



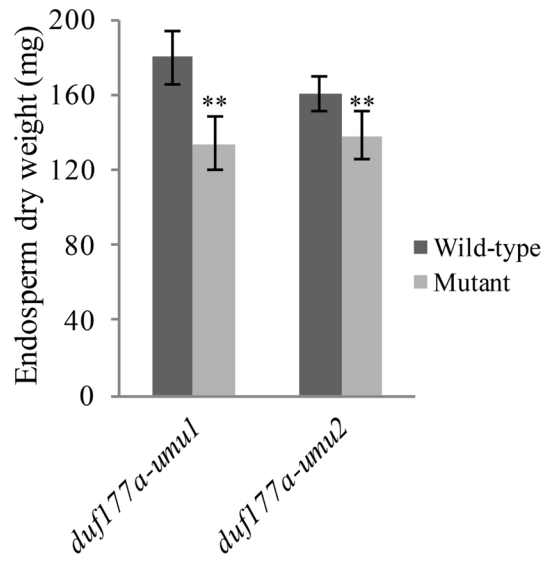
Supplementary Fig. S1. Unrooted tree of DUF177 protein sequences.

Amino acid sequences of DUF177 domain from representative species of plant, algae, and bacteria (Data S2) were aligned by MUSCLE and used to construct a maximum likelihood tree based on the JTT matrix-based model by MEGA6 (Tamura et al., 2013). Bootstrap support was based on 1000 iterations. Bootstrap support values are shown for nodes with 50% or greater. Plant species include *Physcomitrella patens*, *Amborella trichopoda*, *Medicago truncatula*, *Arabidopsis thaliana*, *Vitis vinifera*, *Oryza sativa*, *Sorghum bicolor*, and *Zea mays*. Bacterial DUF177 domains from Proteobacteria, Actinobacteria, Deinococcus Thermus, Thermotogae, Aquificae, Dictyoglomia, Firmicutes, Clostridia, Cyanobacteria, and Choroflexi, and DUF177 domains from *Guillardia theta*, red algae (*Galdieria sulphuraria* and *Porphyridium cruentum*) and green algae (*Bathycoccus prasinus*) are included in the tree.

