RESEARCH PAPER

CUP-SHAPED COTYLEDON1 (CUC1) and CUC2 regulate cytokinin homeostasis to determine ovule number in Arabidopsis

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Abstract

Seeds derive from ovules upon fertilization and therefore the total number of ovules determines the final seed yield, a fundamental trait in crop plants. Among the factors that co-ordinate the process of ovule formation, the transcription factors CUP-SHAPED COTYLEDON 1 (CUC1) and CUC2 and the hormone cytokinin (CK) have a particularly prominent role. Indeed, the absence of both CUC1 and CUC2 causes a severe reduction in ovule number, a phenotype that can be rescued by CK treatment. In this study, we combined CK quantification with an integrative genome-wide target identification approach to select Arabidopsis genes regulated by CUCs that are also involved in CK metabolism. We focused our attention on the functional characterization of UDP-GLUCOSYL TRANSFERASE 85A3 (UGT85A3) and UGT73C1, which are up-regulated in the absence of CUC1 and CUC2 and encode enzymes able to catalyse CK inactivation by O-glucosylation. Our results demonstrate a role for these UGTs as a link between CUCs and CK homeostasis, and highlight the importance of CUCs and CKs in the determination of seed yield.

Keywords: Arabidopsis, cytokinin, hormones, ovule development, pistil development, transcriptional regulation.

Introduction

Ovule primordia emerge as lateral organs from meristematic placental tissue following a series of periclinal cell divisions (Schneitz et al., 1995). The placenta, in turn, is derived from the carpel margin meristem (CMM), whose formation is known to be controlled by the interaction of genetic and hormonal networks (reviewed in Reyes-Olalde et al., 2013). In recent decades, the fundamental role of hormones such as cytokinins (CKs), auxins, and brassinosteroids in determination of ovule number has been studied, and several transcription factors modulating the hormonal responses have been identified (reviewed in Cucinotta et al., 2014). The transcription factors CUP SHAPED COTYLEDON1 (CUC1) and CUC2 have been found to be involved in the establishment of boundaries between organs. Indeed, the characteristic phenotype of aux1aux2 double-mutants is the presence of fused cotyledons (Aida et al., 1997). During ovule development, they form part of a network that regulates ovule primordia initiation (Galbiati et al., 2013). CUC1 and CUC2 are broadly expressed in the placental tissue but, after primordia formation, their expression is limited to the boundaries between ovules (Nahar et al., 2012; Galbiati et al., 2013). Pistils in which CUC2 is mutated and CUC1 is knocked down with...
an RNAi construct under the control of the ovule/placenta-specific SEEDSTICK promoter (uc2 pSTK::RNAi-CUC1) have a reduced number of ovules relative to the wild-type (Galbiati et al., 2013). In uc2 pSTK::RNAi-CUC1 the pistil size is not affected but the space between adjacent primordia increases, leading to a reduced ovule density.

In addition, CUCs promote expression of the auxin efflux carrier PIN1, which is necessary for auxin distribution in the placenta (Galbiati et al., 2013). PIN1 expression in the pistil is also activated by CKs through CYTOKININ RESPONSE FACTOR 2 (CRF2) and CRF6 (Bencivenga et al., 2012; Cucinotta et al., 2016), and CK treatment rescues the uc2 pSTK::RNAi-CUC1 ovule-number phenotype by restoring PIN1 expression (Galbiati et al., 2013), suggesting that CUCs could regulate CK abundance. Several studies have clearly demonstrated that CKs are positive regulators of ovule number (Barrtrina et al., 2011; Bencivenga et al., 2012; Galbiati et al., 2013). Mutants impaired in CK signalling and response have a drastically reduced number of ovules (Riefler et al., 2006; Kinoshita-Tsujimura and Kakimoto, 2011; Bencivenga et al., 2012; Cucinotta et al., 2016). In contrast, plants with higher CK content form more ovules. For instance, the total seed yield of a double-mutant for two CK degradation enzymes, the cytokinin oxidase/dehydrogenases CKX3 (encoded by AT5G56780) and CKX5 (encoded by AT1G75450), is twice the of the wild-type (Barrtrina et al., 2011). Here, we report that mutation of CUC1 and CUC2 alters CK homeostasis in Arabidopsis. We quantified the concentration of CKs in the double-mutant and performed an RNA-seq experiment to identify deregulated genes involved in CK homeostasis that have an effect on ovule number determination. In particular, we focused our attention on the role of two members of the UDP-GLYCOSYL TRANSFERASE family (UGT85A3 and UGT73C1) that catalyse the reversible inactivation of zeatin-type CKs by O-glucosylation (Hou et al., 2004), showing that both factors are involved in ovule number determination.

