Supplementary Material

1 Supplementary Data

1.1 Protocol 1

1-Seed Germination (1st day)

A) Using sterile forceps, distribute the sterilized seeds in a metal tray on a wet sterile paper towel leaving 2 cm between the seeds.

B) Cover the tray with aluminum foil and incubate in a growth chamber, in the dark at 25–28°C for 2 days.

2-Inoculum Preparation (*A. rhizogenes* K599) (2nd day)

A) Spread 100-150 µl of the *Agrobacterium* strain carrying the corresponding vector, a glycerol stock containing 50% (v/v) LB medium stored at -80°C, on LB plates with the appropriate antibiotic selection.

B) Incubate the plates for approximately 30 hours at 30°C

3-Infection with *A. rhizogenes* K599 (3rd day)

A) Select healthy and robust germinated seeds to infect.

B) Using a sterile needle tip (0.4 mm), carefully puncture the hypocotyl area several times and apply penetrating the vascular tissue. Make sure the tissue is not severely damaged.

C) Apply the inoculum on the wounded zone, directly from the plates, using a tip of a sterile micropipette.

4-Plantlet Incubation and Hairy Root Generation

A) After infection, place the germinated seeds on the top of conical tubes, i.e., 15 ml Corning™ Falcon™ containing B&D medium.

B) Place conical tubes inside glass tubes containing sterile water and cover them with plastic caps to prevent water evaporation and thus mantain high humidity inside tubes.

C) Place the glass tubes on racks and incubate in the growth chamber at 28°C, 16 h light/8 h dark until hairy roots emerge (13-15 days post infection). When the first pair of leaves came into contact with the caps (3-5 days post infection), remove the caps and seal the tube hole with parafilm or with adhesive plastic. During this period, make sure that the level of water and B&D media contained within the glass tubes and Corning™ Falcon™ tubes, respectively, is adequate.

D) Once the hairy roots have emerged, remove the main root by cutting the stem 2 cm below the hairy roots.

E) Transfer the seedling to sterile glass tubes containing B&D medium, seal the tube hole and incubate for 3-5 days under the same conditions described in C) in order to increase the biomass of hairy roots.

