Research Paper

CsKDO is a candidate gene regulating seed germination lethality in cucumber

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Seed germination plays an important role in the initial stage of plant growth. However, few related studies focused on lethality after seed germination in plants. In this study, we identified an Ethyl methanesulfonate (EMS) mutagenesis mutant Csleth with abnormal seed germination in cucumber (Cucumis sativus L.). The radicle of the Csleth mutant grew slowly and detached from the cotyledon until 14 d after sowing. Genetic analysis showed that the mutant phenotype of Csleth was controlled by a single recessive gene. MutMap+ and Kompetitive Allele Specific PCR (KASP) genotyping results demonstrated that Csa3G104930 encoding 3-deoxy-manno-octulonosonate cytidylyltransferase (CsKDO) was the candidate gene of the Csleth mutant. The transition mutation of aspartate occurred in Csa3G104930 co-segregated with the phenotyping data. CsKDO was highly expressed in male flowers in wild type cucumbers. Subcellular localization results showed that CsKDO was located in the nucleus. Overall, these results suggest CsKDO regulates lethality during seed germination in cucumber.

Key Words: cucumber, EMS mutation, genotyping, MutMap+, seed germination.

Introduction

The growth process after seed germination is critical to crops. After absorbing water, dried seeds swell with water, marking the first stage of seed germination (Bewley 1997). In the second stage, the seed size increases and metabolism begins, and protein storage mobilization is essential in the germination of dicotyledons (Tiedemann et al. 2000). In the last stage, the endosperm is ruptured and the radicle becomes prominent (Manz et al. 2005, Müller et al. 2006).

At present, great progress has been achieved in the research of plant seed germination. In specific, previous studies identified that genes GID1, MAIN, MAILI, ga-1 OsTPP7, OsIPMS1, Zm00001d026317, and Zm00001d014814 regulate seed germination in Arabidopsis, rice, and maize (De Luxán-Hernández et al. 2020, Gallardo et al. 2002, Han et al. 2020, Hauvermale and Steber 2020, Kretzschmar et al. 2015). In Arabidopsis, mutations of the MAIN or MAIL1 gene plays a role in the protein complex. PROTEIN PHOSPHATASE 7-LIKE in Arabidopsis can cause meristematic cell death and prevent primary root growth after germination (De Luxán-Hernández et al. 2020). Hauvermale and Steber (2020) found that the transition from embryo to seedling in Arabidopsis requires GA and GA receptor GA-INSSENSITIVE DWARF1 (GID1). In ga1-3 GA biosynthesis mutant, GID1 overexpression causes abnormal seed germination, such that the cotyledons appear before the radicle and primary roots fail to develop, leading to plant death. He et al. (2019) showed that disruption of the isopropylmalate synthase gene OsIPMS1 in rice can reduce seed vigor and that OsIPMS1 can affect the vigor, starch hydrolysis, glycolytic activity, and energy amount of germinated seeds. Han et al. (2020) showed that the expression levels of two bHLH TFs in maize increase during seed germination and identified other TF family proteins related to seed germination. The above studies provide a reference to understand the general mechanism underlying plant seed germination, but the molecular mechanism behind cucumber seed germination remains unknown.

Dumont et al. (2016) found that 3-deoxy-D-manno-oct-2-ulosonic acid (KDO) is a monosaccharide found only in the pectin rhamnogalacturan-II (RG-II) of the plant cell wall. CMP-KDO is a nucleotide sugar that can integrate KDO into lipopolysaccharide and capsular polysaccharide transferase, which acts as a substrate in this series of reactions (Jelakovic and Schulz 2002). KDO is found in the core of lipopolysaccharide of Gram-negative bacteria. After being dephosphorylated, KDO-8-P is coupled with CMP by
CMP-KDO synthase to form an activated CMP-KDO sugar nucleotide (Royo et al. 2000). Then, it transported to the Golgi apparatus and participates in the RG-II backbone (Deng et al. 2010, Dumont et al. 2014). Dumont et al. (2016) showed that KDO is associated with pectin synthesis in the cell wall. Kobayashi et al. (2011) characterized CTP:KDO cytidylyltransferase (CMP-KDO synthetase; CKS) in Arabidopsis and found that the cks mutation causes pollen infertility by inhibiting pollen tube elongation. Despite the progress in KDO-related research, the mechanism by which the KDO gene regulates plant seed germination and development remains unclear.

The classic technique for cloning candidate genes in plants is map-based cloning (Wing et al. 1994). With the development of genome sequencing, other techniques, such as MutMap, have been developed for the rapid cloning of mutant genes (Abe et al. 2012). However, regardless of whether map-based cloning or MutMap is used, cross between mutants and wild type (or different ecological types) is necessary to build genetic groups, making causal genes hard to identify from mutants with early lethality or cross-pollination difficulties. With MutMap technology, cross between wild type and mutant is unnecessary; thus, this method is suitable for identifying infertility lethal traits or hampering crossing mutants (Fekih et al. 2013). Using MutMap, Fekih et al. (2013) identified the causal genes of two prematurely dead rice mutants. Liu et al. (2019) also identified CsPlD, a gene that can regulate the morphogenesis of cucumber lateral organs and the development of the ovule.

