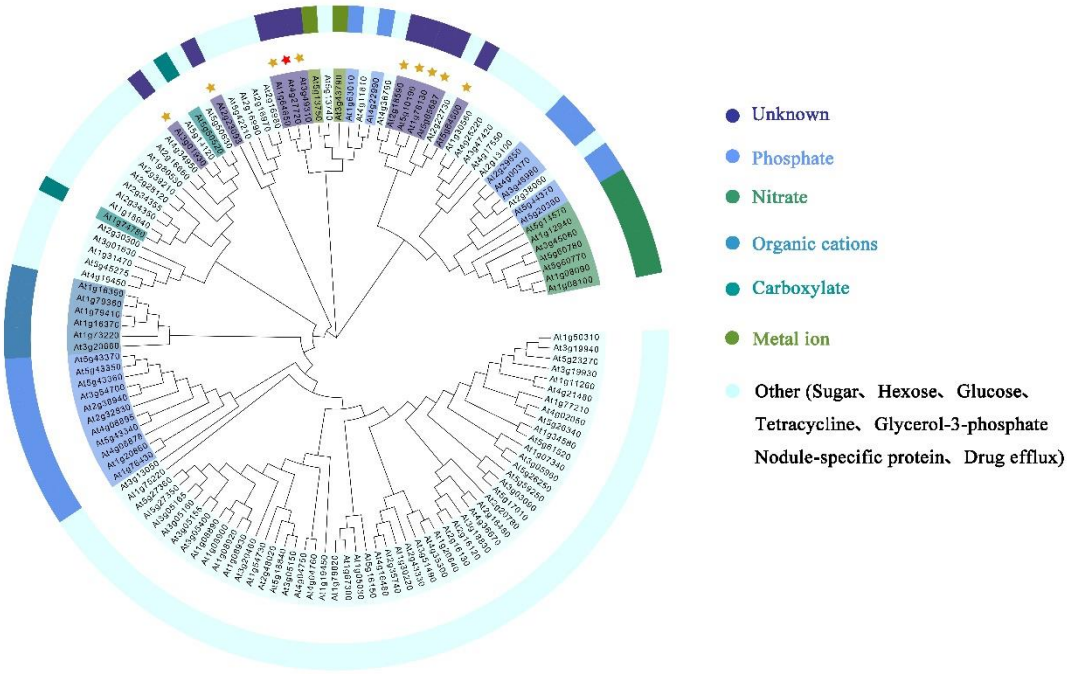


**Figure S1 Phylogenetic tree of the MFS members in *Arabidopsis thaliana*.**
MFS proteins with different transport substrates are represented with different colors. MFS proteins with unclassified substrates are represented with stars. AtCUP1 is marked with red stars. The gene symbol IDs of these unclassified genes are as follows: A: At1G64650; B: At1G78130; C: At3G49310; D: At4G27720; E: At5G10190; F: At5G65687; G: At2g23093; H: At3g01930; I: At5g64500; J: At2g18590.

(a)

| Protein | Plasma membrane | Tonoplast | Endoplasmic reticulum membrane |
|---|---|---|---|
| AtCUP1 | 9 | 3 | 2 |

(b)