Materials and methods

Plant material and growth conditions

Arabidopsis thaliana ecotype Columbia-0 (Col-0, wild-type) and transgenic lines were grown in soil at 22 °C under long-day conditions (16:8 h light/dark). uc2 pSTK::RNAi-CUC1 has been described previously (Galbiati et al., 2013). The mutant line SAIL_241_G09 renamed here as ugt85a3-1 carries a T-DNA insertion in the first exon of the UGT85A3 gene (AT1G22380); SALK_070258, renamed here as ugt85a3-2, carries a T-DNA insertion in the second exon of UGT85A3 that generates rearrangement of the gene and of the T-DNA itself (Supplementary Fig. S2 at JXB online). Mutant ugt73c1 corresponds to SALK_077212C and carries an insertion at the beginning of the coding sequence of UGT73C1 (AT2G36750), which is an intron-less gene (Supplementary Fig. S2). Seeds of ugt85a3-1, ugt85a3-2, and ugt73c1 were obtained from NASC (http://arabidopsis.info/) and a list of primers used for genotyping is available in Supplementary Table S5. To construct 35S::UGT85A3 and 35S::UGT73C1, a genomic fragment was amplified with the primers AtP_4903/AtP_4904 and AtP_4907/AtP_4908 and cloned into the Gateway® destination vector pB2GW7. All constructs were verified by sequencing and used to transform wild-type plants using the floral dip method (Clough and Bent, 1998).

Protoplast transfection

Protoplast preparation and transient expression experiments were performed as described by Galbiati et al. (2013).

RNA extraction, cDNA library preparation, and sequencing for RNA-seq

Total RNA was extracted from three biological replicates (0.5 g) from both wild-type and uc2 pSTK::RNAi-CUC1 mutant pre-fertilization pistils, using a NucleoSpin RNA Plant kit (Macherey-Nagel) according to the manufacturer’s instructions. RNA integrity was analysed by gel electrophoresis and spectrophotometrically before submission to the sequencing facility. Sequencing libraries were prepared with a TrueSeq RNA Sample Prep kit (Illumina Inc.) and subsequently sequenced on an Illumina HiSeq2000 (50 bp single-read) by iGATEch (http://www. igatechnology.com/).

Mapping of short reads, quality analysis, and assessment of gene expression were performed as described by Mizzotti et al. (2014). Evaluation and treatment of raw data was performed using CLC Genomics Workbench v.4.7.1 (https://www.qiagenbioinformatics.com/products). After filtering, high-quality reads were mapped onto the Arabidopsis genome (TAIR10). Approximately 20 million reads of each sample that mapped with ≤2 mismatches were used for further analyses. The fold-change and differential expression values between the wild-type and the uc2 pSTK::RNAi-CUC1 mutant were calculated in terms of the reads per kilobase of million mapped reads (RPKM) of the corresponding transcripts. To obtain statistical confirmation of the differences in gene expression, P-values were computed. We applied a threshold value of P=0.05 to ensure that differential gene expression was maintained at a significant level for the individual statistical tests. Transcripts that exhibited an estimated Absolute Fold-Change ≥1.5 (i.e. ≥2 mapped reads per kilobase of mRNA) were considered to be significantly differentially expressed.

Expression analysis

Total RNA was extracted from pistils or inflorescences at pre-fertilization stages using a NucleoSpin RNA Plant kit (Macherey-Nagel) and equal amounts of each sample were reverse-transcribed using the GoScript™ Reverse Transcription System (Promega). The cDNAs were standardized relative to ACTIN2-8 (ACT2-8) and UBIQUITIN10 (UBI10) transcripts, and gene expression analysis was performed using a Bio-Rad iQ5 Multicolor real-time PCR detection system with GeneSpin SYBR Green PCR Master Mix. RT-PCR primers are listed in Supplementary Table S5.