In the present study, we identified a lethal mutant (Csleth) from our previous ethylmethylsulfone (EMS) mutagenized library (Xue et al. 2016). The Csleth mutant grew slowly and detached from the cotyledon until 14 d after sowing. Csa3G104930 (CsKDO) was identified as the causal gene regulating lethality during seed germination in cucumber. CsKDO was highly expressed in male flowers, and CsKDO protein was localized in the nucleus. This study provided insights into the function of CsKDO in regulating the seed germination of cucumber.

Materials and Methods

Plant materials

The lethality mutant Csleth and wild type of M3 generation were isolated from the selfing of heterozygous individuals of the M2 generation in our previous EMS mutagenized library. All plants were grown under 12 h light/12 h dark at 25°C.

Mutant selection and phenotypic characterization

EMS-mutagenized M2 seeds (10 seeds/M2 line × 295 M2 lines; a total of 2950 seeds) were screened and grown on an acrylic plate culture system with vermiculite as a substrate for cultivation, and wild type seeds were sown into the same acrylic plate as a control. The acrylic plate was placed at an angle of 30° for 15 d. The radicle length was measured with a ruler. The Csleth mutant was identified as the radicle grew slowly and then detached from the cotyledon after growth. In consideration of the early development lethality of the Csleth mutant, M2 generation individuals were selfed with the wild type phenotype and produced the seeds with segregated traits of the wild type and mutant phenotypes (Csleth) in the M3 line. Then, the seeds were sown on an acrylic plate culture system for phenotype identification. The Csleth mutant was obtained after the second screening.

Whole genome re-sequencing

The DNA of M3 plants was extracted using the CTAB method (Clark and Edwards 1997). In the M3 population, the DNA of 11 plants with the mutant phenotype and 34 plants with the wild type phenotype were mixed equally to construct a mutant bulk and a wild type bulk, respectively. Genome re-sequencing for the bulks was performed at Annoroad Genomics (Beijing) by using an Illumina Hi-Seq 2000 sequencer (100 bp).

SNP calling and filtering

Short reads of large amounts of DNA were aligned with the reference genome of cucumber (http://cucurbigenomics.org/organism/2, version 2i) (Huang et al. 2009) by using Burrows-Wheeler Aligner software (Li and Durbin 2009). As described by Abe et al. (2012), the SNP call filter “Coval” was applied to improve the accuracy of SNP. SNPs with SNP index >0.3 detected in only two large pieces of DNA were selected to calculate 𝛿(SNP-index). R scripts were used to apply sliding window analysis in 1 Mb window size and 100 kb increments (Takagi et al. 2013). Finally, causal mutations were mapped in the window, with an average 𝑝 value <0.1.

SNP genotyping with KASP

M3 individual plants were used for KASP genotyping. Primers for all candidate SNPs were designed on the basis of the SNP mutation information (Supplemental Table 1). Specific genotyping was conducted in accordance with the method described by Hao et al. (2018). After completing the reaction, the fluorescence data were read using the LGC Genomics genotyping platform (Zhao et al. 2017).

RNA extraction and quantitative reverse transcription PCR (qRT-PCR)

The total RNA of male flowers, roots, shoot apices, and stems of wild type plants was isolated by using TRIZOL reagent (Invitrogen, USA). First-strand cDNA was synthesized using a cDNA synthesis kit (Toyoobo, Japan). Expression data were calculated using the 2ΔΔCt method (Livak and Schmittgen 2001). CsACTIN (Csa2G139820) was selected as an internal reference to standardize the expression data (Wan et al. 2010). The primers for qRT-PCR analysis are listed in Supplemental Table 2.
Bioinformatics analysis of candidate genes

The gene structure and the full length of DNA were searched in the Cucurbit genomic database (http://cucurbitgenomics.org/organism/20, version 3i) (Li et al. 2019). Protein sequences and homologs were obtained from GenBank (https://www.ncbi.nlm.nih.gov) to study the phylogenetic relationship between CsKDO in cucumber and other species, and then CsKDO was compared with homologous protein sequences in different species by using DNAMAN 8.0 software. To build a phylogenetic tree, we used Clustal W in MEGA 6.0 for multi-sequence alignment and built an adjacent tree based on the bootstrap test of MEGA 6.0 through 1000 repeated tests.