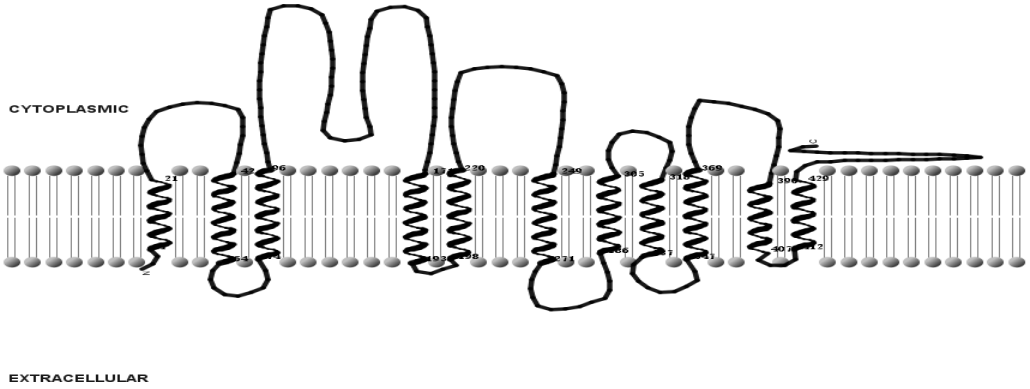


**Figure S2 Prediction of subcellular localization and transmembrane domain of AtCUP1**
(a) Subcellular localization of AtCUP1 protein, predicted by using WoLF PSORT (https://wolfpsort.hgc.jp) website. (b) Transmembrane domain of AtCUP1 protein, predicted by using TMHMM and showed by TMRPres2D.

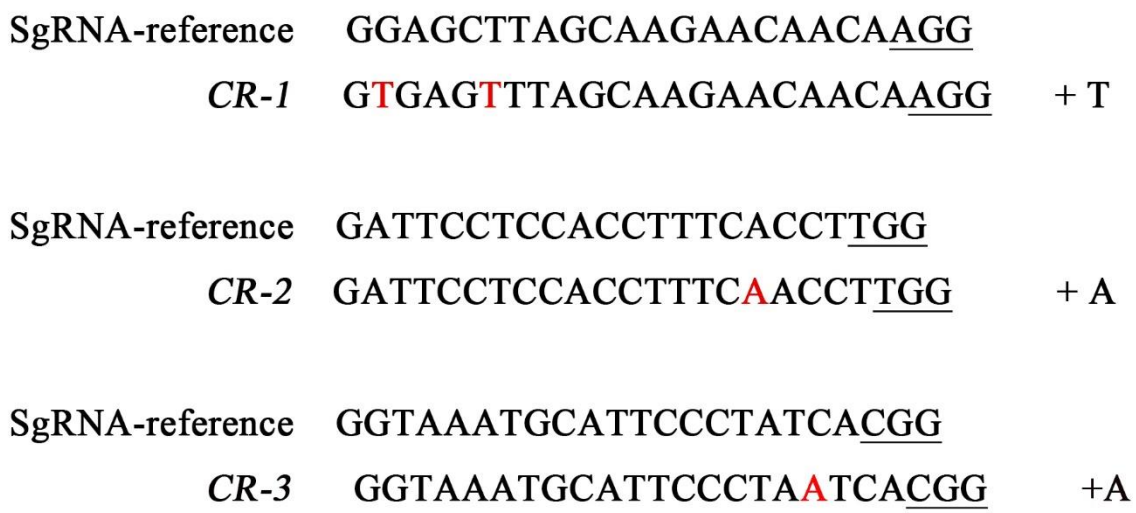


**Figure S3 Display of editing results of *AtCUP1*-editing lines (*CR-1*, *CR-2* and *CR-3*)**
The genome sequence of the wild-type is represented by reference sequence in *A. thaliana.* The underlined part represents the sgRNA and the red part represents PAM. The sequences of *CR-1*, *CR-2* and CR-3 represent the gene edited sequences of *CRISPR-AtCUP1.*

