements transduces the signal to the nucleus. The responses of *ucu1* mutants to exogenous plant hormones and the genetic analyses of double mutants involving *ucu1* alleles indicate that *UCU1* is a key component in at least two signalling pathways controlling cell expansion and overall plant growth, including those of auxins and brassinosteroids. Further analyses of the *ucu1* mutants will shed light on the role of *UCU1* in the transduction of plant hormone signals as well as on plant morphogenesis.

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).

Acknowledgements

This research was supported by grants from the Ministerio de Educación y Cultura of Spain (PB95-0685 and PB98-1389). J. M. Pérez-Pérez was fellow of the Conselleria de Cultura, Educació i Ciència of the Generalitat Valenciana.

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.

CLOUSE, S.D., LANGFORD, M. and McMORRIS, T.C. (1996). A brassinosteroid-insensitive mutant in *Arabidopsis thaliana* exhibits multiple defects in growth and development. *Plant Physiol.* 111: 671-678.

DORNELAS, M.C., LEJEUNE, B., DRON, M. and KREIS, M. (1998). The *Arabidopsis* SHAGGY-related protein kinase (ASK) gene family: structure, organization and evolution. *Gene* 212: 249-257.

KIM, L. and KIMMEL, A.R. (2000). GSK3, a master switch regulating cell fate specification and tumorigenesis. *Curr. Opin. Genet. Dev.* 10: 508-510.

LI, J., NAGPAL, P., VITART, V., McMORRIS, T.C. and CHORY, J. (1996). A role for brassinosteroids in light-dependent development of *Arabidopsis. Science* 272: 398-401.

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.

TAKAHASHI, T., GASCH, A., NISHIZAWA, N. and CHUA, N.H. (1995). The *DIMINUTO* gene of *Arabidopsis* is involved in regulating cell elongation. *Genes Dev.* 9: 97-107.

WILSON, A.K., PICKETT, F.B., TURNER, J.C. and ESTELLE, M. (1990). A dominant mutation in *Arabidopsis* confers resistance to auxin, ethylene and abscisic acid. *Mol. Gen. Genet.* 222: 377-383.