Subcellular localization

To study the subcellular localization of CsKDO protein, we cloned the coding region of the CsKDO gene without a stop codon and then fused it with eGFP in pSUPER-1300 to form the pSuper1300::CsKDO-eGFP vector. pSuper1300::eGFP served as the blank vector control. Bacterial solution was injected on the back of tobacco (Nicotiana benthamiana) leaves (5–6 weeks old). After 48–72 h of injection, the subcellular localization was measured under a laser confocal microscope. The fluorescence signal was detected using a Zeiss LSM 7100 laser scanning confocal microscope.

Results

Isolation and phenotypic characterization of the lethality mutant Csleth

A total of 2950 seeds from 295 EMS-mutagenized M₂ lines were sown on an acrylic plate culture system with vermiculite as a substrate for cultivation to identify novel mutants related to seed germination in cucumber. M₂ lines were selected as the radicle grew slowly and then detached from the cotyledon after growth. In consideration of the early development lethality of the Csleth mutant, individual heterozygous M₂ plants were self-pollinated, and seeds of an M₃ line with segregation were obtained and then confirmed another time. After this screening, the Csleth mutant was obtained (Fig. 1A). The radicle growth rate of the mutant was significantly lower than that of the wild type (Fig. 1B). The radicle of the Csleth mutant developed slowly in the early stage. Compared with that of the wild type, the radicle length of the mutant did not show obvious change about 5 d after sowing. The radicle of the wild type developed normally, and the length was 14.8 ± 0.16 cm at 14 d after sowing. However, the Csleth mutant remained at 0.83 ± 0.11 cm, and the radicle grew slowly throughout the growth period. True leaves of wild type plants emerged at 14 d after sowing, whereas the cotyledon of the Csleth mutant stopped growing and the radicle separated from the cotyledon, which was a lethal phenotype (Fig. 1).

Identification of the candidate genes for Csleth in cucumber

Given the seed early development lethality of the Csleth mutant, an M₃ line with segregation was obtained from selfing an individual heterozygous M₂ plant. Among the M₃ progeny, eight plants showed lethality phenotype, and 42 plants showed wild type phenotype, which fitted Mendel’s segregation ratio of 1:3 ($\chi^2 = 1.707$, $p > 0.05$). These data revealed that the phenotype of Csleth mutant was regulated by a single recessive gene.

In order to identify the candidate gene of Csleth mutants with more mutant plants, another large number M₃ generation was used. Two bulks, one wild type bulk with 34 progeny and one mutant bulk of 11 progeny, were established to identify the candidate gene by MutMap $^+$ (Fekih et al. 2013). The DNA copies of two bulks were re-sequenced. A total of 84,241,922 (94.77%) and 86,131,106 (94.29%) clean reads were obtained for the wild type and mutant bulks, respectively. In addition, 76,893,170 (91.28%) and 74,568,719 (86.58%) reads were mapped to the cucumber reference genome (http://cucurbitgenomics.org/organism/2,
version 2) in the two bulks (Supplemental Table 3). The difference in SNP-index values of the mutant and wild type bulks was the ΔSNP-index. From this, scatter plots and graphs of the mutant SNP-index, the wild type SNP-index, and the ΔSNP-index as a function of chromosome position were obtained (Fig. 2). The ΔSNP-index should be 0 in most regions of the genome, but its value should be significantly positive in regions containing mutation sites that cause mutation phenotypes.

According to the filter conditions: (1) The SNP-index in the mutant is close to 1; (2) G to A or C to T mutations; (3) Whether it is a non-synonymous mutation or a stop codon mutation. Five candidate SNP mutation sites located in chromosome 3 of cucumber were screened out, including four non-synonymous mutation sites in the exon region of the gene and one mutation site in the stop codon region of the gene (Table 1). Gene annotation indicated that the candidate genes containing these five SNPs were involved in 3-deoxy-manno-octulosonate cytidylyltransferase (SNP 5265242), receptor-like kinase (SNP 5985717), tetratricopeptide-like helical (SNP 7327506), Arabidopsis At2G44640 genes related to lipid transporter from the ER to the chloroplast (SNP 8204046), and embryo sac development arrest 6 protein (SNP 9037163).

To identify the candidate SNP, we performed the KASP technique to genotype the five SNPs in a M3 population accompanied with phenotypic data. KASP genotyping was conducted twice. A small number of M3 plants containing five mutant plants and 34 wild type plants were used in the first KASP genotyping analysis. Results showed that two (SNP 5265242 and SNP 7327506) of the five SNPs showed co-segregation of genotyping and phenotyping data (Supplemental Table 4). To distinguish the two SNPs, we performed another genotyping test using a large M3 population with 68 individuals (57 wild type, 11 mutants). Results showed that SNP 5265242 of Csa3G104930 co-segregated with the phenotyping data in the M3 population. Plants with mutant phenotypes had A:A genotypes, whereas those with wild type phenotypes had A:G or G:G genotypes. However, later results (Supplemental Table 5) showed that SNP...