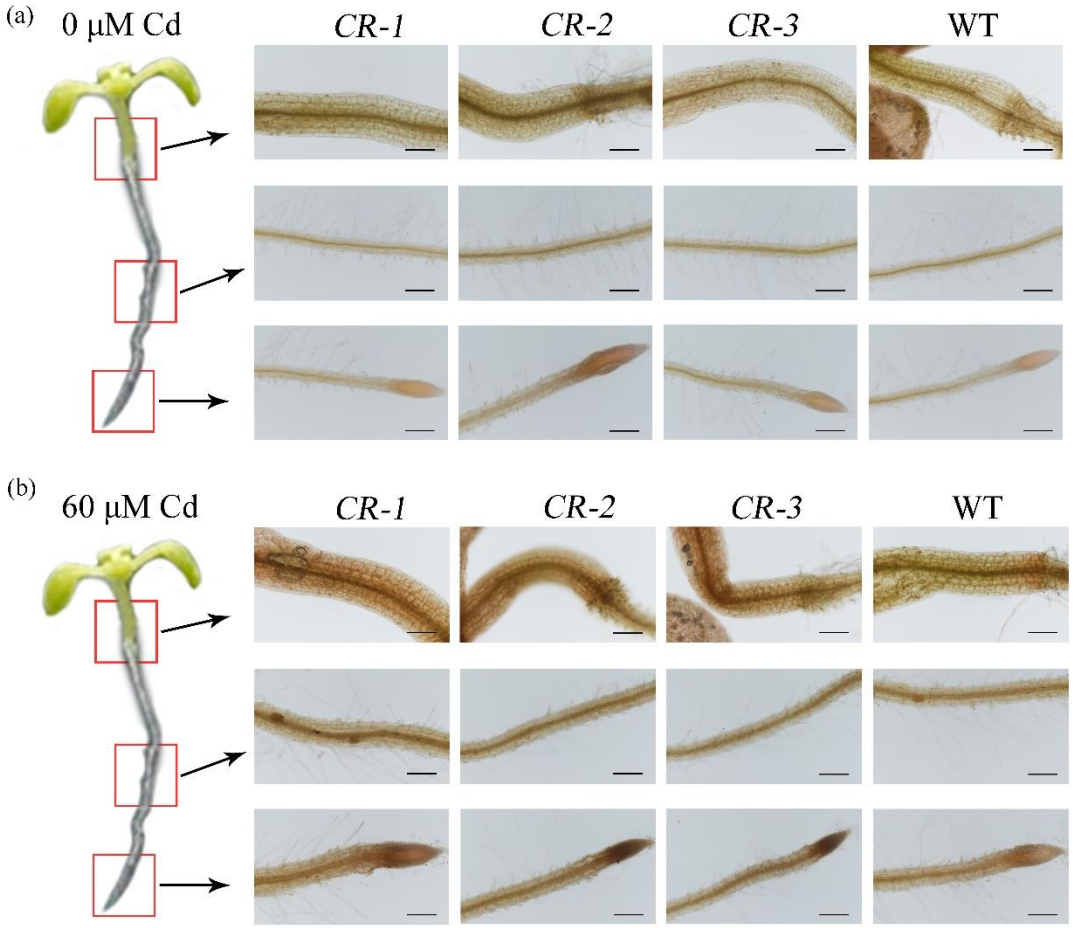


**Figure S4 Cadmium localization in the root and hypocotyl tissues of *CRISPR-AtCUP1* lines exposed to 0 or 60 μM Cd for 7 d**

Cadmium - dithizone precipitate was reddish-brown precipitate. Bars= 500 μm.