Cytokinin quantification

Quantification of CK metabolites was performed as described by Nudimov et al. (2012), including the modifications of Antoniadis et al. (2015). Fresh plant tissue (20 mg) was homogenized and extracted in 1 ml of 80% methanol together with a cocktail of stable isotope-labelled internal standards (0.25 pmol of CK bases, ribosides, N-glucosides, and 0.5 pmol of CK O-glucosides, nucleotides added per sample). The extracts were purified using an Oasis MCX column (30 mg 1 ml⁻¹; Waters, Milford, MA, USA) conditioned with 1 ml each of 100% methanol, and 2 ml of 0.35M NH₄OH in 60% (v/v) MeOH solution. The eluates were quantified using diagnostic MRM transitions of precursor and product ions and the effluent was introduced into the electrospray ion source of a triple quadrupole mass spectrometer Xevo™ TQ-S (Waters). Analytes were quantified using diagnostic MRM transitions of precursor and

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appropriate product ions using stable isotope-labelled internal standards for reference (Novák et al., 2008). Four independent biological replicates were performed.

Results

CUC1 and CUC2 induce a CK response in vivo

CUCs promote expression of the auxin efflux carrier PIN1, which is necessary for auxin distribution in the placenta, and when both CUC1 and CUC2 are silenced in the placenta the number of ovules formed is significantly reduced (Galbiati et al., 2013). CK treatment (benzyl adenine, BAP) restores ovule number in aw2 pSTK::RNAi-CUC1 plants by re-establishing the correct level of PIN1 expression (Galbiati et al., 2013; Cucinotta et al., 2016). BAP treatment does not have any effect if PIN1 is mutated (Bencivenga et al., 2012; Cucinotta et al., 2016), as is the case in pin1-5 plants. One possible explanation is that CKs act downstream of CUC1 and CUC2 to induce PIN1 expression.

To elucidate whether CUC1 and CUC2 are able to induce a CK response in vivo, we performed transient expression assays in BY-2 tobacco (Nicotiana benthamiana) protoplasts. We used the ‘two-component system (TCS) signalling sensor’ promoter fused to the luciferase (LUC) gene as a CK-response reporter (Müller and Sheen, 2008). The TCS is a synthetic promoter that reflects the transcriptional activity of type-B response regulators, the final targets of the CK signalling cascade. Protoplasts were transformed either with a 3SSs::CUC1/2 construct or a 3SSs::GUS construct as a negative control. Protoplasts overexpressing CUC1 or CUC2 showed a significant increase of TCS::LUC expression compared to controls (Fig. 1). We used the induction of pPIN1::LUC by both CUC1 and CUC2 as a positive control and pPIN7::LUC as negative control (Galbiati et al., 2013). The results of the protoplast assay suggested that CUC1 and CUC2 were positive regulators of CK response.

CUC1 and CUC2 influence CK homeostasis

The increased expression of the TCS::LUC reporter in protoplasts overexpressing CUC1 and CUC2 could be explained by the action of the two transcription factors on metabolism of, signalling by, or the response to CK. To investigate whether CUC1 and CUC2 modulated CK homeostasis, we quantified CK levels with LC-MS. Pre-fertilization inflorescences of aw2 pSTK::RNAi-CUC1 plants were compared to those of the wild-type. aw2 pSTK::RNAi-CUC1 plants showed a significant reduction in total active CK bases, particularly cis-zeatin (tZ) and isopentenyladenine (iP) relative to the wild-type (Fig. 2A).

In addition, in the aw2 pSTK::RNAi-CUC1 background a consistent increase in the level of three types of O-glucosylated CK ribosides (trans-zeatin ROG, cis-zeatin ROG, and dihydrozeatin ROG) was detected (Fig. 2B). O-glucosylation is a reversible reaction that occurs at the hydroxyl group on the side-chains of zeatin or dihydrozeatin. CK O-glucosides (tZROG, cZROG, and DHZROG) are inactive in bioassays and play a role in maintaining CK homeostasis (Mok and Mok, 2001; Spichal et al., 2004; Smehilová et al., 2016). Moreover, in aw2 pSTK::RNAi-CUC1 plants the content of inactive N-glucosylated trans-zeatin (tZ7G and tZ9G) was also higher than wild-type levels, together with a significant increase in total CK N-glucosides (Supplementary Table S1). N-glucosylation of cytokinin occurs on the nitrogen at positions N7 or N9 of the purine ring and, unlike O-glucosylation, is thought to be irreversible. The level of the other CK metabolites did not change between the wild-type and mutant (Supplementary Table S1). Taken together, the results of CK quantification indicated that in aw2 pSTK::RNAi-CUC1 plants there was an increase in the amount of inactive CK glucosides at the expense of active CK bases, suggesting that CUCs might be involved in the regulation of CK homeostasis.