Fig. 2. Scatter plots of all candidate sites generated by the MutMap+ method. A: SNP-index plots for wild type plants. B: SNP-index plots for Cseth mutant (Five candidate SNP mutation sites with SNP-index equal to 1 were represented in the black dashed box). C: ΔSNP-index obtained by subtracting mutant SNP-index bulk from wild type bulk (The point represents the SNP-index, the red line represents the average SNP-index in the sliding window. The black dashed box indicates the five candidate SNP mutation sites).

Table 1. List of candidate SNPs

| Chromosome | Position | Gene       | Reference | Alteration | SNP_index | Type   | Amino Acid change | Annotation                                                |
|------------|----------|------------|-----------|------------|-----------|--------|-------------------|-----------------------------------------------------------|
| 3          | 5265242  | Csa3G104930| C         | T          | 1         | Exonic | G to D            | the Glyco-trans-GTA-type superfamily protein              |
| 3          | 5985717  | Csa3G114500| C         | T          | 1         | Exonic |                   |                                                           |
| 3          | 7327506  | Csa3G122560| C         | T          | 1         | Exonic |                   |                                                           |
| 3          | 8204046  | Csa3G128880| C         | T          | 1         | Exonic |                   |                                                           |
| 3          | 9037163  | Csa3G134830| G         | A          | 1         | Exonic |                   |                                                           |
7327506 did not co-segregate with the phenotype of M3 individuals. These results indicated that Csa3G104930, a gene encoding 3-deoxy-manno-octulosonate cytidylyltransferase, which harbors SNP 5265242, is the causal gene of the Csleth mutant. In this study, the causal gene of the Csleth mutant is hereafter referred to as CsKDO.

**Sequence analysis of CsKDO**

Gene sequence analysis revealed that CsKDO is 4238 bp in length, containing 8 exons and 9 introns by consulting Cucurbit Genomics Database (http://cucurbitgenomics.org/organism/20, version 3i). A transition from glycine (Gly) to aspartate (Asp) was occurred in the first exon of CsKDO gene due to a G to A substitution at 252 bp (Fig. 3B). CsKDO encodes the 3-deoxymannose cytidyl acetyltransferase, which catalyzes the production of sugar nucleotide CMP-3-deoxy-D-mannose (CMP-KDO). KDO is activated during the biosynthesis of Rhamnogalacturonan II (RG-II), a pectin polysaccharide with a complex primary cell wall. RG-II is essential for cell wall integrity of fast-
growing tissues and for pollen tube growth and elongation (O’Neill et al. 1996).

CsKDO encoding a protein with 894 amino acids, with a G to A mutation at 252 bp (Fig. 3B). According to the structural properties indicated by the InterPro program (http://www.ebi.ac.uk/interpro/), the protein contains seven ligand binding sites, located at the 57th, 58th, 59th, 98th, 125th, 147th, and 149th amino acids. Conserved domain analysis of the amino acid encoded by CsKDO was performed using the NCBI database. Protein sequences of Cucurbita moschata, Cucumis melo, and Arabidopsis thaliana in the NCBI database were compared to obtain alignment scores in different species. The homology of the proteins was as high as 93.91%, with no highly conserved domains (Fig. 3C).

Using the protein sequence of CsKDO as the search sequence, we searched for homologous proteins by BLASTP and constructed a phylogenetic tree for analysis to explore further the genetic relationship of the protein in different species. Results showed that the CsKDO protein had high homology with the KDO proteins in Manihot esculenta, Cucumis melo, Nicotiana attenuata, Arachis ipaensis, and other plants. The highest and lowest homologies were observed with Cucumis melo (89.23%) and Punica granatum (78.55%), respectively (Fig. 4).

Expression and subcellular localization analysis

The expression of CsKDO was also investigated in different organs of wild type plants. The expression level of CsKDO was higher in male flowers than in other organs, such as shoot apices, root, and stems (Fig. 5). Subcellular localization results showed that CsKDO was located in the nucleus (Fig. 6).

Discussion

Lethal phenotypes are ubiquitous in nature. They are closely related to the growth and development of plants and include the initial growth processes, such as seed germination and nutrient absorption. In this study, we obtained a cucumber lethality mutant and identified a gene (CsKDO) encoding 3-deoxy-manno-octulosonate cytidylyltransferase. This gene is homologous to Arabidopsis At1g53000 and is a candidate to the Csleth mutant.