2 Supplementary Figures and Tables

2.1 Supplementary Figures

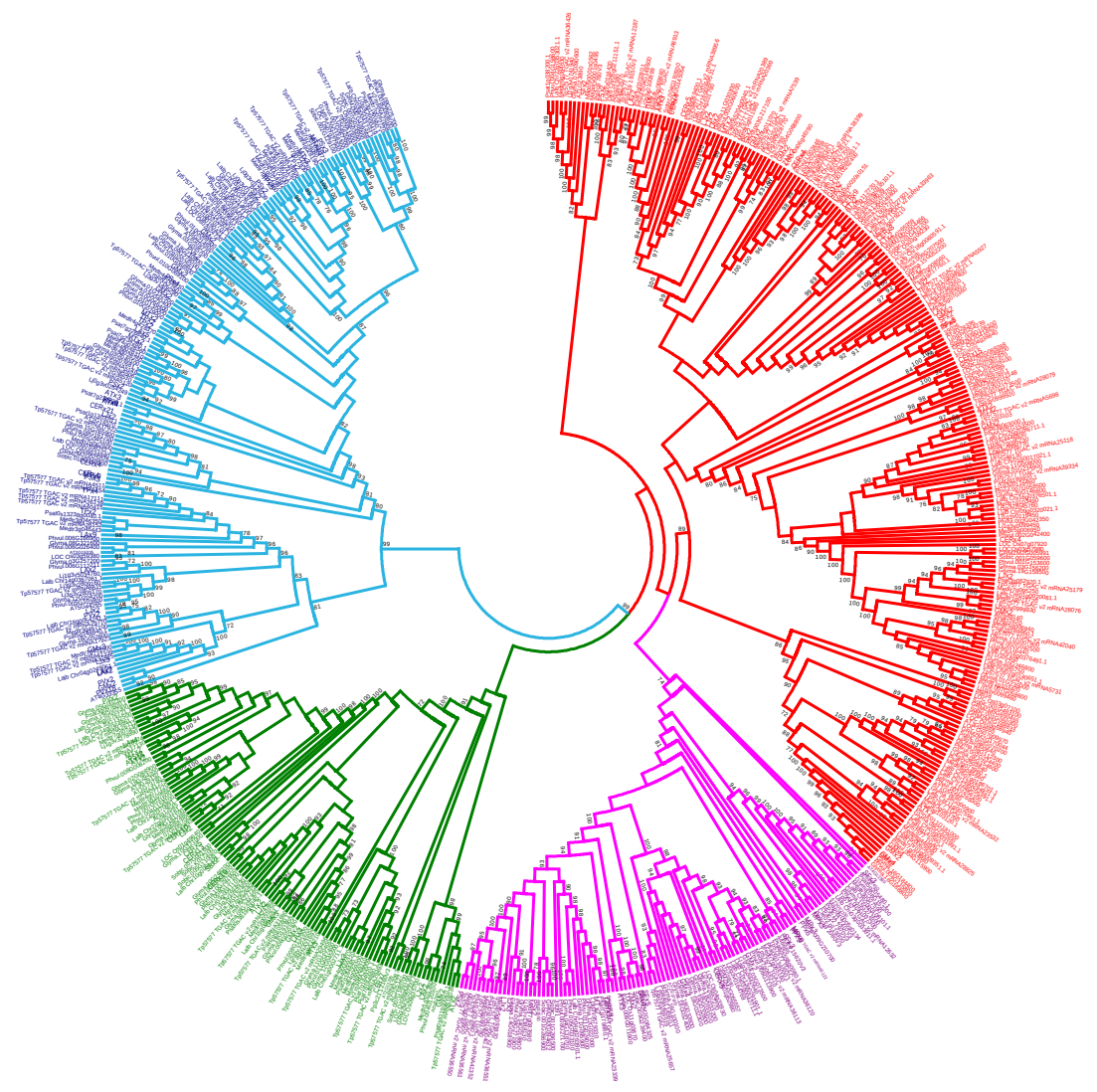

Figure S1. Evolutionary relationships of LTPs (including gene names). Unrooted approximately maximum-likelihood phylogenetic tree inferred from 960 LTPs identified in 15 plant species: *C. reinhardtii*, *P. patens*, *M. polymorpha*, *S. moellendorffii*, *O. sativa*, *S. bicolor*, *Z. mays*, *A. thaliana*, *G. max*, *P. vulgaris*, *L. japonicus*, *M. truncatula*, *L. albus*, *P. sativum*, and *T. pratense*. The clades are shown in different colors: LTP 1 class (light blue), LTP2, c, and d classes (green), LTPd and e classes (lilac), and LTPg class (red). The phylogenetic tree was constructed using IQ-TREE software with the VT+R9 substitution model with 1,000 bootstrap iterations.

