

# Arabidopsis ribosomal proteins RPL23aA and RPL23aB are functionally equivalent

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## Research article

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# Abstract

**Background:** In plants, each ribosomal protein (RP) is encoded by a small gene family but it is largely unknown whether the family members are functionally diversified. There are two *RPL23a* paralogous genes (*RPL23aA* and *RPL23aB*) found in *Arabidopsis thaliana*. Knock-down of *RPL23aA* using RNAi impeded growth and led to morphological abnormalities, whereas knock-out of *RPL23aB* had no observable phenotype, thus these two RPL23a paralogous proteins have been used as examples of ribosomal protein paralogues with functional divergence in many published papers.

**Results:** In this study, we characterized T-DNA insertion mutants of *RPL23aA* and *RPL23aB*. A rare non-allelic non-complementation phenomenon was found in the F1 progeny of the *rpl23aa* X *rpl23ab* cross, which revealed a dosage effect of these two genes. Both of *RPL23aA* and *RPL23aB* were found to be expressed almost in all examined tissues as revealed by GUS reporter analysis. Expression of *RPL23aB* driven by the *RPL23aA* promoter can rescue the phenotype of *rpl23aa*, indicating these two proteins are actually equivalent in function. Interestingly, based on the publicly available RNA-seq data, we found that these two *RPL23a* paralogues were expressed in a concerted manner and the expression level of *RPL23aA* was much higher than that of *RPL23aB* at different developmental stages and in different tissues.

**Conclusions:** Our findings suggest that RPL23aA and RPL23aB proteins actually have equal function and presence of paralogous genes for the RPL23a protein in plants might be necessary to maintain its adequate dosage.

## Background

Ribosome, a ribonucleoprotein complex formed from a large and a small subunit, is responsible for polypeptide synthesis in all living cells. In plants, the large ribosomal subunit is composed of 28S, 5.8S and 5S rRNAs together with 48 RPL (Ribosomal Protein of Large subunit) proteins, whereas the small subunit is composed of 18S rRNA and 33 RPS (Ribosomal Protein of Small subunit) proteins [1]; [2]. In *E. coli*, genes encoding RPs are arranged in about 20 operons, with approximately half of the genes mapping to a single locus [3]; [4]. In mammals, although there are about 2,000 sequences which may encode RPs, most of them are predicted to be pseudogenes, and most functional RPs are encoded by a single copy [5]. In yeast *Saccharomyces cerevisiae*, two-thirds of the RPs are encoded by gene families with more than one member. Although there is substantial redundancy between duplicate genes, there is a degree of non-redundant functions for some RPs [6]; [7]; [8].

Plants have even more gene members encoding a single RP than yeast. In *Arabidopsis thaliana*, each RP is encoded by a small gene family with several family members, which share between 65% and 100% amino acid sequence identity [9]. Assessment of cognate EST (expressed sequence tag) numbers of RP genes suggested that RP gene family members were differentially expressed in *Arabidopsis* [9]. Microarray data also revealed that transcripts of genes encoding RPs within the same family were

accumulated at different levels in Arabidopsis [10]. Under various stimuli, while the transcript level for most ribosomal protein genes remain unchanged, some RP genes show significantly increased or decreased transcript levels [10]. Many studies have investigated the functional consequence of absent/reduced expression of a single RP paralogue in Arabidopsis. Disruptions in any one of the RP protein genes, RPL3A, RPL8A, RPL19A, RPL23C, RPL40B, and RPS11A, is embryo lethal [11]. Less severe phenotypes were reported for mutations in several other RPs. Morphological changes of early vegetative leaves from the spatulate wild type shape to a pointed, narrow shape were found in mutants of some RP genes, including RPL5A, RPL5B, RPL9C, RPL10aB, RPL24B, RPL28A, RPS13B, and RPS18A ; [12]; [13]; [14]; [15]; [16]. Despite these studies on RPs, it remains unknown why RPs are encoded by paralogues in plants or whether RP paralogues have specialized functions.

In Arabidopsis, the RPL23a family consists of two paralogues (RPL23aA and RPL23aB), which encode proteins with 95% amino acid identity. Both paralogues are transcribed and translated, and protein products of either paralogue can be incorporated into the ribosome [17]; [18]. Knock-down of the RPL23aA gene through RNAi results in severe developmental defects, whereas knock-down, or even knock-out, of RPL23aB has no phenotypic consequences [19], which could be the basis for the argument that RPL23aA and RPL23aB had specialized functions [10]; [11]; [19]; [20]; [21].

With the general question of why plant RPs are encoded by paralogous genes in mind, we sought to study the functional relationship between RPL23aA and RPL23aB. With T-DNA insertion mutants in RPL23aA and RPL23aB, we found a rare non-allelic non-complementation phenomenon, indicating that two (out of the four) gene copies in a diploid plant being functional is not sufficient to produce a wild-type phenotype. We showed that expression of RPL23aB driven by the RPL23aA promoter can rescue the phenotype of *rpl23aa*, demonstrating that RPL23aA and RPL23aB proteins are functionally equivalent. Furthermore, interrogation of RNA-seq data from several developmental stages and in different organs showed that although the level of RPL23aA transcripts is much higher than that of RPL23aB, the fluctuations in expression of the two genes were well matched, suggesting that these two genes were coordinately regulated. These results revealed that duplicated RPL23a genes contribute to ribosome dosage necessary for plant growth and development.