Strohmaier et al. (1995) found two Escherichia coli regulatory genes, kdsA and kdsB, which are involved in 3-deoxy-D-manno-octulosonic acid metabolism and biosynthesis of enterobacterial lipopolysaccharide and expressed in the growth phase regulated primarily at the transcriptional level in Escherichia coli K-12. In other crop, Royo et al. (2000) first discovered the maize root development-related gene ZmCKS, which is involved in the synthesis of corn CMP-KDO enzymes. In Arabidopsis, CMP-KDO synthesis gene has also been reported, the CKS gene is expressed in mature pollen and pollen tube, and its expression is also high in the shoot tip (Winter et al. 2007). Analysis of the cks mutant showed that the gene is
expressed in the leaves, stems, roots, and cilia of *Arabidopsis thaliana*, but the highest expression level can be found in the roots. In 2009, a report pointed that KDO is an essential component of the plant cell wall, and this gene may affect the catalytic performance of KDO synthetase (Misaki et al. 2009). Séveno et al. (2010) pointed that Kdo transferase (KDTA) deletion mutants do not affect the phenotypic changes and structural changes of RG-II in Arabidopsis and that AtKDTA is involved in mitochondrial molecular synthesis rather than cell wall RG-II. Smyth et al. (2013) reported that the 2β-deoxy-Kdo as an in vivo inhibitor of AtkdsB can affect the root cell elongation even lead to the growth and development defect in Arabidopsis. The study showed that this phenomenon may be attributed to the specific inhibition of AtkdsB enzyme by 2β-deoxidized KDO in Arabidopsis seedlings, resulting in structural changes or decreased RG-II abundance, which can also give us a new method for identification of RG-II/KDO related genes. In the meantime, a previous study reported that CMP-KDO is related to the synthesis of pectin in the cell wall (Dumont et al. 2016). However Kobayashi et al. (2011) found that the Arabidopsis *At1g53000* gene encodes a mitochondrial-associated CMP-KDO (3-deoxy-D-mannose-octasulfonate) synthetase and incorporated RG II (type II rhamnogalacturonan) in KDO previously responsible for activating KDO; the heterozygous mutant of the gene is defective in pollen development and pollen tube elongation (Kobayashi et al. 2011). They also pointed out that the *At1g53000* gene encodes a *ck* mutation of *Arabidopsis thaliana*, subcellular localization of the gene indicates that it is expressed in the mitochondria of plant cells and is related to mitochondrial inner membrane synthesis (Kobayashi et al. 2011). The results obtained in the present study showed that cucumber CsKDO was a nuclear localization, which is different from its homologous from other crops including Arabidopsis *AT1G53000* gene, indicating that functions between CsKDO and its homologous might be different, which might be caused by their localization differences.

RG-II is a pectin molecule that is present in the primary cell wall of plants, it exist in the form of dimer, which is cross-linked by the borate diester bond between two arachidononic residues, containing 12 different glycosyl residues including aceric acid, apiose, 3-deoxy-D-lyxo-hept-2ulosaric acid 3-deoxy-D-manno-oct-2-ulosonic acid (KDO) and so on (Bar-Peled et al. 2012, Kobayashi et al. 1996, O’Neill et al. 1996, 2004, Zhao et al. 2020). The synthesis of dimer promotes the formation of pectin network, which promotes the mechanical properties of the primary wall and is necessary for the normal growth and development of plants, the absence of this dimer will lead to abnormal biochemical and biomechanical properties of the cell wall, even affect the productivity of plants (Julien et al. 2018). Many evidence shows that the alteration and cross-linking of RG-II structure have serious effects on the growth, development and viability of plants (Sechet et al. 2018). Defects in RG-II biosynthesis lead to changes in plant growth and cell wall structure (Dumont et al. 2014, Fleischer et al. 1999, O’Neill et al. 2001, Voeux et al. 2011). However, the exact function of RG-II is unknown. In plants, 3-deoxy-D-manno-oct-2ulosonic acid (KDO) is a monosaccharide that is only found in the cell wall pectin, rhamnogalacturonan-II (RG-II) (Dumont et al. 2016). The inhibited mutant of CMP-KDO synthesis is lethal, which further indicates the important role of RG-II in plant growth (Ahn et al. 2006, Delmas et al. 2003, 2008, Molhøj et al. 2003). Therefore, we speculate that the mutations of Csa3G104930 and its homologous genes are likely to inhibit the synthesis of CMP-KDO during the germination of cucumber seeds. CMP-KDO is related to RG-II synthesis, and the inhibition of its synthesis may affect plant primary cell wall growth, as a result, the plant is stunted early in the stage, leading to plant death. This hypothesis further confirms that the cucumber lethality mutation phenotype is caused by the CsKDO gene mutation which identified in our study.

In conclusion, we obtained the stable dysplasia mutant Cseleth by screening the EMS-mutated M3 generation pedigree and found the CsKDO gene. The mutant significantly differed from the wild type in terms of lethality during seed germination. Predecessor analysis results suggest that mutations of the CsKDO gene block the synthesis of CMP-KDO in plant cells, affecting plant development and leading to cucumber lethal during seed germination. This study may serve as a theoretical reference for cucumber development during seed germination and promote the research of crop growth in the future.

**Author Contribution Statement**

TW and NH conceived the experiment. CW performed the research. CW and NH constructed the mutant and M3 populations for experiments. YX, YD, and KH collected data for experiments. CW and TW wrote the manuscript. All authors reviewed and approved this submission.