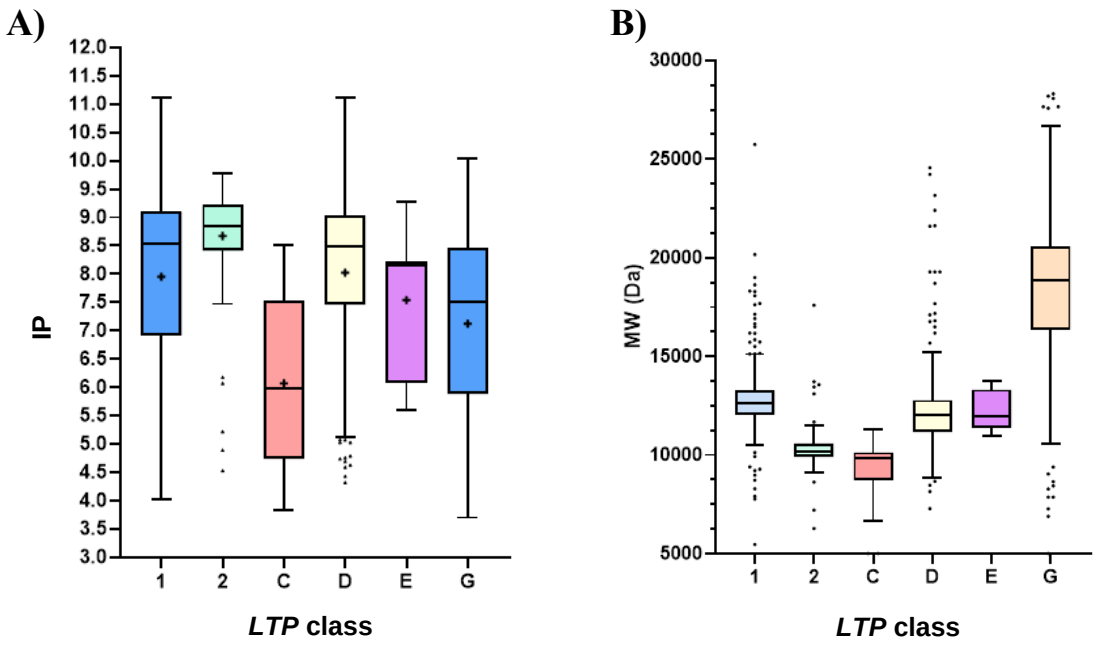

Figure S2. Graphical representation of IP and MW data. Global (A) IP and (B) MW data for each class of LTPs in the 15 species studied.

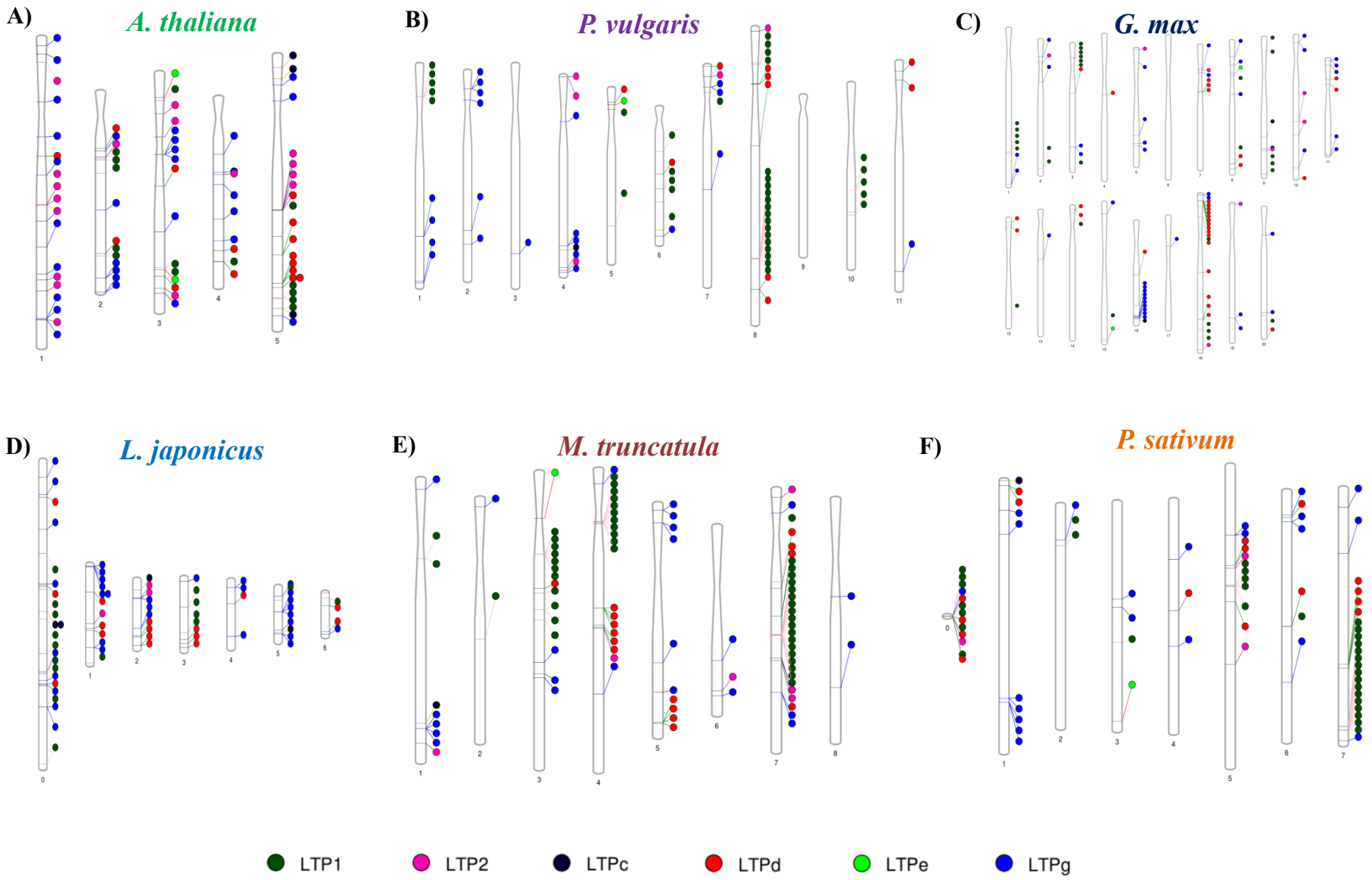
A)
A. thaliana
B)
P. vulgaris
C)
G. max
D)
L. japonicus
E)
M. truncatula
F)
P. sativum
LTP1
LTP2
LTPc
LTPd
LTPe
LTPg