## Methods

### Plant material and growth conditions

*Arabidopsis thaliana* wild type Columbia-0 (Col-0) and the T-DNA insertion lines, SALK\_005448 (named here *rpl23aa*) and SAIL\_597\_B08 (named here as *rpl23ab*), were obtained from the Arabidopsis Biological Resource Center (ABRC). Seeds were first treated for 2 minutes in 75% ethanol, then treated for 6 minutes in commercial bleach and rinsed at least 3 times with sterile distilled water. Solid medium consisted of 2.2 g/L Murashige and Skoog basal salt mixture (Phyto Tech Labs), 10 g/L sucrose, and 8 g/L agar. pH was adjusted to 5.6 with KOH before autoclaving. When required, BASTA (GOLDBIO) was added at a final concentration of 125 µg/L. Seeds were sown in a water suspension, using a 1.5 mL pipette tip, in 150 mm

Petri dishes filled with 120 ml of solid culture medium, at a density of 150 regularly spaced seeds per plate. Once inoculated, the Petri dishes were sealed with Micropore Scotch 3M surgical tape, which prevented contamination but allowed gaseous exchange, and placed in 4°C for 24 h. Growth was allowed to proceed at 22°C in Percival tissue culture chambers under long day conditions (16 hours light and 8 hours dark). 10-day seedlings were then transplanted to pots containing a 1:2:2 mixture of perlite, vermiculite and soil at 22°C under long day conditions from a combination of incandescent and fluorescent lamps (10000 lux). Plants were watered twice a week with nutrient solution.

#### RNA isolation and RT-PCR

50 mg seedlings from 14-day-old Col-0, *rpl23aa*, and *rpl23ab* were harvested and immediately frozen in liquid nitrogen. RNA was extracted using RNAiso Plus (TAKARA BIO INC). In the elution step, RNA was resuspended in DEPC-treated water. cDNA was obtained by reverse transcription of 1 µg of RNA with the PrimeScript<sup>TM</sup> RT reagent Kit with gDNA Eraser (TAKARA BIO INC).

#### Plasmid construction and generation of transgenic plants

In order to construct the pRPL23aA::RPL23aA and pRPL23aB::RPL23aB plasmids, we amplified the full-length coding sequences (including the promoter region) of RPL23aA (AT2G39460) and RPL23aB (AT3G55280) genes from Col-0 genomic DNA using Phusion polymerase (Thermo Scientific). The primers used are shown in Table S1 (Additional file 10). The amplified DNA sequences were cloned in pEG301 [22] to result in pRPL23aA::RPL23aA and pRPL23aB::RPL23aB. The plasmids were used to transform *rpl23aa*. For pRPL23aA::RPL23aB construction, the promoter region (about 1.5 kb) of RPL23aA plus the coding region of RPL23aB were synthesized by a commercial company (GENEWIZ SuZhou), then the synthesized DNA fragment was sequenced and was cloned in pEG301. The promoter regions of RPL23aA (AT2G39460) and RPL23aB (AT3G55280) were cloned into pMDC162 [22] to generate the plasmids pRPL23aA::GUS and pRPL23aB::GUS, which were then used to transform Col-0 plants. T1 transgenic plants were screened on solid 1/2 Murashige & Skoog (MS) medium with 25 mg/L Hygromycin B and verified by PCR. GUS staining was carried out with plants in the T2 generation.

#### GUS staining assay

8-days-old seedlings and 36-days-old inflorescences, immature and mature flowers, immature and mature siliques of Col-0, pRPL23aA::GUS and pRPL23aB::GUS were subjected to histochemical GUS staining according to the standard protocol [23].

#### Transcripts profiling

RNA-seq data was obtained from a public website (<http://travadb.org/browse/DeSeq/>), and the average value of normalized absolute read counts from two biological replicates was extracted. We also downloaded the original RNA-seq data of *A. thaliana* different organs and developmental stages from NCBI Sequence Read Archive (project ID PRJNA314076 for samples except meristem and project ID PRJNA268115 for the meristem samples). The RPKM (Reads Per Kilobase per Million mapped reads) value of RPL23aA (AT2G39460), RPL23aB (AT2G39460), and ACT2 (AT3G18780) were calculated. Our

calculated RPKM value is consistent with the value of normalized absolute read counts obtained from the public website (<http://travadb.org/browse/DeSeq/>).

## Results

### Characterization of *rpl23aa* and *rpl23ab* mutants

The Arabidopsis genome contains two RPL23a paralogous genes RPL23aA (At2g39460) and RPL23aB (At3g55280), which encode proteins with 95% amino acids identity (see Additional file 1). We acquired T-DNA insertion lines of RPL23aA and RPL23aB, namely SALK\_005448 and SAIL-597-B08, respectively (hereafter referred to as *rpl23aa* and *rpl23ab*). PCR-genotyping confirmed that both *rpl23aa* and *rpl23ab* are homozygous T-DNA insertion alleles (see Additional file 2). Sequencing results revealed that *rpl23aa* contains a T-DNA insertion in the 3' UTR region, 10 bp downstream of the stop codon of the RPL23aA gene (Fig. 1A), while *rpl23ab* contains a T-DNA insertion in the second exon of RPL23aB (Fig. 1B). A semi-quantitative RT-PCR assay was used to detect transcripts from RPL23aA and RPL23aB in these T-DNA lines. As shown in Fig. 1C, the 3' region around the stop codon of the RPL23aA mRNA was disrupted in the mutant. Because the majority of the RPL23aA mRNA from the T-DNA line was intact, we suspect that SALK\_005448 is a hypomorphic allele. *rpl23ab* is likely null mutant, because no RPL23aB mRNA was detected (Fig. 1D). Absence of dosage compensation by RPL23aA in Arabidopsis was reported following loss of RPL23aB [24]. As shown in Fig. 1D, there is also no dosage compensation by RPL23aB in the *rpl23aa* mutant.

The *rpl23aa* mutant exhibits pleiotropic defects, including pointed leaves, retarded root growth, and reduced plant size (Fig. 2B). These phenotypes are similar to those of a previously reported RNAi line [19]. An incompletely penetrant tricotyledon phenotype (less than 5% of the total population) was observed in *rpl23aa* mutant plants (see Additional file 3). However, we didn't observe appreciable defects in terms of growth rate, morphology, flowering or fertility in the *rpl23ab* mutant (Fig. 2D), which is consistent with published work [24]. We amplified genomic DNA encompassing the promoter plus the coding region of RPL23aA from wild-type plants and fused it to the sequence encoding the HA epitope tag. When this transgene was introduced into *rpl23aa*, the developmental defects were fully rescued (Fig. 2C), suggesting that dysfunction of RPL23aA was responsible for the developmental defects in *rpl23aa*.