**Acknowledgments**

We are thankful to the National Key Research and Development Program of China (2018YFD1000800), National Natural Science Foundation of China (31972429, 31972407, 3201154003), and Hunan Provincial Natural Science Foundation of China (2020JJ4363).

**Literature Cited**

Abe, A., S. Kosugi, K. Yoshida, S. Natsume, H. Takagi, H. Kanzaki, H. Matsumura, K. Yoshida, C. Mitsuoka, M. Tamiru et al. (2012) Genome sequencing reveals agronomically important loci in rice using MutMap. Nat. Biotechnol. 30: 174–178.

Ahn, J.W., R. Verma, M. Kim, J.Y. Lee, Y.K. Kim, J.W. Bang, W.D. Reiter and H.S. Pai (2006) Depletion of udp-D-apiose/udp-D-
xylose synthases results in rhamnogalacturonan-II deficiency, cell wall thickening, and cell death in higher plants. J. Biol. Chem. 281: 13708–13716.

Bar-Peled, M., B.R. Urbanowicz and M.A. O’Neill (2012) The synthesis and origin of the pectic polysaccharide rhamnogalacturonan II—insights from nucleotide sugar formation and diversity. Front. Plant Sci. 3: 92.

Bewley, J.D. (1997) Seed germination and dormancy. Plant Cell 9: 1055–1066.

Clark, M.S. and K.J. Edwards (1997) Plant molecular biology. A laboratory manual. Plant Growth Regul. 23: 210.

De Luxán-Hernández, C., J. Lohmann, W. Hellmeyer, S. Seanpong, K. Wölte, Z. Magyar, A. Pettö-Szandner, T. Pélissier, G. De Jaeger, S. Hoth et al. (2020) PPTL is essential for MAIL1-mediated transposable element silencing and primary root growth. Plant J. 102: 703–717.

Delmas, F., J. Petit, J. Joubès, M. Séveno, T. Paccalé, M. Hernould, P. Lerouge, A. Mouras and C. Chevalier (2003) The gene expression and enzyme activity of plant 3-deoxy-D-manno-2-octulosonic acid-8-phosphate synthase are preferentially associated with cell division in a cell cycle-dependent manner. Plant Physiol. 133: 348–360.

Delmas, F., M. Séveno, J.G.B. Northey, M. Hernould, P. Lerouge, P. McCourt and C. Chevalier (2008) The synthesis of the rhamnogalacturonan II component 3-deoxy-D-manno-2-octulosonic acid (Kdo) is required for pollen tube growth and elongation. J. Exp. Bot. 59: 2639–2647.

Deng, Y., W. Wang, W.Q. Li, C. Xia, H.Z. Liao, X.Q. Zhang and D. Ye (2010) MALE GAMETOPHYTE DEFECTIVE 2, encoding a sialyltransferase-like protein, is required for normal pollen germination and pollen tube growth in Arabidopsis. J. Integr. Plant Biol. 52: 829–843.

Dumont, M., A. Lehner, S. Bouton, M.C. Kiefer-Meyer, A. Voxeur, J. Pelloux, P. Lerouge and J.C. Mollet (2014) The cell wall pectin polymer rhamnogalacturonan-II is required for proper pollen tube elongation: implications of a putative sialyltransferase-like protein. Ann. Bot. 114: 1177–1188.

Dumont, M., A. Lehner, B. Vauzelles, J. Malassie, A. Marchant, K. Smyth, B. Linclau, A. Baron, J.M. Pons, C.T. Anderson et al. (2016) Plant cell wall imaging by metabolic click-mediated labelling of rhamnogalacturonan II using azido 3-deoxy-D-manno-2-octulosonic acid. Plant J. 85: 437–447.

Fekih, R., H. Takagi, M. Tamiru, A. Abe, S. Natsume, H. Yaegashi, S. Sharrma, S. Sharma, H. Kanzaki, H. Matsumura et al. (2013) MutMap: Genetic mapping and mutant identification without crossing in rice. PLoS ONE 8: e68529.

Fleischer, A., M.A. O’Neill and R. Ehwald (1999) The pore size of non-graminaceous plant cell walls is rapidly decreased by borate cross-linking of the pectic polysaccharide rhamnogalacturonan II. Plant Physiol. 121: 829–838.

Gallardo, K., C. Job, S.P.C. Groot, M. Puype, H. Demol, J. Vandekerckhove and D. Job (2002) Proteomics of Arabidopsis seed germination. A comparative study of wild-type and gibberellin-deficient seeds. Plant Physiol. 129: 823–837.

Han, Z., B. Wang, L. Tian, S. Wang, J. Zhang, S. Guo, H. Zhang, L. Xu and Y. Chen (2020) Comprehensive dynamic transcriptome analysis at two seed germination stages in maize (Zea mays L.). Physiol. Plant. 168: 205–217.