Figure S3. *In silico* mapping of *LTP* gene loci. Chromosomal locations of the *LTP* genes in **(A)** *A. thaliana*, **(B)** *P. vulgaris*, **(C)** *G. max*, **(D)** *L. japonicus*, **(E)** *M. truncatula*, and **(F)** *P. sativum*. The colored dots and lines indicate the chromosomal locations of the genes.

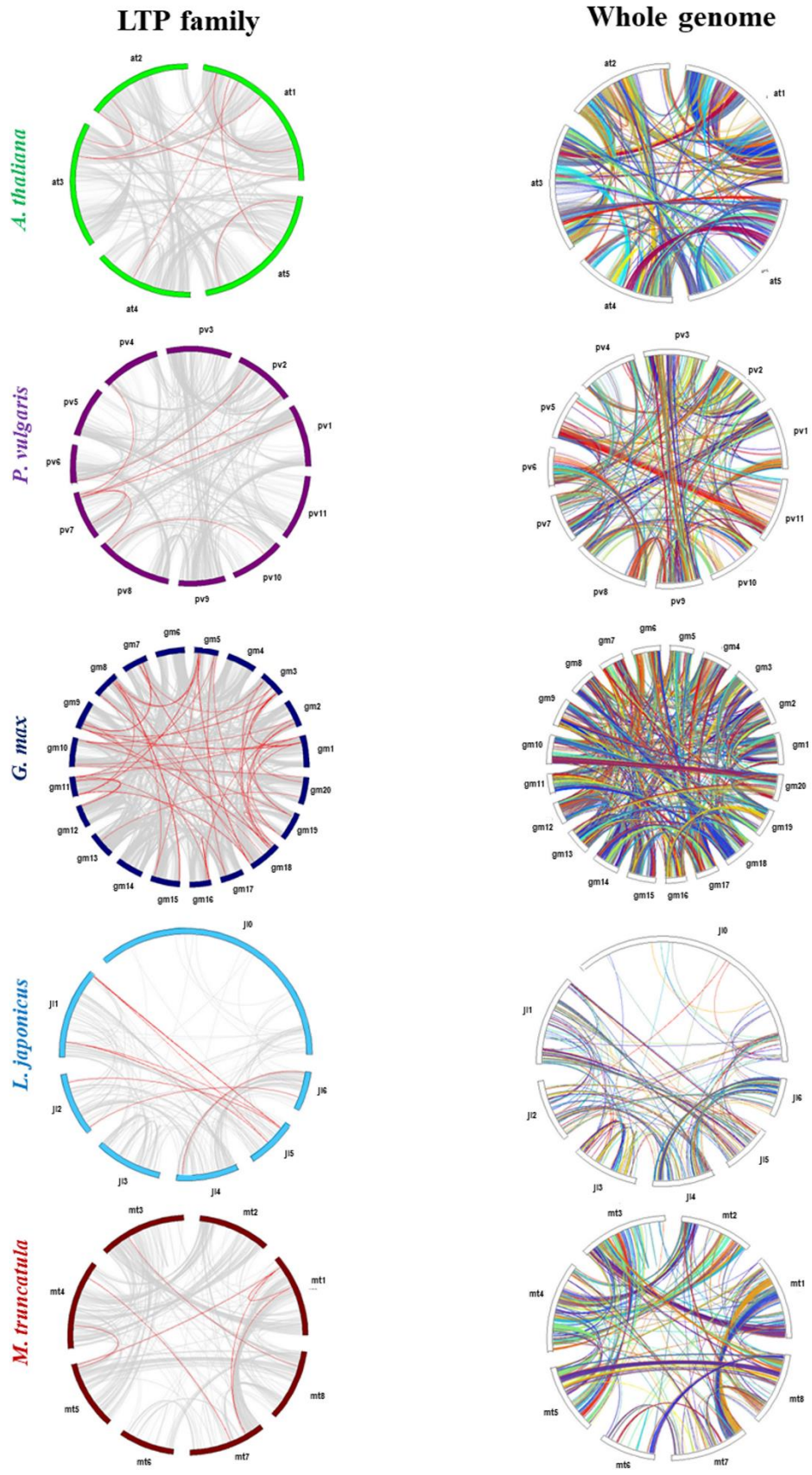
LTP family
Whole genome
A. thaliana
P. vulgaris
G. max
L. japonicus
M. truncatula
at1
at2
at3
at4
at5
pv1
pv2
pv3
pv4
pv5
pv6
pv7
pv8
pv9
pv10
pv11
gm1
gm2
gm3
gm4
gm5
gm6
gm7
gm8
gm9
gm10
gm11
gm12
gm13
gm14
gm15
gm16
gm17
gm18
gm19
gm20
lj0
lj1
lj2
lj3
lj4
lj5
lj6
mt1
mt2
mt3
mt4
mt5
mt6
mt7
mt8