### RPL23aA and RPL23aB are dosage-dependent genes

In order to study the genetic interaction between RPL23aA and RPL23aB, we crossed *rpl23aa* with *rpl23ab*. To our surprise, the doubly heterozygous plants (RPL23aA/*rpl23aa*; RPL23aB/*rpl23ab*) in the F1 progeny all have pointed first true leaves (Fig. 3B). Siliques of the doubly heterozygous plants are much shorter than siliques of *rpl23aa* or *rpl23ab* (Fig. 3I). We dissected siliques from RPL23aA/*rpl23aa*; RPL23aB/*rpl23ab* plants and found many aborted ovules (Fig. 3G and 3H). An F2 population was generated by selfing the above F1 plants. We genotyped 144 F2 plants but did not find double homozygous (*rpl23aa* /*rpl23aa*; *rpl23ab* /*rpl23ab*) plants. In fact, we did not even detect any genotypes with a single functional allele from either gene (RPL23aA/*rpl23aa*; *rpl23ab* /*rpl23ab* or *rpl23aa* /*rpl23aa*;

RPL23aB/rpl23ab) (Table 1), although these genotypes are collectively expected to appear in 31.25% (5 out of 16) of the F<sub>2</sub> plants. We suspected this non-allelic non-complementation phenomenon between rpl23aa and rpl23ab is probably due to gene dosage effects.

Table 1  
Genetic interactions between rpl23aa and rpl23ab.

Genotype	First leaf phenotype	
	Pointed	Normal
RPL23aA/RPL23aA RPL23aB/RPL23aB	0	15
RPL23aA/RPL23aA RPL23aB/rpl23ab	0	37
RPL23aA/RPL23aA rpl23ab/rpl23ab	0	19
RPL23aA/rpl23aa RPL23aB/RPL23aB	0	26
RPL23aA/rpl23aa RPL23aB/rpl23ab	38	0
RPL23aA/rpl23aa rpl23ab/rpl23ab	0	0
rpl23aa/rpl23aa RPL23aB/RPL23aB	9	0
rpl23aa/rpl23aa RPL23aB/rpl23ab	0	0
rpl23aa/rpl23aa rpl23ab/rpl23ab	0	0
rpl23aa and rpl23ab were crossed and the F <sub>2</sub> plants were subjected to genotyping at the RPL23aA and RPL23aB loci. Leaf phenotype of the plants was classified into pointed or normal. Primers for genotyping are listed in Table S1 (Additional file 10).		
(A), (B) Structure of the RPL23aA and RPL23aB paralogous genes, with the positions of the T-DNA insertions in rpl23aa and rpl23ab mutants indicated by black triangles. Black boxes and lines between black boxes indicate exons and introns, respectively. White boxes correspond to the 5' and 3' untranslated regions. Long arrows indicate promoters. Short arrows represent primers used in RT-PCR in (C) and (D). (C), (D) Semi-quantitative RT-PCR analysis of RPL23aA and RPL23aB transcripts in the corresponding mutant background. The full-length gel of (C) is presented in Supplementary Figure S6 (Additional file 6), and the full-length gel of (D) is presented in Supplementary Figure S7 (Additional file 7).		

### RPL23aA and RPL23aB genes are ubiquitously expressed

In order to investigate the expression pattern of RPL23aA and RPL23aB genes, we fused the promoter regions of RPL23aA and RPL23aB genes to the GUS reporter and generated transgenic plants in the Col-0 background. GUS staining of 14 pRPL23aA:GUS and 5 pRPL23aB:GUS independent transgenic lines uncovered a ubiquitous expression pattern for both genes with particularly intense GUS staining in young and actively proliferating tissues, such as developing leaves, floral buds and root apices (Fig. 4). Similar expression patterns of RPL23aA and RPL23aB support our hypothesis that the non-allelic non-

complementation phenomenon between these two genes is the consequence of overlap in expression (and function) of RPL23aA and RPL23aB in the same cells.

RPL23aA and RPL23aB proteins are functionally equivalent

It has been reported that some paralogous ribosomal proteins have evolved specialized functions in yeast [7]. As mentioned above, dysfunction of RPL23aA results in severe developmental defects, whereas knock-out of RPL23aB has no phenotypic consequences in Arabidopsis. It's natural to assume that these two paralogous ribosomal proteins have undergone functional specialization.

We designed gene complementation experiments to explore whether RPL23aA and RPL23aB have distinct functions. If RPL23aA and RPL23aB have specialized functions, RPL23aB is not expected to complement the *rpl23aa* mutation. We fused the promoter regions of RPL23aA to the coding region of RPL23aB. The pRPL23aA:RPL23aB transgene was introduced into *rpl23aa* plants, and 21 independent pRPL23aA:RPL23aB transgene lines were obtained, among which 15 lines rescued the phenotype of *rpl23aa* (Fig. 5C), indicating that RPL23aA and RPL23aB have equivalent function. The pRPL23aB:RPL23aB transgene was also introduced into *rpl23aa* plants, and 8 out of 15 independent, homozygous transgenic lines exhibited near wild type morphology (Fig. 5D). However, a portion (about 2%) of the transgenic plants of each line exhibited the tricotyledon phenotype (see Additional file 4). Thus, the pRPL23aB:RPL23aB transgene can largely but not fully rescue the phenotype of *rpl23aa*.