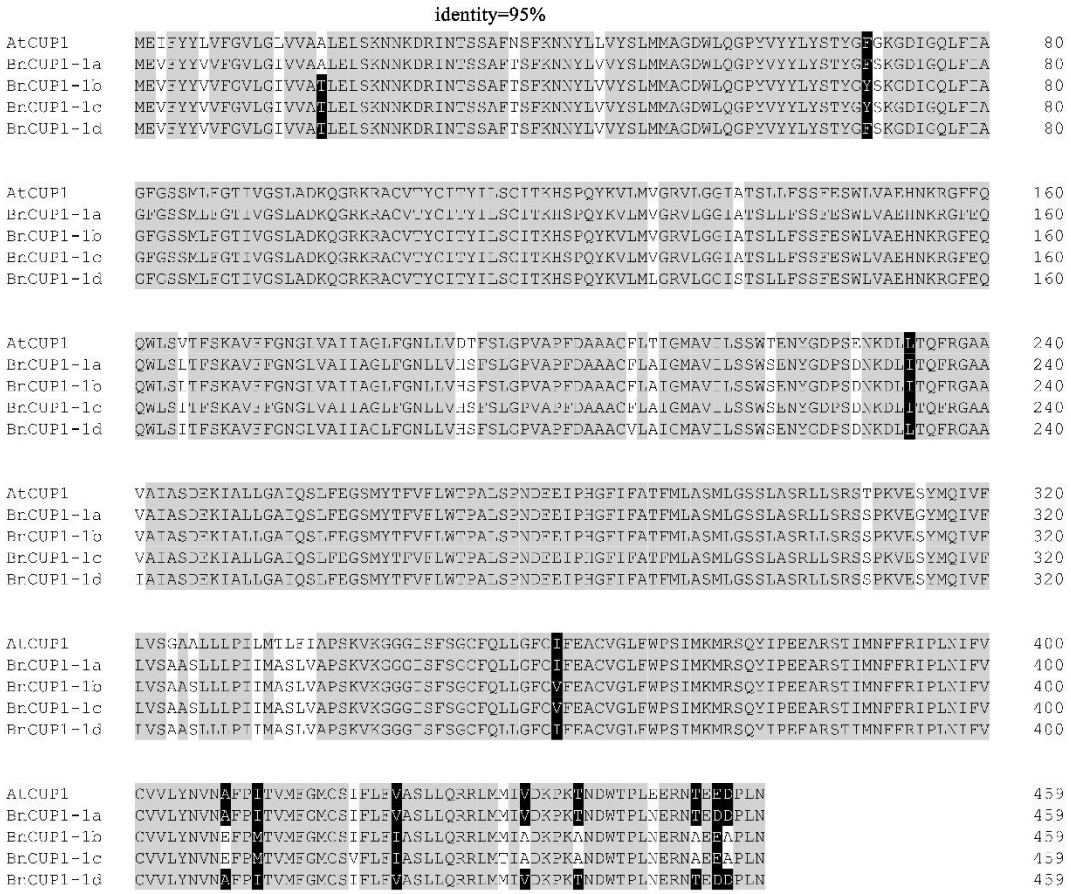


**Figure S5 Alignment of CUP1 homolog sequences identified from *A. thaliana* (*AtCUP1*) and *B. napus* (*BnCUP1-1a*, *BnCUP1-1b*, *BnCUP1-1c* and *BnCUP1-1d*). The amino acid sequence differences are highlighted in White and black boxes.**