Figure S4. Synteny of all *LTP* genes in *P. vulgaris, L. japonicus, G. max, M. truncatula,* and *A. thaliana* and the complete genome of each species. On the left, the syntenic *LTP* genes are connected by red lines. On the right, all syntenic genes in the complete genome of each species are connected by colored lines. Chromosomes are indicated by color code: purple, *P. vulgaris*; light blue, *L. japonicus*; dark blue, *G. max*; burgundy, *M. truncatula*; and green, *A. thaliana*.

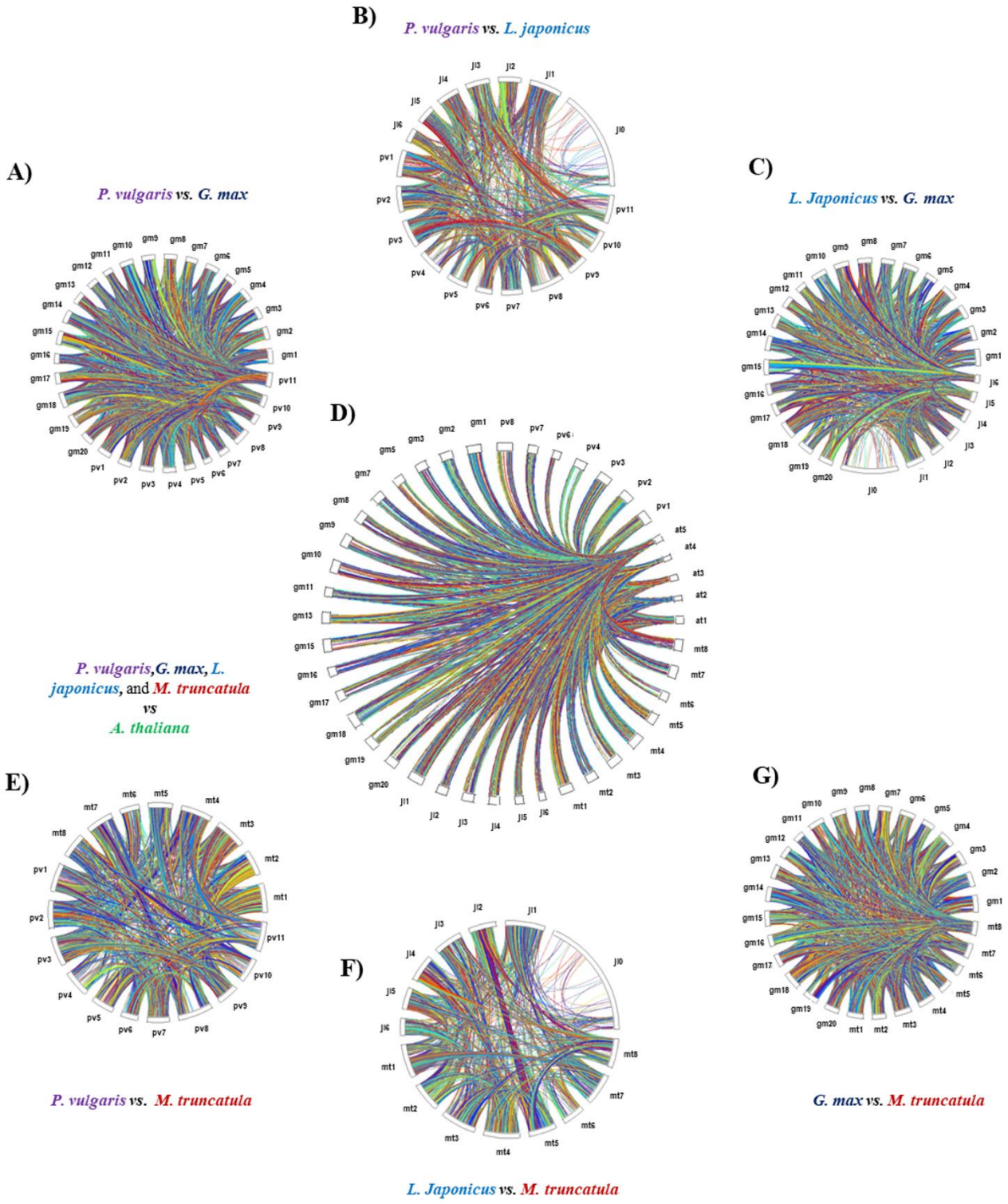

Figure S5. Synteny of all *LTP* genes in the analyzed species. Syntenic genes in the complete genomes of **(A)** *P. vulgaris* and *G. max*, **(B)** *P. vulgaris* and *L. japonicus*, **(C)** *L. japonicus* and *G. max*, **(D)** the four legumes against *A. thaliana*, **(E)** *P. vulgaris* and *M. truncatula*, **(F)** *L. japonicus* and *M. truncatula*, and **(G)** *G. max* and *M. truncatula*.