RPL23aA and RPL23aB genes are transcribed in a concerted manner with higher expression level of RPL23aA than RPL23aB

Since the above results indicated that RPL23aA and RPL23aB proteins have equivalent function, we suspected that the difference in phenotype between *rpl23aa* and *rpl23ab* is due to the difference in the expression levels of these two genes. The expression of RPL23aA may be much higher than RPL23aB, so the impacts on ribosomes by the *rpl23aa* mutation are higher than the *rpl23ab* mutation thus leading to much severe morphological defects. We compared the transcript levels of RPL23aA and RPL23aB at different developmental stages and in different organs by analyzing published RNA-seq data [25]. As shown in Fig. 6 and Figure S5(Additional file 5), transcript levels of RPL23aA are much higher than those of RPL23aB at all developmental stages and in all the examined tissues. Strikingly, the spatial and temporal patterns of expression of these two paralogous genes are well matched, suggesting that they are similarly regulated at differently developmental stages in all examined tissues. ACT2, which is a house keeping gene, was included as a control. Transcript levels of RPL23aB are higher than ACT2 in some organs, and total amount of PRL23a transcripts is much higher than ACT2 in most examined organs (Fig. 6C, 6E), indicating that RPs are in great demand for plant development.

## Discussion

RPs are encoded by small gene families in plants. Some of the paralogous RPs are identical in amino acid sequences such as RPL36aA and RPL36aB, but many of the paralogues display sequence variations

and are differentially expressed during development. The presence of multiple gene members for each RP in plants might be necessary to maintain adequate RP doses or to maintain some degree of ribosome heterogeneity and functional specialization.

In this study, we characterized the RPL23a gene family containing two highly homologous family members. The hypomorphic T-DNA insertion allele of RPL23aA exhibits pleiotropic defects. However, knock-out of RPL23aB has no appreciable phenotypic impacts. We crossed mutants of RPL23aA and RPL23aB and found a non-allelic non-complementation phenomenon in their F1 progeny. This phenomenon is also found in other RP coding gene families such as RPL5 [26], RPL36a [27], and RPS6 [28]. However, mutations in the paralogues within RPL5, RPL36a, and RPS6 families caused almost the same phenotype, indicating that the paralogues are functionally equivalent. In the case of the RPL23a family, phenotypes of the single mutants suggest unequal functions of the two paralogues. The non-allelic non-complementation phenomenon may be due to a dosage problem - reduced dosage at one of the paralogues still supports the wild phenotype but simultaneous reduction of dosage at both paralogues could not sustain the wild phenotype. For the dosage effect hypothesis to be true, there must be at least some overlap in the expression of the gene family members. Indeed, promoter-GUS experiments demonstrated that both RPL23aA and RPL23aB were ubiquitously expressed.

Phenotypical differences between members of an RP within a family might result from diversification of protein function or variation in levels and patterns of expression. We demonstrated that RPL23aA and RPL23aB proteins had equal function, as expression of RPL23aB driven by the RPL23aA promoter could rescue the phenotype of the *rpl23aa* mutant. We found that the expression level of RPL23aA was much higher than that of RPL23aB according to the publicly available RNA-seq data. Thus, the difference in expression levels might be the reason why disruption of RPL23aA and RPL23aB had different consequences. It is interesting that despite the difference in expression levels, the temporal and spatial patterns of expression of the two paralogous genes were nearly identical. These results suggested that RPL23aA and RPL23aB genes are transcribed in a coordinated manner. Posttranscriptional and translational regulation may also play a role in RPL23aA and RPL23aB expression [29]. Subcellular localization specialization could be another factor that causes differences in functional effects between paralogous RPs [30]. Previous studies revealed that both of RPL23aA and RPL23aB are targeted to the nucleolus with RPL23aA targeting being a bit more efficient than RPL23aB [10]; [19]. Targeting of RP to the nucleolus is an essential step in eukaryotic ribosome biogenesis [31]; [32], so the efficiency of RPL23aA assembly into ribosomes may be higher than that of RPL23aB. Although posttranscriptional differences between RPL23aA and RPL23aB may exist, the fact that expression of RPL23aB with the RPL23aA promoter rescues the *rpl23aa* phenotypes indicates that differences in expression level underlie the different functional contributions of the paralogues as exemplified by the single mutant phenotypes. Our findings suggest that the two paralogous RPL23a proteins had equivalent function and the presence of multiple genes for individual RPs in plants might be necessary to maintain adequate ribosome dosage at least for some ribosomal protein families.

# Conclusions

Ribosomal protein RPL23a paralogues (RPL23aA and RPL23aB) have been used as examples of paralogues with functional divergence in many published papers. In this study, our findings provided four convincing evidences demonstrating duplicated RPL23a genes actually have redundant function (without functional specialization), thus are necessary to provide a threshold dose: 1) The non-allelic non-complementation phenomenon between *rpl23aa* and *rpl23ab* suggests RPL23aA and RPL23aB are dosage dependent genes; 2) RPL23aA and RPL23aB genes are expressed in the same tissues; 3) RPL23aB could rescue the phenotype of *rpl23aa*, demonstrating RPL23aA and RPL23aB protein have equal function; 4) RPL23aA and RPL23aB genes are transcribed in a concerted manner without spatiotemporal divergence. Since the presence of multiple genes for individual RP widely exists in plants, our findings will have implications to study other paralogous RP genes.

# Abbreviations

RP: ribosomal protein; RPL: ribosomal protein of large subunit; RPS: ribosomal protein of small subunit; RPKM: Reads Per Kilobase per Million mapped reads

# Declarations

## Ethics approval and consent to participate

Not applicable

## Consent for publication

Not applicable

## Availability of data and materials

The original RNA-seq data of *A. thaliana* different organs and developmental stages were downloaded from NCBI Sequence Read Archive (project ID PRJNA314076 for samples except meristem and project ID PRJNA268115 for the meristem samples).

## Competing interests

The authors declare that they have no competing interests.