Identification of CUC1/2 downstream targets related to CK metabolism

To gain insight on how CUCs regulate CK homeostasis at the genetic level, we performed a transcriptomic analysis by RNA-seq. The transcriptome of wild-type pistils was compared to those of the wild-type aw2 pSTK::RNAi-CUC1 plants in order to identify genes downstream of CUC1 and CUC2 linked to CK metabolic pathways during pistil and ovule development.

In order to enrich the dataset in genes involved in ovule development, unfertilized pistils from developmental stages 8 to 12 (according to Roeder and Yanofsky, 2006) were dissected and utilized for RNA extraction.

Bioinformatic analysis revealed varying levels of silencing of CUC1 in the biological replicates, probably due to the fact that we used RNAi, which typically leads to variable levels of silencing that tend to decrease over time. Nonetheless, the analysis (see Methods for details) identified 416 differentially expressed genes (DEGs) in the aw2 pSTK::RNAi-CUC1

Fig. 1. CUC overexpression enhances cytokinin response. Luciferase activity in tobacco BY-2 protoplasts transiently transformed with pPIN1::LUC, pPIN7::LUC, or pTCS::LUC expressing p35s::GUS, p35s::CUC1, or p35s::CUC2. TCS activity, as well as PIN1, is enhanced by CUC1 and CUC2 overexpression compared to the p35s::GUS control, while PIN7 expression remains at basal levels. The fold-change is normalized to the activity of luciferase (LUC) in p35s::GUS protoplasts. Data are means ±SE, n=8 separate transfection events and measurements. Significant differences between mock and effector constructs (35s::CUC1, 35s::CUC2) were determined using Student’s t-test (P<0.01).
mutant with a fold-change higher than 1.5 and a P-value of 0.05 or less. Of these, 251 genes were significantly up-regulated (Supplementary Table S2), whereas 165 were found to be significantly down-regulated (Supplementary Table S3).

There are few known direct targets of CUCs during pistil and ovule development that could be used to validate the dataset. However, LATERAL SUPPRESSOR (LAS) is known to be a direct target of CUCs in the initiation of axillary meristems (Raman et al., 2008), and this was indeed down-regulated almost 5-fold (~4.723) in our dataset, supporting the validity of the data.

To gain more insight into the data and the potential functions of genes downstream of CUC1 and CUC2, a functional categorization of the gene ontology (GO) terms (Ashburner et al., 2000; The Gene Ontology Consortium, 2017) associated with the DEGs was performed using version 1.8 of the AmiGO tool (Carbon et al., 2009) and the PlantGoSlim vocabulary (Supplementary Table S4). The categorization of the ‘biological process’ terms is shown in Fig. 3. Generic terms such as ‘biological process’ (GO:0008150) and ‘cellular process’ (GO:0009987) have been removed from the graph to highlight other more relevant terms (the complete list of terms is shown in Supplementary Table 4). The categories observed fitted with the tissues sampled and with previously described roles of CUC1 and CUC2 in these processes. For example, categories such as ‘reproduction’ (GO:0000003) and ‘flower development’ (GO:0009908) were abundantly represented. Categories related to hormonal responses such as ‘response to endogenous stimulus’ (GO:0009719) and ‘signal transduction’ (GO:0007165) were also represented, but terms directly related to the response to specific hormones were absent.

After removing generic terms, ‘nucleobase-containing compound metabolic process’ (GO:0006139) was the most abundant. This term is defined as any cellular metabolic process...
involving nucleobases, nucleosides, nucleotides, and nucleic acids. This includes metabolic precursors or derivatives of cytokinins, in agreement with the possible role of CUCs in control of CK homeostasis as revealed by CK quantification.

Overall, the categorization of the terms associated with the DEGs fitted with the role of CUCs and the tissue sampled, and revealed alterations in genes related with CK metabolism. None of these terms were found to be statistically over-represented in an enrichment analysis performed with the PANTHER 13.0 software against the whole Arabidopsis genome (Mi et al., 2017). This was probably because we could only identify a moderate number of DEGs due to the intrinsic variability of the samples lowering the statistical power of the analysis. Nonetheless, these data provide insight and directions for future studies.