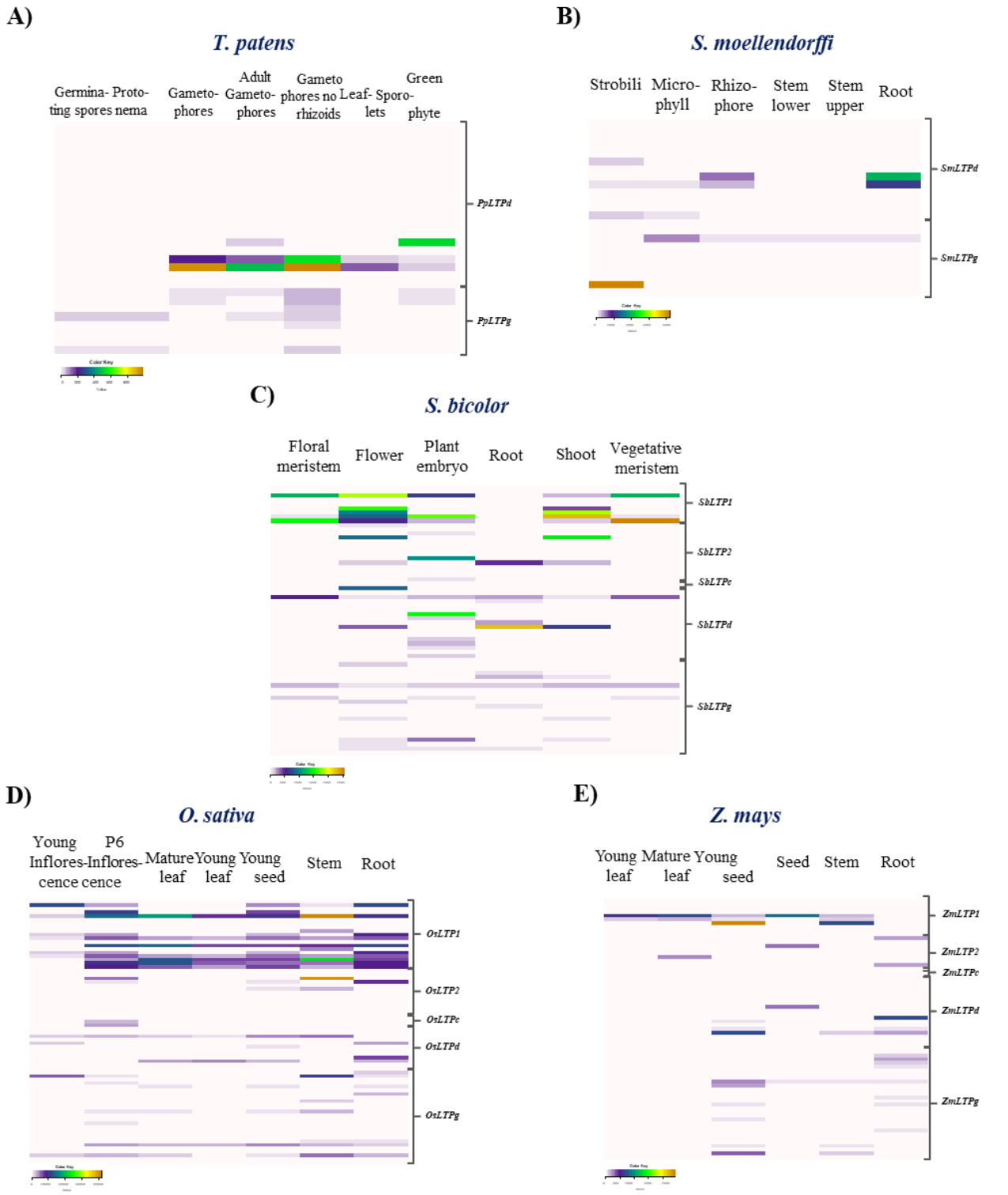

Figure S6. Expression patterns of *LTP* genes. Heat maps of the expression values of the *LTPs* in (**A**) *T. patens*, (**B**) *S. moellendorffii*, (**C**) *S. bicolor*, (**D**) *O. sativa*, and (**E**) *Z. mays*. The expression data were retrieved from the PEATmoss and BAR databases. RPKM values are represented as color key codes below each heat map. The classes of *LTPs* on the right indicate the corresponding sequences for each class and species.

2.2 Supplementary Tables

Table S1. Species used to identify LTPs in the current study and the corresponding databases used.

Species	Lineage	Database
Chlamydomonas reinhardtii P.A.Dang.	Algae	Phytozome (https://phytozome.jgi.doe.gov)
Physcomitrium patens	Bryophyte, moss	
Marchantia polymorpha L.	Bryophyte, liverwort	
Selaginella moellendorffii	Lycophytes, fern	
Oryza sativa L.	Monocotyledons	
Sorghum bicolor (L.), Moench.		
Zea mays L.		
Arabidopsis thaliana (L.), Heynh	Non-legume dicotyledon	
Glycine max (L.), Merr.	Legume, forms determinate nodules	
Phaseolus vulgaris L.		
Medicago truncatula Gaertn.		
Trifolium pratense L.	Legume, forms indeterminate nodules	
Lotus japonicus L.		LotusBASE (https://lotus.au.dk)