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## Author contributions

XC and BM designed experiments; WX, XZC, and CZ carried out experiments; WX, JZ, and TL analyzed the RNA-seq data; XW and LL analyzed experimental results; XW, XZC, XC and BM wrote the manuscript. All authors agree to be accountable for the content of the work.

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## Additional Files

**Additional file 1: Figure S1.** Amino acid sequence alignment between RPL23aA and RPL23aB.

**Additional file 2: Figure S2.** Genotyping of *rpl23aa* and *rpl23ab*.

**Additional file 3: Figure S3.** Images of wild type and *rpl23aa* plants.

**Additional file 4: Figure S4.** Images of *pRPL23aB:RPL23aB rpl23aa* plants.

**Additional file 5: Figure S5.** Transcript profiles of RPL23aA and RPL23aB in different organs.

**Additional file 6: Figure S6.** Full-length gel of Figure 1C.

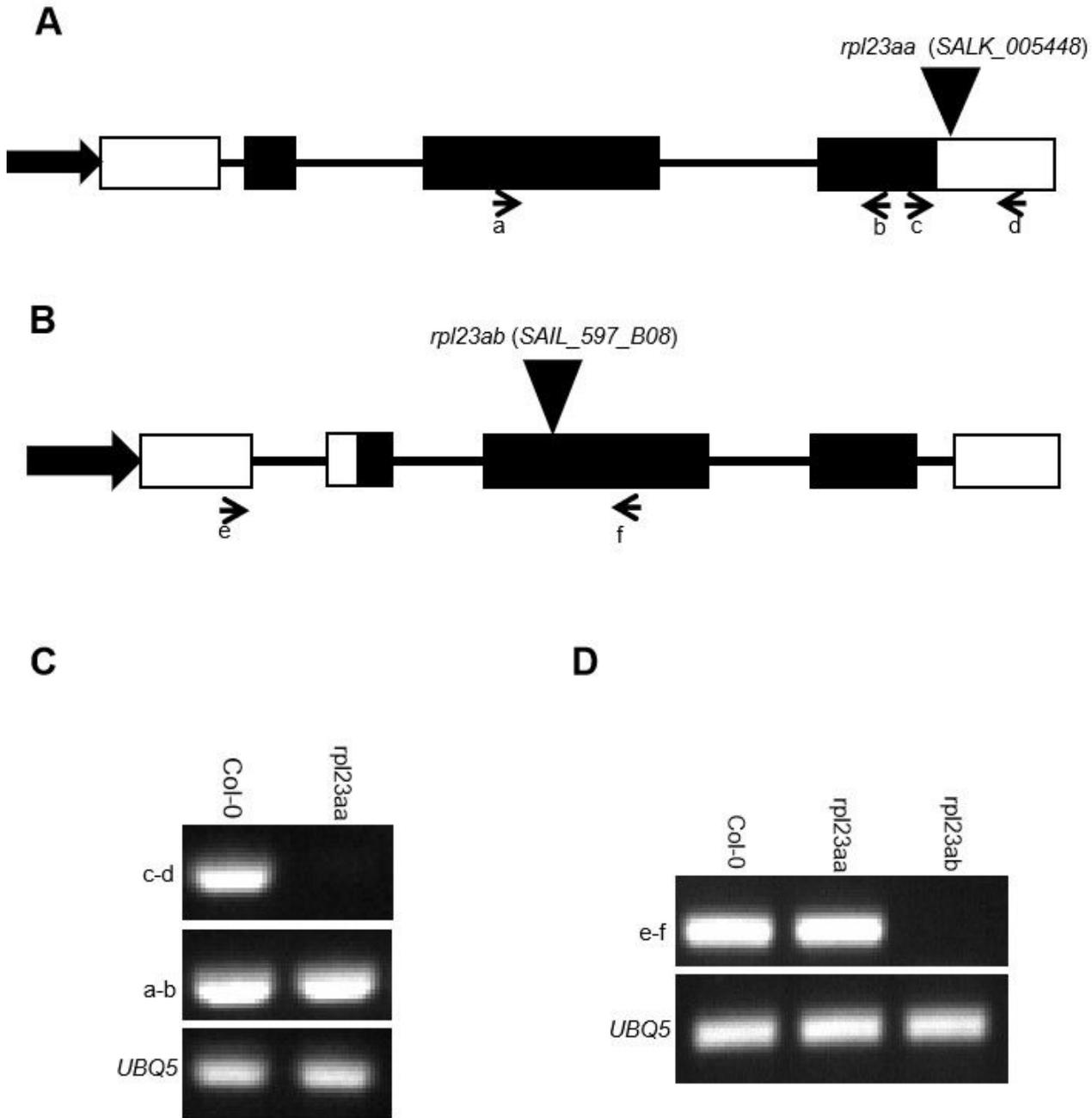
**Additional file 7: Figure S7.** Full-length gel of figure 1D.