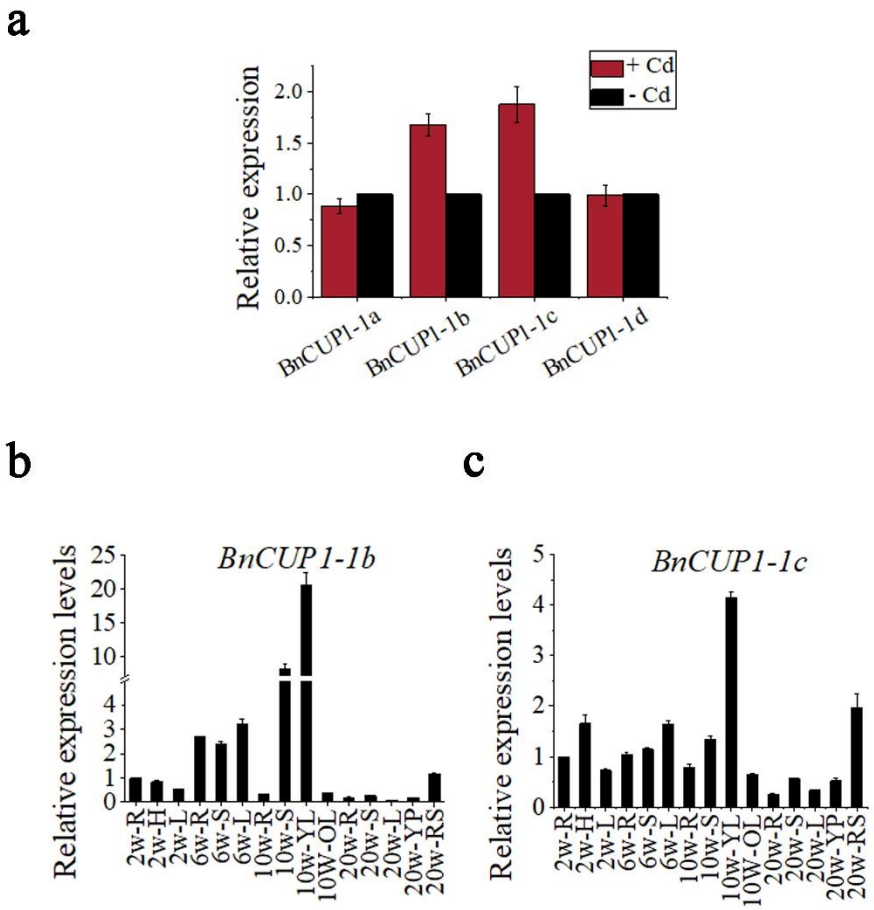


**Figure S6 Relative expression levels of each copy of *BnCUP1***

(a) Relative expression levels of *BnCUP1* copies before and after Cd stress. The relative expression level after Cd treatment is shown in red, and the relative expression level without Cd treatment is shown in black. (b) The relative expression level of *BnCUP1* copies in different tissues at different stages of rapeseed development were detected via qPCR. 2W, 6W, 10W and 20W represent *B. napus* aged 2 weeks, 6 weeks, 10 weeks and 20 weeks, respectively. R indicates the root, H indicates the hypocotyl, L indicates the leaf, S indicates the stem, YL indicates the young leaf, OL indicates the old leaf, YP indicates the young silique, RS indicates the rape seed.


**Figure S7 Display of editing results of *BnCUP1* gene editing lines (*CRISPR-S12* and *CRISPR-S34*)**

The genome sequence of the wild-type is represented by reference sequence in *B. napus.* The underlined part is PAM. The red font represents base changes in the edited lines.

## Table S1 Primers used in the present study

| role | primers | Sequences (5' to 3') |
|---|---|---|
| qPCR assay of Cd uptake candidate genes in Arabidopsis | At1G64650 -qpcr-F | TACTTCGTGGTGTTCGGTGG |
| | At1G64650 -qpcr-R | TCCCCAGCCATCATGAGAGA |
| | At1G78130-qpcr-F | GATTTGGTCCGGGAAGCTGA |
| | At1G78130-qpcr-R | CCCGTGTGAGAACCCAATGA |
| | At2g18590-qpcr-F | CGTCCCACAGTTTTTGCGTT |
| | At2g18590-qpcr-R | TGCATGTCCAACCCCATTGA |
| | At2g23093-qpcr-F | TGCGTGGAGGGATGATAAGC |
| | At2g23093-qpcr-R | TGTGGTGACTTTCCCCATCG |
| | At3g01930-qpcr-F | CTCTTCTCGTCGGCTCTGTC |
| | At3g01930-qpcr-R | TGAGGATACACATAGCCCATAAAGG |
| | At3G49310-qpcr-F | CCAGCTCTTAGCCCAAACGA |
| | At3G49310-qpcr-R | GAAGTGAAGCAGCCGAGACT |
| | At4G27720-qpcr-F | TTGGGGTTTTGGGTCTCGTC |
| | At4G27720-qpcr-R | AACCAATCACCCGCCATCAT |
| | At5G10190-qpcr-F | TAACCGAGCTCACGTCATCG |
| | At5G10190-qpcr-R | GACTAGGGACTGAATCGCGG |
| | At5g64500-qpcr-F | AGGGGTCACAGTTGTATGCG |
| | At5g64500-qpcr-R | ACCCTGTGTGGCAAAGACAA |
| | At5G65687-qpcr-F | GTCTCCATTGCGTGAGACCA |
| | At5G65687-qpcr-R | AGCTGCGAGGAAGAGGATTG |
| Yeast functional experiments | PYES2-A-F | CGGGGTACCATGGAGATCTTCTACTTCGTGGT |
| | PYES2-A-R | CCGGAATTCTTATGGGTTTAGAGGGTCAGC |
| | PYES2-B-F | CGGGGTACCATGAAGGCGGAGACGATGAC |
| | PYES2-B-R | CCGGAATTCTTATCTAGCTTGTAAATTGGGGT |
| | PYES2-C-F | CGGGGTACCATGGAGGTTTTCTACTACTTGG |
| | PYES2-C-R | CCGGAATTCTCAGAGGGTAAGAGGATCAACTT |
| | PYES2-D-F | CCCAAGCTTATGGAGATTTTCTACTACTTGGT |
| | PYES2-D-R | CCGGAATTCTCATATGTTGAGGGGATCTTCTT |
| | PYES2-E-F | CGGGGTACCATGAAGTCGGAGACTTTAACATT |
| | PYES2-E-R | CCGGAATTCTTAACTCTCATTCTGATGCTG |
| | PYES2-F-F | CGGGGTACCATGACGAGAGTTGGCCAGAGA |
| | PYES2-F-R | TGCTCTAGATTAGGCGAGAGTAGAGTTCTCT |
| | PYES2-G-F | CGGGGTACCATGGGTGTCGTTATTGAGACCT |
| | PYES2-G-R | CCGGAATTCTCATAATTTGTGCCAATCAT |
| | PYES2-H-F | CGGGGTACCATGGCGAGGACGACGCGAGAAAGAG |
| | PYES2-H-R | CCGGAATTCTCAGTTGCGGGTTTTGCCGTAGAGA |
| | PYES2-I-F | CGGGGTACCATGGATGTTGACGGAGAAGGTG |
| | PYES2-I-R | CCGGAATTCTCATGCTTCCTGGAGAAGAGG |
| Construction of *AtCUP1* overexpression vector | 35s::AtCUP1-F | GCTCTAGAATGGAGATTTTCTACTACTTGGT |
| | 35s::AtCUP1-R | CCCCCGGGTATGTTGAGGGGATCTTCTT |
| | SALK-0454-LP | ATGTAGCTGGATTGAGCATGG |