Combining the information from the RNA-seq and CK quantification, we searched for DEGs that might be related to CK homeostasis pathways for further study. From the list of up-regulated genes, we selected UDP-GLUCOSYL TRANSFERASE 85A3 (UGT85A3) because it is the closest homolog to UGT85A1 that has been biochemically characterized as a CK-specific UGT that is able to catalyse O-glucosylation of CKs (Hou et al., 2004). Another protein with similar biochemical properties is UGT73C1. UGT73C1 was also up-regulated in our dataset (fold-change +2.904), although initially it was excluded from the list of DEGs due to a P-value higher than 0.05 (0.064) (Supplementary Table S2). Given the sample variability and the possible functional relevance of UGT73C1, we re-evaluated its expression by qRT-PCR. The expression analysis of UGT85A3 and UGT73C1 in the mutant and in the wild-type indeed confirmed the up-regulation of both genes in the cu2 pSTK::RNAi-CUC1 plants in three different biological replicates (Supplementary Fig. S1).

In Arabidopsis, the UGT85A family consists of six members, of which A1, A2, A3, A5, and A7 are clustered near each other on Chromosome 1 (Woo et al., 2007). The UGT73C family is comprised of seven genes, six of which are clustered in tandem on Chromosome 2 (UGT73C1-6) (Husar et al., 2011). The O-glucosyltransferase activity of UGT73C1 and UGT85A1, a close homolog of UGT85A3, have been proven in vitro, and they are able to recognize iZ, iZ, and DHZ, and to form O-glucosides (Hou et al., 2004). The high protein–sequence similarity between UGT85A1 and A3 suggests that they evolved by gene duplication and may therefore have similar enzymatic properties (Li et al., 2001). To investigate their role in the determination of ovule number, we analysed two independent ugt85a3 mutant alleles, the ugt73c1 mutant (Supplementary Fig. S2) and plants in which UGT85A3 and UGT73C1 were overexpressed with the CaMV35S promoter. Phenotypic analysis showed a moderate but significant increase in ovule and seed number in both the ugt85a3-1 and ugt85a3-2 independent ugt85a3 mutant lines (Fig. 4A, B). In particular, ugt85a3-1 has an increase of 12.5% and 13% in ovules and seeds, respectively, compared to the wild-type, while ugt85a3-2 developed 7% more ovules and 10% more seeds than the wild-type. No significant differences were detected in pistil and silique lengths in the ugt85a3 mutants relative to the wild-type (Fig. 4C, D). These results suggested that UGT85A3 may have a role in determining the density of ovules formed inside the pistil, as already demonstrated for CUC1 and CUC2 (Galbiati et al., 2013). The production of more ovules was not accompanied by a greater length of the pistil and did not affect fertility in the ugt85a3 mutants, which produced a full seed-set and no ovule or seed abortions (Fig. 4D, E). On the contrary, when UGT85A3 was overexpressed, plant fertility was impaired. Indeed, plants of four independent lines, with high levels of UGT85A3 expression (Supplementary Fig. S2), showed strong CK deficiency-related pleiotropic phenotypes, such as smaller stature than the wild-type and significant reductions in ovule number and pistil length (Fig. 4A, C). Moreover, in 35S::UGT85A3 the silique length and the seed number were even more reduced due to a large number of unfertilized ovules (Fig. 4B, D–F).

In parallel, the same phenotypic analysis was performed on the UGT73C1 mutant and overexpression lines. Plants of ugt73c1-1 did not differ from the wild-type (Fig. 5A–D). This might have been due to redundancy of the UGT73C family. Overexpression of UGT73C1 caused a significant reduction in the number of ovules and seeds (Fig. 5A, B) and this variation was linked only to reductions in the length of pistils and siliques (Fig. 5C, D) since in 35S::UGT73C1 fertility was not compromised and seed-set was complete (Fig. 5E, F). In contrast, in 35S::UGT85A3 the reduction in silique length was linked both to a reduction in pistil length and to a high number of unfertilized ovules. These data suggested that UGT73C1 might be involved in processes related to pistil and fruit elongation rather than in the formation of ovules.