**Additional file 8: Figure S8.** Full-length gel of figure S2C.

**Additional file 9: Figure S9.** Full-length gel of figure S2D.

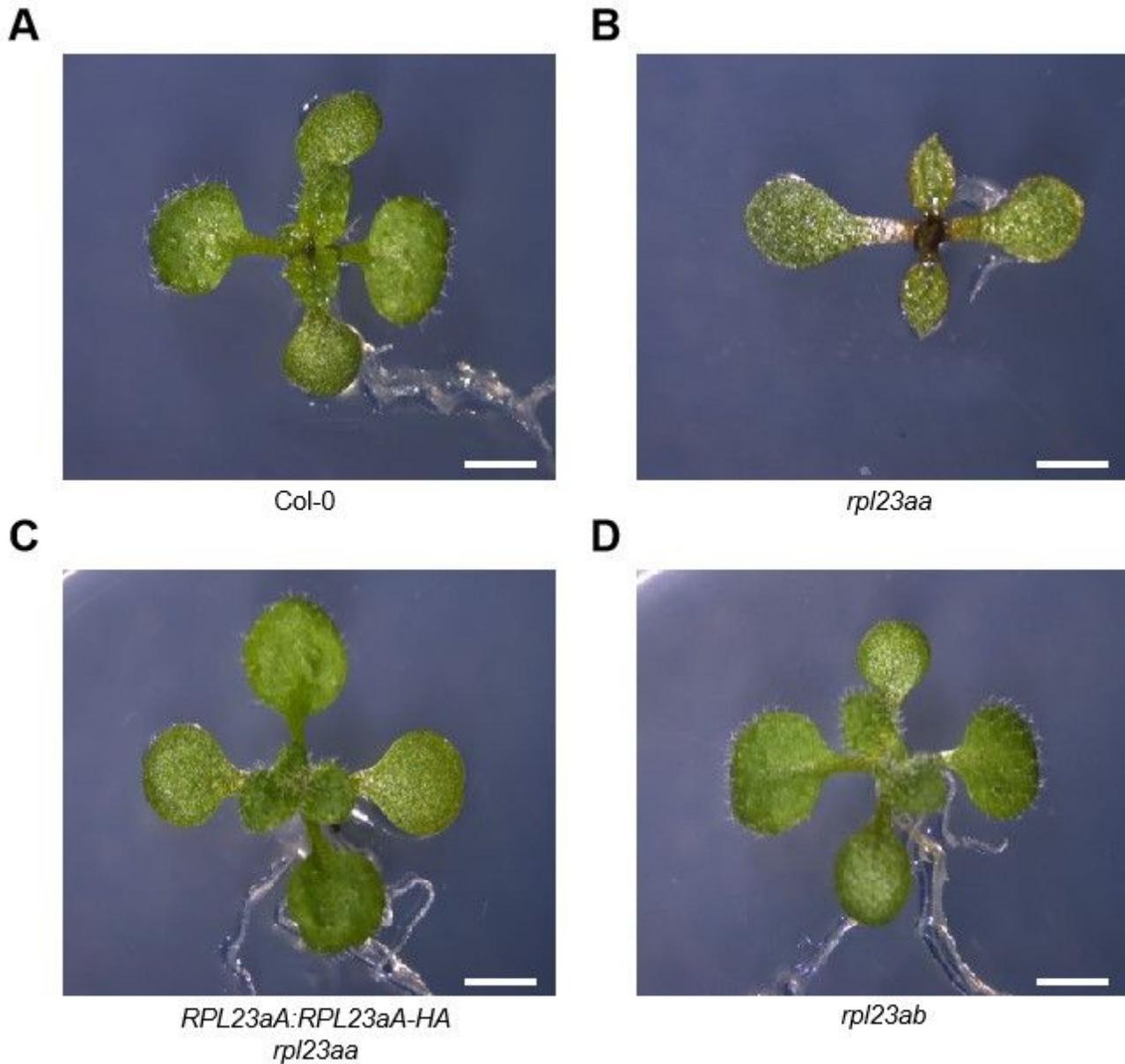
**Additional file 10: Table S1.** Primers used in this work.