Hao, N., Y. Du, H. Li, C. Wang, C. Wang, S. Gong, S. Zhou and T. Wu (2018) CsMYB36 is involved in the formation of yellow green peel in cucumber (Cucumis sativus L.). Theor. Appl. Genet. 131: 1659–1669.

Hauvermale, A.L. and C.M. Steber (2020) GA signaling is essential for the embryo-to-seedling transition during Arabidopsis seed germination, a ghost story. Plant Signal. Behav. 15: 1705028.

He, Y., J. Cheng, Y. He, B. Yang, Y. Cheng, C. Yang, H. Zhang and Z. Wang (2019) Influence of isopropylmalylate synthase OsIPLS1 on seed vigour associated with amino acid and energy metabolism in rice. Plant Biotechnol. J. 17: 322–337.

Huang, S., R. Li, Z. Zhang, L. Li, X. Gu, W. Fan, W.J. Lucas, X. Wang, B. Xie, P. Ni et al. (2009) The genome of the cucumber, Cucumis sativus L. Nat. Genet. 41: 1275–1281.

Jelakovic, S. and G.E. Schulz (2002) Catalytic mechanism of CMP: 2-keto-3-deoxy-manno-octonic acid synthetase as derived from complexes with reaction educt and product. Biochemistry 41: 1174–1181.

Julien, S., H. Soc, U. Breeanna, A. Abigail, F. Wei and I. Toshiki (2018) Suppressing arabidopsis GGLT1 affects growth by reducing the L-galactose content and borate cross-linking of rhamnogalacturonan II. Plant J. 96: 1036–1050.

Kobayashi, M., T. Matoh and J. Azuma (1996) Two chains of Rhamnogalacturonan II are cross-linked by borate-diol ester bonds in higher plant cell walls. Plant Physiol. 110: 1017–1020.

Kobayashi, M., N. Kouzu, A. Inami, K. Toyooka, Y. Konishi, K. Matsuoka and T. Matoh (2011) Characterization of Arabidopsis CTP: 3-deoxy-D-manno-2-octulosonate cytidylyltransferase (CMP-KDO synthetase), the enzyme that activates KDO during rhamnogalacturonan II biosynthesis. Plant Cell Physiol. 52: 1832–1843.

Kretzschmar, T., M.A.F. Pelayo, K.R. Trijatmiko, L.F.M. Gabunada, R. Alam, R. Jimenez, M.S. Mendioro, I.H. Slamat-Loedín, N. Sreenivasulu, J. Bailey-Serres et al. (2015) A trehalose-6-phosphate phosphatase enhances anaerobic germination tolerance in rice. Nat. Plants 1: 15124.

Li, H. and R. Durbin (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754–1760.

Li, Q., H. Li, W. Huang, Y. Xu, Q. Zhou, S. Wang, J. Ruan, S. Huang and Z. Zhang (2019) A chromosome-scale genome assembly of cucumber (Cucumis sativus L.). GigaScience 8: giz072.

Liu, X., N. Hao, H. Li, D. Ge, Y. Du, R. Liu, C. Wen, Y. Li, X. Zhang and T. Wu (2019) PINOID is required for lateral organ morphogenesis and ovule development in cucumber. J. Exp. Bot. 70: 5715–5730.

Livak, K.J. and T.D. Schmittgen (2001) Analysis of relative gene expression data using real-time quantitative PCR and the 2-ΔΔCt method. Methods 25: 402–408.

Manz, B., K. Muller, B. Kucera, F. Volke and G. Leubner-Metzger (2005) Water uptake and distribution in germinating tobacco seeds investigated in vivo by nuclear magnetic resonance imaging. Plant Physiol. 138: 1538–1551.

Misaki, R., H. Kajiura, K. Fujii, K. Fujiyama and T. Seki (2009) Cloning and characterization of cytokine monophosphate-3-deoxy-D-manno-octulosonate synthetase from Arabidopsis thaliana. J. Biosci. Bioeng. 108: 527–529.

Molhøj, M., R. Verma and W.D. Reiter (2003) The biosynthesis of the branched-chain sugar D-apiose in plants: functional cloning and characterization of a UDP-D-apiose/UDP-D-xylose synthase from Arabidopsis. Plant J. 35: 693–703.

Müller, K., S. Tintelnot and G. Leubner-Metzger (2006) Endosperm-limited brassicaceae seed germination: abscisic acid inhibits embryo-induced endosperm weakening of Lepidium sativum (cress) and endosperm rupture of cress and Arabidopsis thaliana.
Plant Cell Physiol. 47: 864–877.