Zea mays	DUF177A	LDGIVRVIILGFRP	REAPQGI	FANF	LLLEDP	VEEE	PD	LGTF	EDDDK	GGAS																												
Sorghum bicolor	DUF177A	LDGIVRVIILGFRP	REAPQGI	FANF	LLLEDP	VEE	PD	VDV	LGTF	FEEDAKGAS																												
Oryza sativa	DUF177A	LDGIVRVIILGFRP	REAPQGI	FANF	LLLEDP	VEE	PD	VDV	LGTF	FEEDAKGAS																												
Populus trichocarpa	DUF177A	LDGILKVTILGFRP	REAPQGI	FANF	LLLEDP	VEE	PD	VDV	LGTF	FEEDAKGAS																												
Medicago truncatula	DUF177A	LDGILKVTILGFRP	REAPQGI	FANF	LLLEDP	VEE	PD	VDV	LGTF	FEEDAKGAS																												
Glycine max	DUF177A	LDGILKVTILGFRP	REAPQGI	FANF	LLLEDP	VEE	PD	VDV	LGTF	FEEDAKGAS																												
Vitis vinifera	DUF177A	LDGILKVTILGFRP	REAPQGI	FANF	LLLEDP	VEE	PD	VDV	LGTF	FEEDAKGAS																												
Arabidopsis thaliana	DUF177A	LDGIVRVIILGFRP	REAPQGI	FANF	LLLEDP	VEE	PD	VDV	LGTF	FEEDAKGAS																												
Amborella trichopoda	DUF177A	LDGILKVTILGFRP	REAPQGI	FANF	LLLEDP	VEE	PD	VDV	LGTF	FEEDAKGAS																												
Physcomitrella patens	DUF177A	VDGILRVLALVGR	REAPQGI	FANF	LLLEDP	VEE	PD	VDV	LGTF	FEEDAKGAS																												
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Cluster 1																																						
Zea mays	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
Sorghum bicolor	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
Oryza sativa	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
Populus trichocarpa	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
Medicago truncatula	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
Glycine max	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
Vitis vinifera	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
Arabidopsis thaliana	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
Amborella trichopoda	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
Physcomitrella patens	DUF177A	LACAMDC	DD	DD	DD	DD	DD	DD	DD	DD																												
.....																																						
Zea mays	DUF177B	-----	P	V	I	V	R	P	G	D	-----	E	V	D	L	S	V	I	Q	E	T	V	L	T	A	S	K	S	E	E	T	E	K	S	T	V	V	W
Sorghum bicolor	DUF177B	-----	P	V	I	V	R	P	G	D	-----	E	V	D	L	S	V	I	Q	E	T	V	L	T	A	S	K	S	E	E	T	E	K	S	T	V	V	W
Oryza sativa	DUF177B	-----	P	V	I	V	R	P	G	D	-----	E	V	D	L	S	V	I	Q	E	T	V	L	T	A	S	K	S	E	E	T	E	K	S	T	V	V	W
Medicago truncatula	DUF177B	-----	P	V	I	V	R	P	G	D	-----	E	V	D	L	S	V	I	Q	E	T	V	L	T	A	S	K	S	E	E	T	E	K	S	T	V	V	W
Vitis vinifera	DUF177B	-----	P	V	I	V	R	P	G	D	-----	E	V	D	L	S	V	I	Q	E	T	V	L	T	A	S	K	S	E	E	T	E	K	S	T	V	V	W
Arabidopsis thaliana	DUF177B	-----	P	V	I	V	R	P	G	D	-----	E	V	D	L	S	V	I	Q	E	T	V	L	T	A	S	K	S	E	E	T	E	K	S	T	V	V	W
Amborella trichopoda	DUF177B	-----	P	V	I	V	R	P	G	D	-----	E	V	D	L	S	V	I	Q	E	T	V	L	T	A	S	K	S	E	E	T	E	K	S	T	V	V	W
Physcomitrella patens	DUF177B	-----	P	V	I	V	R	P	G	D	-----	E	V	D	L	S	V	I	Q	E	T	V	L	T	A	S	K	S	E	E	T	E	K	S	T	V	V	W
.....																																						
Cluster 2																																						
Zea mays	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
Sorghum bicolor	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
Oryza sativa	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
Populus trichocarpa	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
Medicago truncatula	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
Glycine max	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
Vitis vinifera	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
Arabidopsis thaliana	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
Amborella trichopoda	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
Physcomitrella patens	DUF177A	GANL	T	S	S	C	T	C	K	-----	P	N	V	Q	L	S	L	E	L	-----																		
.....																																						
Zea mays	DUF177B	QYGS	Q	R	K	T	S	S	-----	Q	R	S	K	L	L	D	L	K	-----																			
Sorghum bicolor	DUF177B	QYGS	Q	R	K	T	S	S	-----	Q	R	S	K	L	L	D	L	K	-----																			
Oryza sativa	DUF177B	QYGS	Q	R	K	T	S	S	-----	Q	R	S	K	L	L	D	L	K	-----																			
Medicago truncatula	DUF177B	QYGS	Q	R	K	T	S	S	-----	Q	R	S	K	L	L	D	L	K	-----																			
Vitis vinifera	DUF177B	QYGS	Q	R	K	T	S	S	-----	Q	R	S	K	L	L	D	L	K	-----																			
Arabidopsis thaliana	DUF177B	QYGS	Q	R	K	T	S	S	-----	Q	R	S	K	L	L	D	L	K	-----																			
Amborella trichopoda	DUF177B	QYGS	Q	R	K	T	S	S	-----	Q	R	S	K	L	L	D	L	K	-----																			
Physcomitrella patens	DUF177B	QYGS	Q	R	K	T	S	S	-----	Q	R	S	K	L	L	D	L	K	-----																			

Supplementary Fig. S2. Amino acid alignment of plant DUF177 domain sequences.

DUF177 domain sequences from proteins of *Physcomitrella patens*, *Amborella trichopoda*, *Medicago truncatula*, *Arabidopsis thaliana*, *Vitis vinifera*, *Populus trichocarpa*, *Glycine max*, *Oryza sativa*, *Sorghum bicolor*, and *Zea mays*, were aligned by MEGA6 (Tamura et al., 2013). The two clusters of conserved amino-acid residues are indicated by solid lines and the highly conserved cysteine residuals in the two cysteine motifs are color highlighted in yellow and indicated by asterisks. The region underlined by broken line (amino acids 53-76) represents the plant DUF177A-specific insertion missing from plant DUF177B domains. Small nonpolar, orange; hydrophobic, green; polar, magenta; negatively charged, red; and positively charged, blue.



Supplementary Fig. S3. Dry weight of wild-type and *duf177a* mutant endosperm at maturity.

** , P -value <0.01 . Error bars show standard deviation. $n=30$