| | | |
|---|---|---|
| Identification of *AtCUP1* mutants | SALK-0454-RP | CTTAGGAGCCACACGATCTTG |
| | LBb1.3-BP | ATTTTGCCGATTTCGGAAC |
| Subcellular localization | PBI221-AtCUP1-F | GCTCTAGAATGGAGATTTTCTACTACTTGGT |
| | PBI221-AtCUP1-R | GCGGATCCTATGTTGAGGGGATCTTCTT |
| Analysis of *AtCUP1* promoter activity | Pro-4G-F | CCCAAGCTTTAGTTCTGAATGGTGAGGAAGAGA |
| | Pro-4G-R | CGCGGATCCTGTCGTTCGATCCAGAAACTAT |
| CRISPR-*AtCUP1* vector construction | U6-26-T1-F | GATTGGTTTTGGGTCTCGTCGTCG |
| | U6-26-T1-R | AAACCGACGACGAGACCCAAAACC |
| | U6-29-T2-F | GATTGCTTGTCTATTCGCTCATGA |
| | U6-29-T2-R | AAACTCATGAGCGAATAGACAAGC |
| | U6-26-T3-F | GATTGATTCCTCCACCTTTCACCT |
| | U6-26-T3-R | AAACAGGTGAAAGGTGGAGGAATC |
| | U6-29-T4-F | GATTGCTCGAATATACAGAACCCA |
| | U6-29-T4-R | AAACTGGGTTCTGTATATTCGAGC |
| | U6-26-T5-F | GATTGGTAAATGCATTCCCTATCA |
| | U6-26-T5-R | AAACTGATAGGGAATGCATTTACC |
| | U6-29-T6-F | GATTGTGTTGCAAGTCTCCTACAA |
| | U6-29-T6-R | AAACTTGTAGGAGACTTGCAACAC |
| Identification of *AtCUP1*-edited lines | T1-T2edi-F | AGAAGAATTGAGAGATAACAGAGAG |
| | T1-T2edi-R | AGGAAGTAAAATCTAAAAGGGGCAA |
| | T3-T6edi-F | CTATTGGGTGCTATCCAGTCGCTCT |
| | T3-T6edi-R | CTTCTTCTGTGTTTCTTTCT |
| qPCR analysis of *BnCUP1* under Cd stress | BnCUP1-1a-qpcr-F | TCGTTGGATCTCTTGCCGAC |
| | BnCUP1-1a-qpcr-R | CAGCGACAAGCCATGATTCG |
| | BnCUP1-1b-qpcr-F | CCCTGAGGAAGCAAGAAGCA |
| | BnCUP1-1b-qpcr-R | GCCTTTGGCTTGTCAGCAAT |
| | BnCUP1-1c-qpcr-F | GCTCTAAGCCCCAACGATGA |
| | BnCUP1-1c-qpcr-R | AATAGTGAGGCCGCAGACAC |
| | BnCUP1-1d-qpcr-F | GCAATCGCCATAGCCTCTGA |
| | BnCUP1-1d-qpcr-R | CATCGTTGGGGCTTAGAGCA |
| CRISPR-*BnCUP1* vector construction | S12-s1-BsF | ATATATGGTCTCGATTGTTGGTTCCTCTATGCTCTTGTT |
| | S12-s1-F0 | TGTTGGTTCCTCTATGCTCTTGTTTTAGAGCTAGAAATAGC |
| | S12-s2-R0 | AACTGTATTGAGGAGAATGCTTCAATCTCTTAGTCGACTCTAC |
| | S12-s2-BsR | ATTATTGGTCTCGAAACTGTATTGAGGAGAATGCTTCAA |
| | S34-s3-BsF | ATATATGGTCTCGATTGTAGCACCTTCCAAAGTAAAGTT |
| | S34-s3-F0 | TGTAGCACCTTCCAAAGTAAAGTTTTAGAGCTAGAAATAGC |
| | S34-s4-R0 | AACAAACAGTCCTACACACGCCCAATCTCTTAGTCGACTCTAC |
| | S34-s4-BsR | ATTATTGGTCTCGAAACAAACAGTCCTACACACGCCCAA |
| Identification of *BnCUP1*-edited lines | S12-1a-s2-edi-F | TACTCTACTACTATGAGTTAGAGACTGGTTGT |
| | S12-1a-s2-edi-R | AATAAAAGCATCAGAAAGAGAGGAAACTCAC |
| | S12-1a-s1-edi-F | TATGTTCTTGTACTTAATGGCAAATCTCAACA |
| | S12-1a-s1-edi-R | ATAAACCTTTGGTAACTTTACCTTATTGTGCT |
| | S12-1b-s1-edi-F | GTTTATAGGAAGGTGGAGTTTTGGTATATGTC |
| | S12-1b-s1-edi-R | AACATAATAAAAGCATGAGAGAGAGAGAGAGA |