Discussion

In this study we quantified CK and performed a transcriptomic analysis to investigate genes downstream of CUCs related to CK metabolic pathways. Analysis of the levels of different CK forms revealed that in cu2 pSTK::RNAi-CUC1 inflorescences the levels of active forms (iZ and iP) were reduced compared to the wild-type, while those of inactive O-glucosylated and N-glucosylated forms increased. In parallel, by comparing transcription profiles of wild-type and cu2 pSTK::RNAi-CUC1 pistils by RNA-seq, we obtained a list of genes that were deregulated due to the absence of CUC1 and/or CUC2, that might therefore be direct or indirect targets of CUC1/2. We selected genes that had a link to CK homeostasis and tested their possible involvement in ovule number determination. The candidates considered were UGT73C1 and UGT85A3, which code for enzymes that inactivate CKs by O-glucosylation (Hou et al., 2004) and which were up-regulated in cu2 pSTK::RNAi-CUC1. Absence of UGT85A3 positively influenced ovule number, as revealed by phenotypic analysis of two ugt85a3 mutant lines. Indeed, ugt85a3 plants produced more seeds without compromising their viability. This effect could be explained if ugt85a3 mutant plants have a slight increase in active CK bases and thus have higher activity of the meristematic placenta tissue. Indeed, CKs are well known to act by maintaining cell proliferation in meristematic
tissues, in particular through action on D-type cyclins (Riou-Khamlichi et al., 1999; Dewitte et al., 2007). It can be assumed that, in the placenta, CUCs repress the expression of CK-specific UGTs in order to increase the level of active CKs and decrease the glyco-conjugated inactive forms. When overexpressed, UGT85A3 causes severe growth and fertility problems, as reported for mutants strongly impaired in CK response, such as the cre ahk2 ahk3 CK receptor triple-mutant (Higuchi et al., 2004; Nishimura et al., 2004).

The second candidate, UGT73C1, seemed to play a prevalent role in pistil and fruit elongation rather than in ovule formation. Indeed, high levels of UGT73C1 resulted in shorter pistils bearing fewer ovules, but did not compromise their fertility. It should also be noted that the two enzymes, UGT73C1 and UGT85A3, exercise their O-glucosylation function on different types of zeatin, which might affect different developmental processes. iZ and iP are the most abundant CK forms. iZ-types and iP-types accumulate in xylem and phloem, respectively, and generally exhibit higher activities than cZ (Sakakibara, 2006). According to our results, iZ and iP were also the most abundant active CKs in pre-fecundation inflorescences. CK O-glucosides have been shown to be reversibly deglycosylated by the action of β-glucosidase and therefore are thought to serve as inactive storage forms of CK (Šmehilová et al., 2016). Interestingly, in the list of down-regulated genes (Supplementary Table S3) we found BETA GLUCOSIDASE 41 (BGLU41, fold-change –3.992), which codes for an enzyme that may be involved in the de-glucosylation of CK O-glucosides. In accordance with the hypothesis proposed above, it would be interesting to investigate whether CUCs may favor the expression of BGLU41 to increase the ‘reactivation’ of CKs.

In addition, our analysis revealed that N-glucosides were the most abundant form of cytokinin in the inflorescence and that their levels increased even more in the absence of CUC1/2 (Fig. 2). Unlike O-glucosides, N-glucosides are usually resistant to β-glucosidases, making them the terminal products of irreversible deactivation. This suggests that CUCs regulate both reversible and irreversible CK inactivation. However, the putative CUC target(s) responsible for N-glucosylation were not identified in our RNA-seq data and remain to be discovered.

RNA-seq data do not allow discrimination between direct and indirect targets of transcription factors. CUCs have been described as positive regulators of transcription (Raman et al., 2008; Galbiati et al., 2013) and UGT85A3 and UGT73C1 were up-regulated in our dataset. Based on that, it is likely that UGT73C1 and UGT85A3 are indirect targets of CUCs. However, the role of a transcription factor as an activator or repressor is context-dependent, and therefore further experiments are necessary to rule out this possibility.
Judicious selection of CRISPR-Cas9 gRNAs could allow disruption of multiple UGT family members and unravel specific and redundant gene functions. Taken together, our results point to the role of both UGT genes as a link between CUCs and CK homeostasis, and highlight the importance of CUCs and CKs in seed yield determination. Furthermore, UGTs are good candidates for improved seed yield in agronomically important plants.

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Supplementary data
Supplementary data are available at JXB online.

Fig. S1. qRT-PCR validation of RNA-seq data for the two candidate CUC target genes UGT85A3 and UGT73C1.

Fig. S2. Position of the T-DNA insertions in UGT85A3 and UGT73C1 mutants, and expression of UGT85A3 and UGT73C1 in mutant and overexpression lines.

Table S1. Total cytokinin metabolite levels in wild-type and cuc2 pSTK::RNAi-CUC1 inflorescences.

Table S2. List of genes up-regulated in cuc2 pSTK::RNAi-CUC1 versus wild-type pistils.

Table S3. List of genes down-regulated in cuc2 pSTK::RNAi-CUC1 versus wild-type pistils.

Table S4. Functional categorization of biological process GO terms.

Table S5. List of primers.

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