Lupinus albus L.		LIS: Legume Information System (https://legumeinfo.org)
Pisum sativum L.		

Table S2. Primes used for RT-qPCR analysis in this study.

Gene	Class	Tag	Primer sequence (5' →3')	Amplicon size (bp)	Efficiency (%)
Phvul.004G154200	LTP2.3	1542-Fw	GGGTTTCTGTGCCTGATTATG	128	103
		1542Rv	CAAAGAGACGTTAAACCACACC		
Phvul.005G045100	LTP1.6	451Fw	CTTGGAATTGGAGGGTGATG	113	104
		451Rv	GTGAAGAAATCCACGTAGGTAG		
Phvul.007G173500	LTPg.20	1735Fw	CTTTTAGAGGGTTTGTCTTGTGT	131	90
		1735Rv	CCATTATGTAGTTGAGGCAAGG		
Phvul.008G008200	LTP2.5	082Fw	CCTATAAACCATCTTCACACGG	133	108
		082Rv	ACTCAGACAAAAGTTCATCACG		
Phvul.008G112900	LTPd.4	1129Fw	CAGGTGTTTCATTACCACTGAAG	103	99
		1129Rv	ACATCTGCTTACAAACTTATGGAC		
Phvul.008G137100	LTPd.6	1371_Fw	CCCGTGACTCCATATACTGC	150	99
		1371_Rv	CTGATCTCTCTAACTCAATCGC		
Phvul.011G047400	LTPd.10	474_Fw	GTTATGTACTCTTTAATTGCCCTG	146	101
		474_Rv	GTACACAGGAACTAGCCAAAAC		

Table S3. Sequence spacing patterns in *LTP* genes grouped by classes.

Table S4. Features of LTPs in the 15 species studied.

Table S5: Collinear *LTP* genes identified by species and between species.

Table S6: Expression profile data of PvLTP genes in home transcriptome data of *P. vulgaris* at 7 dpi with *R. tropici*.