## Figures



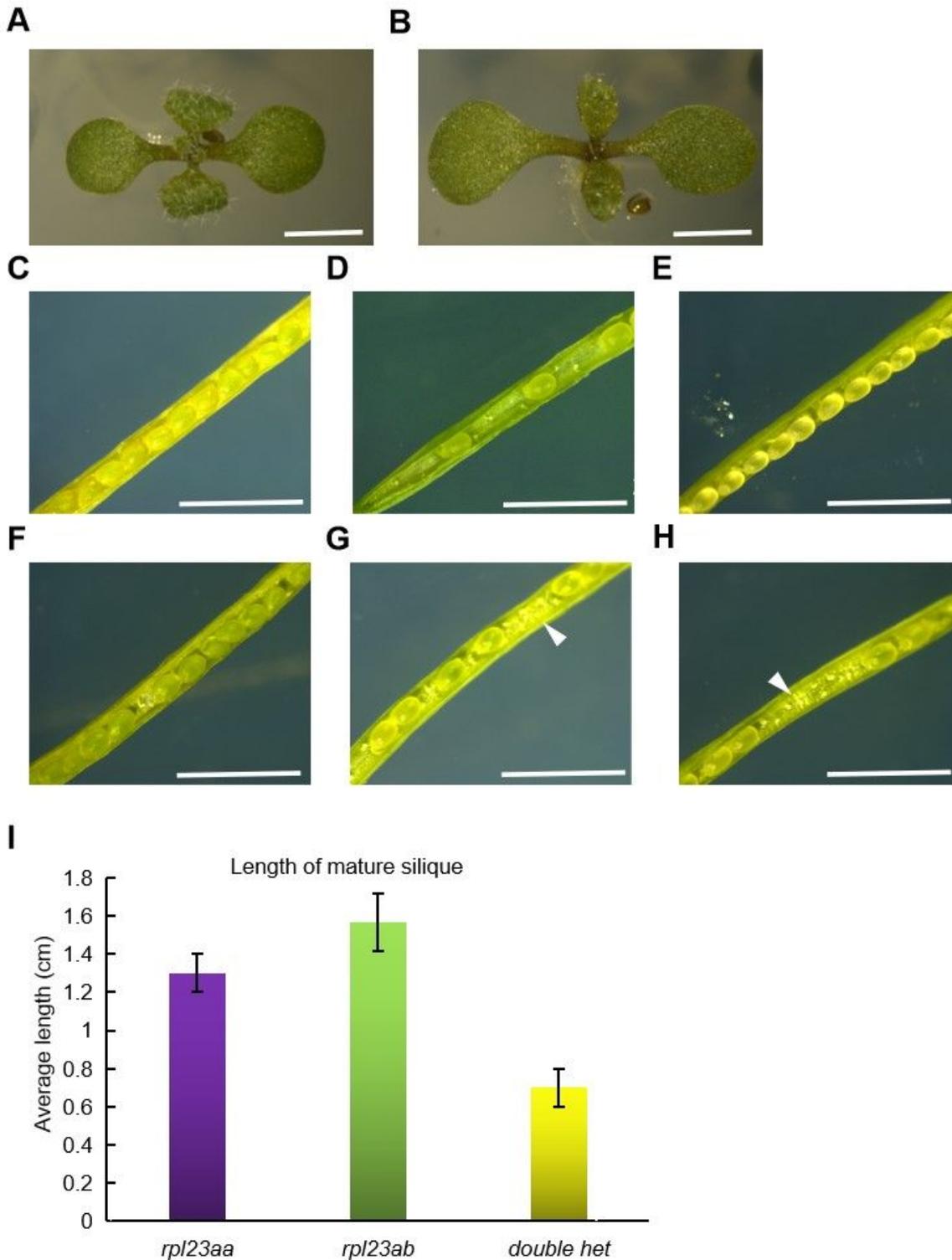
**Figure 1**

Characterization of *rpl23aa* and *rpl23ab* mutants. (A), (B) Structure of the RPL23aA and RPL23aB paralogous genes, with the positions of the T-DNA insertions in *rpl23aa* and *rpl23ab* mutants indicated by black triangles. Black boxes and lines between black boxes indicate exons and introns, respectively. White boxes correspond to the 5' and 3' untranslated regions. Long arrows indicate promoters. Short arrows represent primers used in RT-PCR in (C) and (D). (C), (D) Semi-quantitative RT-PCR analysis of RPL23aA and RPL23aB transcripts in the corresponding mutant background. The full-length gel of (C) is presented in Supplementary Figure S6 (Additional file 6), and the full-length gel of (D) is presented in Supplementary Figure S7 (Additional file 7).



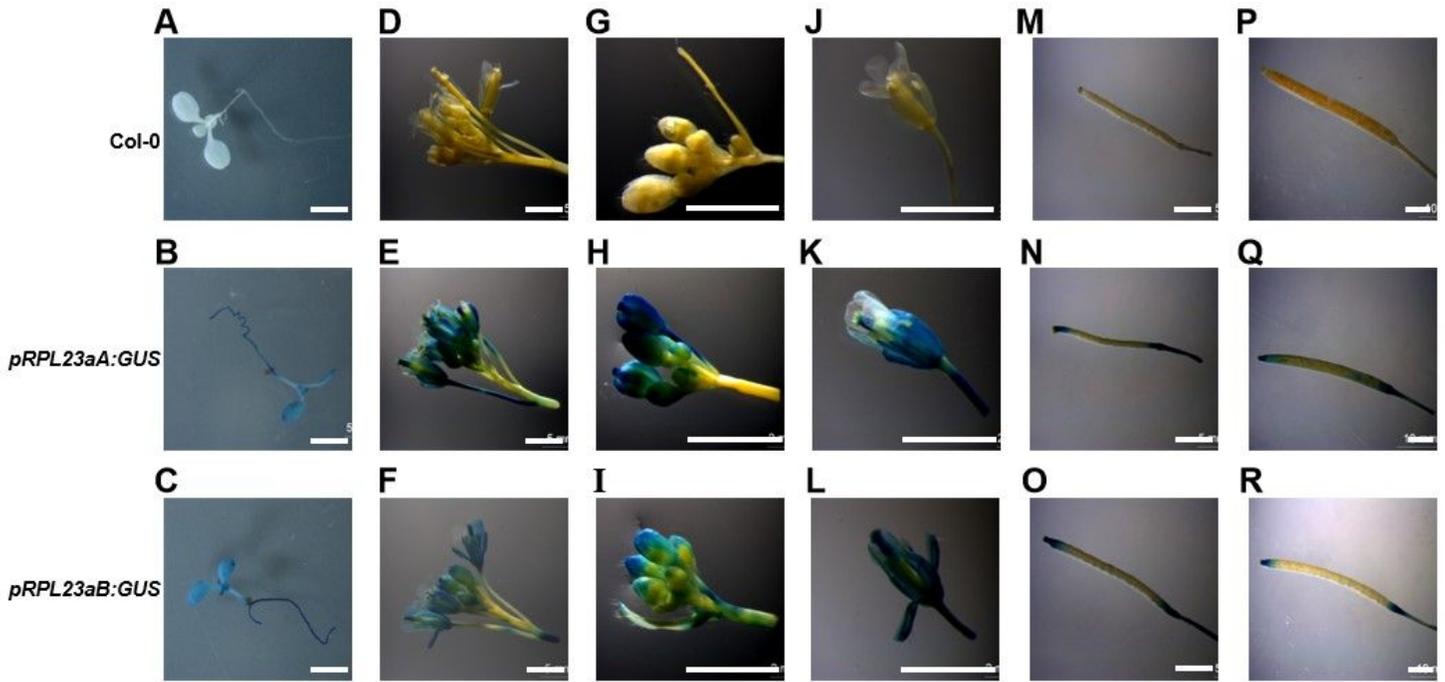
**Figure 2**

Plant phenotypes. 14-day-old plants of (A) Col-0, (B) *rpl23aa*, (C) *pRPL23aA::RPL23aA-HA/rpl23aa*, (D) *rpl23ab*. *rpl23aa* exhibits pleiotropic defects, including pointed leaves, retarded root growth, and reduced plant size; *pRPL23aA::RPL23aA-HA* fully rescued the morphological defects of *rpl23aa*; *rpl23ab* had no observable phenotype. Size bar, 2 mm.