O’Neill, M.A., D. Warrenfeltz, K. Kates, P. Pellerin, T. Doco, A.G. Darvill and P. Albersheim (1996) Rhamnogalacturonan-II, a pectic polysaccharide in the walls of growing plant cell, forms a dimer that is covalently cross-linked by a borate ester: in vitro conditions for the formation and hydrolysis of the dimer. J. Biol. Chem. 271: 22923–22930.

O’Neill, M.A., S. Eberhard, P. Albersheim and A.G. Darvill (2001) Requirement of borate cross-linking of cell wall rhamnogalacturonan II for Arabidopsis growth. Science 294: 846–849.

O’Neill, M.A., T. Ishii, P. Albersheim and A.G. Darvill (2004) Rhamnogalacturonan II: structure and function of a borate cross-linked cell wall pectic polysaccharide. Annu. Rev. Plant Biol. 55: 109–139.

Royo, J., E. Gómez and G. Hueros (2000) A maize homologue of the bacterial CMP-3-deoxy-D-manno-2-octulosonate (KDO) synthetases. Similar pathways operate in plants and bacteria for the activation of KDO prior to its incorporation into outer cellular envelopes. J. Biol. Chem. 275: 24993–24999.

Sechet, J., S. Htwe, B. Urbanowicz, A. Agyeman, W. Feng, T. Ishikawa, M. Colomes, K.S. Kumar, M. Kawai-Yamada, J.R. Dinneny et al. (2018) Suppression of Arabidopsis GGLT1 affects growth by reducing the L-galactose content and borate cross-linking of rhamnogalacturonan-II. Plant J. 96: 1036–1050.

Séveno, M., E. Séveno-Carpentier, A. Voeux, L. Menu-Bouaouiche, C. Rihouey, F. Delmas, C. Chevalier, A. Driouich and P. Lerouge (2010) Characterization of a putative 3-deoxy-D-manno-2-octulosonic acid (Kdo) transferase gene from Arabidopsis thaliana. Glycobiology 20: 617–628.

Smyth, K.M., H. Mikolajek, J.M. Werner and A. Marchant (2013) 2β-deoxy-Kdo is an inhibitor of the Arabidopsis thaliana CMP-2-Keto-3-deoxymanno-octulosonic acid synthetase, the enzyme required for activation of Kdo prior to incorporation into rhamnogalacturonan II. Mol. Plant 6: 981–984.

Strohmaier, H., P. Remler, W. Renner and G. Högenauer (1995) Expression of genes kdsA and kdsB involved in 3-deoxy-D-manno-octulosonic acid metabolism and biosynthesis of enterobacterial lipopolysaccharide is growth phase regulated primarily at the transcriptional level in Escherichia coli K-12. J. Bacteriol. 177: 4488–4500.

Takagi, H., A. Abe, K. Yoshida, S. Kosugi, S. Natsume, C. Mitsuoka, A. Uemura, H. Utsushi, M. Tamiru, S. Takuno et al. (2013) QTL-seq: Rapid mapping of quantitative trait loci in rice by whole genome resequencing of DNA from two bulked populations. Plant J. 74: 174–183.

Tiedemann, J., B. Neubohn and K. Müntz (2000) Different functions of vicilin and legumin are reflected in the histopattern of globulin mobilization during germination of vetch (Vicia sativa L.). Planta 211: 1–12.

Voeux, A., L. Gilbert, C. Rihouey, A. Driouich, C. Rothan, P. Baldest and P. Lerouge (2011) Silencing of the GDP-D-mannose 3,5-epimerase affects the structure and cross-linking of the pectic polysaccharide rhamnogalacturonan II and plant growth in tomato. J. Biol. Chem. 286: 8014–8020.

Wan, H., Z. Zhao, C. Qian, Y. Sui, A.A. Malik and J. Chen (2010) Selection of appropriate reference genes for gene expression studies by quantitative real-time polymerase chain reaction in cucumber. Anal. Biochem. 399: 257–261.

Wing, R.A., H.B. Zhang and S.D. Tanksley (1994) Map-based cloning in crop plants. Tomato as a model system: I. Genetic and physical mapping of jointless. Mol. Gen. Genet. 242: 681–688.

Winter, D., B. Vinegar, H. Nahal, R. Ammar, G.V. Wilson and N.J. Provart (2007) An “Electronic Fluorescent Pictograph” browser for exploring and analyzing large-scale biological data sets. PLoS ONE 2: e718.

Zhao, X., B. Ebert, B. Zhang, H. Liu, Y. Zhang, W. Zeng, C. Rautengarten, H. Li, X. Chen, A. Bacie et al. (2020) UDP-Api/UDP-Xyl synthases affect plant development by controlling the content of UDP-Api to regulate the RG-II-borate complex. Plant J. 104: 252–267.

Zhao, Y., X. Liu, H. Zhao, C. Yuan, G. Qi, Y. Wang and Y. Dong (2017) Comparison of methods for SNP genotyping in soybean. Mol. Plant Breed. 15: 3540–3546.