| | | |
|---|---|---|
| | S12-1c-s1-edi-F | TATGGGTATAGCAAAGGGGACATTGG |
| | S12-1c-s1-edi-R | AATATGTAAGTAATACAGTACGTAACGCACGC |
| | S12-1d-s1-edi-F | ATGATCTAACCGGAGTTTGATATTTTGTGAAT |
| | S12-1d-s1-edi-R | TTGTTGAGATTTGCCATTTAGTAGTGTAAGAA |
| | S34-1a-edi-F | TGTGGGTTCTTCTTTGCGTGC |
| | S34-1a-edi-R | GATTGTGCTTCTGGCTTCCTCA |
| | S34-1b-edi-F | TGCTGCCTCACTATTGCTTCCA |
| | S34-1b-edi--R | GTGCTTCTTGCTTCCTCAGGG |
| | S34-1d-edi-F | CGCTTCGATGCTCGGCAG |
| | S34-1d-edi-R | TGGCTTCCTCAGGTATGTATTGGG |
| | S34-1c-edi-F | GCATCTCGTCTGTTGTCTCGC |
| | S34-1c-edi-R | TGGCTTCCTCAGGGATGTATTGG |
| | S12-C07-F | CCTCGACTTGATCCGACTTGT |
| | S12-C07-R | CTCGGCGATCCCTTTTACCT |
| | S12-C08-F | TACGTCCTCGTCTTCTCCGT |
| | S12-C08-R | TGCTCAGCAATGAGCCAAGA |
| | S12-CNN-F | CACGTCAGAATGTTGATTTCCTCA |
| | S12-CNN-R | TGCAACCTTGTACCTACACAA |
| | S12-C03-F | TCGCTTATCTTGCGCCTCAC |
| | S12-C03-R | CCGGAGAAACAGAGGCTGAG |
| Off-target detection of | S12-A03-F | ACAAGCTCCTGTAACCGGC |
| *BnCUP1*-edited lines | S12-A03-R | TTGCCGGAAAGTCAGAGGTC |
| | S34-A10-F | TGCGTGTCCTACTTGTGCTT |
| | S34-A10-R | GTCACAACATTTCCCAATGGCT |
| | S34-C03-F | TTGGTCCCTACAGGCCAAC |
| | S34-C03-R | TCCCATCCAAGGAACTGGCTA |
| | S34-C04-F | TGAGGAAGAACCAGCAGCAG |
| | S34-C04-R | CATCCAAGGAGCTAGCCAGG |
| | S34-C08-F | AGTGCCGACAGTGAAAACCA |
| | S34-C08-R | CCGAGCTTCTGACGCTACTA |