**Figure 3**

The non-allelic non-complementation phenomenon between *rpl23aa* and *rpl23ab*. 9-day-old plants of (A) *rpl23aa* × Col-0 (F1 generation), (B) *rpl23aa* × *rpl23ab* (F1 generation). Dissected mature siliques from (C) Col-0, (D) *rpl23aa*, (E) *rpl23ab*, (F) *rpl23aa* × Col (F1 generation), (G) *rpl23aa* × *rpl23ab* (F1 generation), (H) *rpl23ab* × *rpl23aa* (F1 generation). (I) The length of mature silique from *rpl23ab*, *rpl23aa*, and the double heterozygote (double het). Arrowheads indicate aborted embryos. Size bar, 5 mm.



**Figure 4**

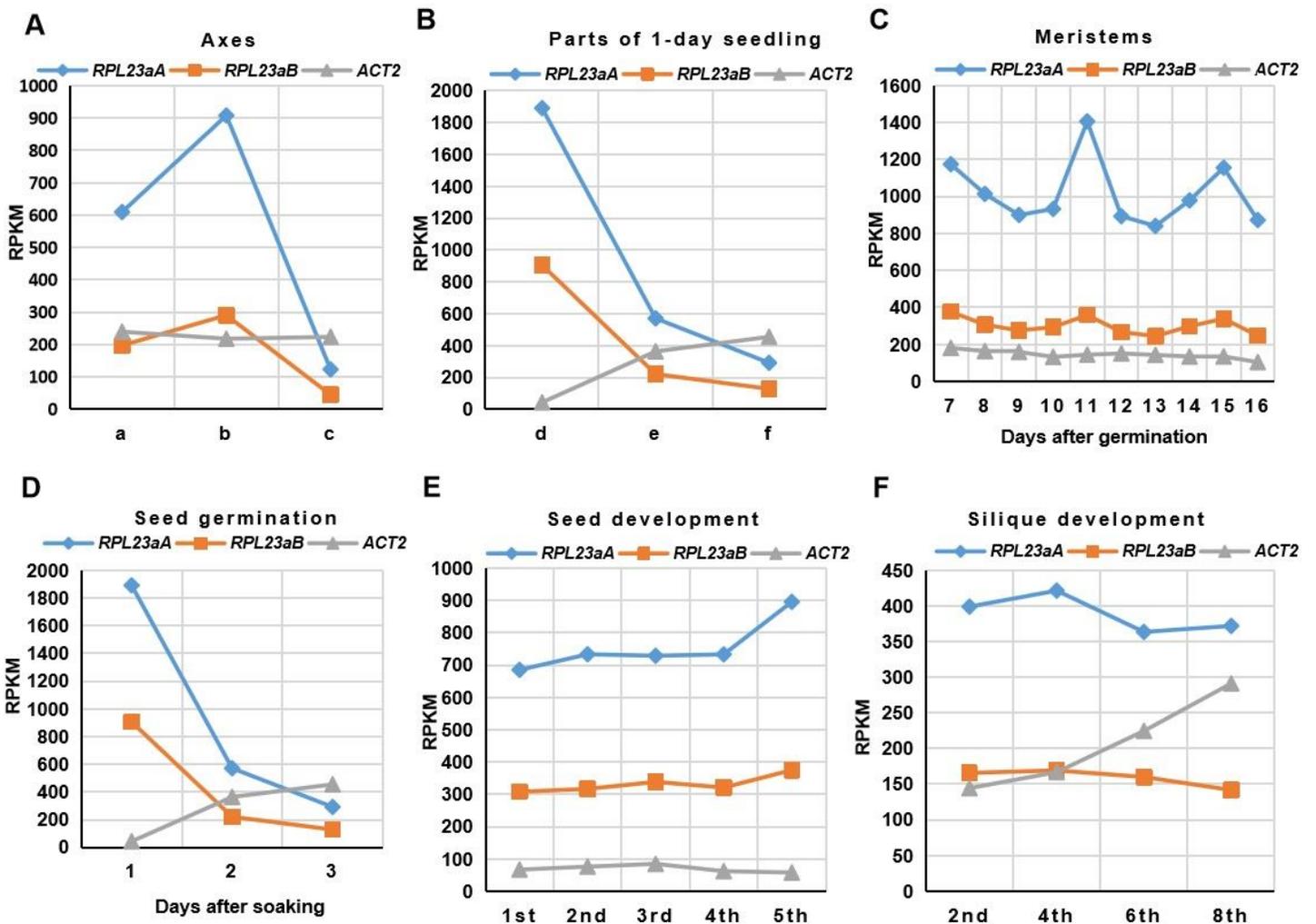
Promoter-GUS reporter analysis of RPL23aA and RPL23aB. (A), (B), (C) Seedling. (D), (E), (F) Inflorescences. (G), (H), (I) Immature and (J), (K), (L) mature flowers. (M), (N), (O) Immature and (P), (Q), (R) mature siliques. Pictures were taken at 8 days (A)-(C) and 36 days (D)-(R). Size bar, 5 mm.



**Figure 5**

Phenotypes of representative 17-day-old plants. Upper left: *rpl23aa*; upper right: *pRPL23aA::RPL23aA/rpl23aa*; lower left: *pRPL23aA::RPL23aB/rpl23aa*; lower right: *pRPL23aB::RPL23aB/rpl23aa*.

pRPL23aB::RPL23aB/rpl23aa; central: Col-0.



**Figure 6**

Transcript profiles of RPL23aA and RPL23aB at different developmental stages and in different organs. Y axis: the average RPKM (Reads Per Kilobase per Million mapped reads) value of two biological replicates. (A) Different parts of axes. a, peduncles; b, inflorescence axis; c, the first elongated internode; (B) Parts of 1-day-old seedling. d, hypocotyl; e, cotyledons; f, apical meristem with adjacent tissues. (C) Meristems after germination. (D) Seed germination after soaking. (E) Seed development. The X-axis represents the siliques from which ovules were taken at the moment when the first silique was 1 cm long. (F) Silique development. The X-axis represents siliques (seeds not removed) sampled at the moment when the first silique was 1 cm long.

## Supplementary Files

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