**Table S2 Detection of potential off-target sequences**

| Sequence name | Sequence | putative off-target locus | off-target (Y/N) |
|---|---|---|---|
| CRISPR-S12-sgRNA1 | TTTGGTTCCTCTATGCTCTTTGG | - | - |
| OFF_1 | ATTGGTCCCTCCATACTCTTCGG | BnaC07g43370D | N |
| OFF_2 | TTCGGCTCCTCTATGCTCTTTGG | BnaC08g21010D | N |
| CRISPR-S12-sgRNA2 | ATGGTGGGCCGTGTGCTGGGAGG | - | - |
| OFF_1 | ATAGTGGCCCGTGTGCTTCGAGG | BnaCnng06240D | N |
| OFF_2 | AAGGTGAGCCGTAAGCTGGGAAG | BnaC03g35330D | N |
| OFF_3 | AGGGTGAGCCGTAAGCTGGGAAG | BnaA03g30060D | N |
| CRISPR-S34-sgRNA3 | GTAGCACCTTCCAAAGTAAAGGG | - | - |
| OFF_1 | GTAACACCTTCCAAAATATATGG | BnaA10g08980D | N |
| OFF_2 | GTAGCACAAACCGAAGTAAACGG | BnaC03g22100D | N |
| OFF_3 | GTAGCACAAACCGAAGTAAACGG | BnaC04g06140D | N |
| OFF_4 | GGAGCACCTTCCACAATATATGG | BnaC08g36380D | N |
| CRISPR-S34-sgRNA4 | AGGCGTGTGTAGGACTGTTTTGG | - | - |
| OFF_ | None | - | - |

**Table S3 Detection of physical and chemical properties of the soil**

| Soil type | PH | Total N (g/kg) | Total P (g/kg) | Total K (g/kg) | Organic matter (g/kg) | Cd concentration (mg/kg) |
|---|---|---|---|---|---|---|
| soil 1 | 5.80±0.07 | 1.35±0.05 | 0.78±0.05 | 6.03±0.01 | 21.68±0.77 | 5.12±0.31 |
| control | 6.62±0.09 | 1.57±0.05 | 0.62±0.01 | 8.45±0.06 | 26.05±0.83 | 0.14±0.02 |

Note: Soil 1 is the experimental soil; control is the field soil with low-Cd concentration.

**Table S4 Statistics of agronomic characters of three lines grown in the control soil**

| Material | Branch number | Plant height (cm) | silique number per plant | number of seeds per silique | 1000-seed weight (g) | Yield/plant (g) |
|---|---|---|---|---|---|---|
| Westar | 9.67±0.67 | 157.80±2.54 | 352.47±12.91 | 19.60±0.50 | 3.90±0.05 | 26.91±1.23 |
| *S12* | 9.53±0.48 | 156.53±3.14 | 348.27±11.84 | 19.92±0.49 | 3.72±0.08 | 25.62±0.82 |
| *S34* | 9.80±0.56 | 160.27±2.95 | 351.40±16.02 | 20.356±0.35 | 3.69±0.07* | 26.22±1.12 |

Note: The data represent the mean ± SD; Significant differences were tested using the least significant difference (LSD) method (*P < 0.05, **P < 0.01).