

Supplementary Information

Flower induction, microscope-aided cross-pollination, and seed production in the duckweed *Lemna gibba* with discovery of a male-sterile clone

Lili Fu^{1,*}, Meng Huang^{1,*}, Bingying Han¹, Xuepiao Sun¹, K. Sowjanya Sree², Klaus-J Appenroth³, Jiaming Zhang¹

¹*Institute of Tropical Bioscience and Biotechnology, MOA Key Laboratory of Tropical Crops Biology and Genetic Resources; Hainan Bioenergy Center, CATAS, Haikou, Hainan Province, 571101, China;*

²*Department of Environmental Science, Central University of Kerala, RSTC, Padanakkad-671314, Kerala, India;*

³*Institute of Plant Physiology, University of Jena, Dornburger Str. 159, 07743 Jena, Germany*

*These authors contributed equally to this work.

Correspondence and requests for materials should be addressed to J.Z. (zhangjiaming@itbb.org.cn)

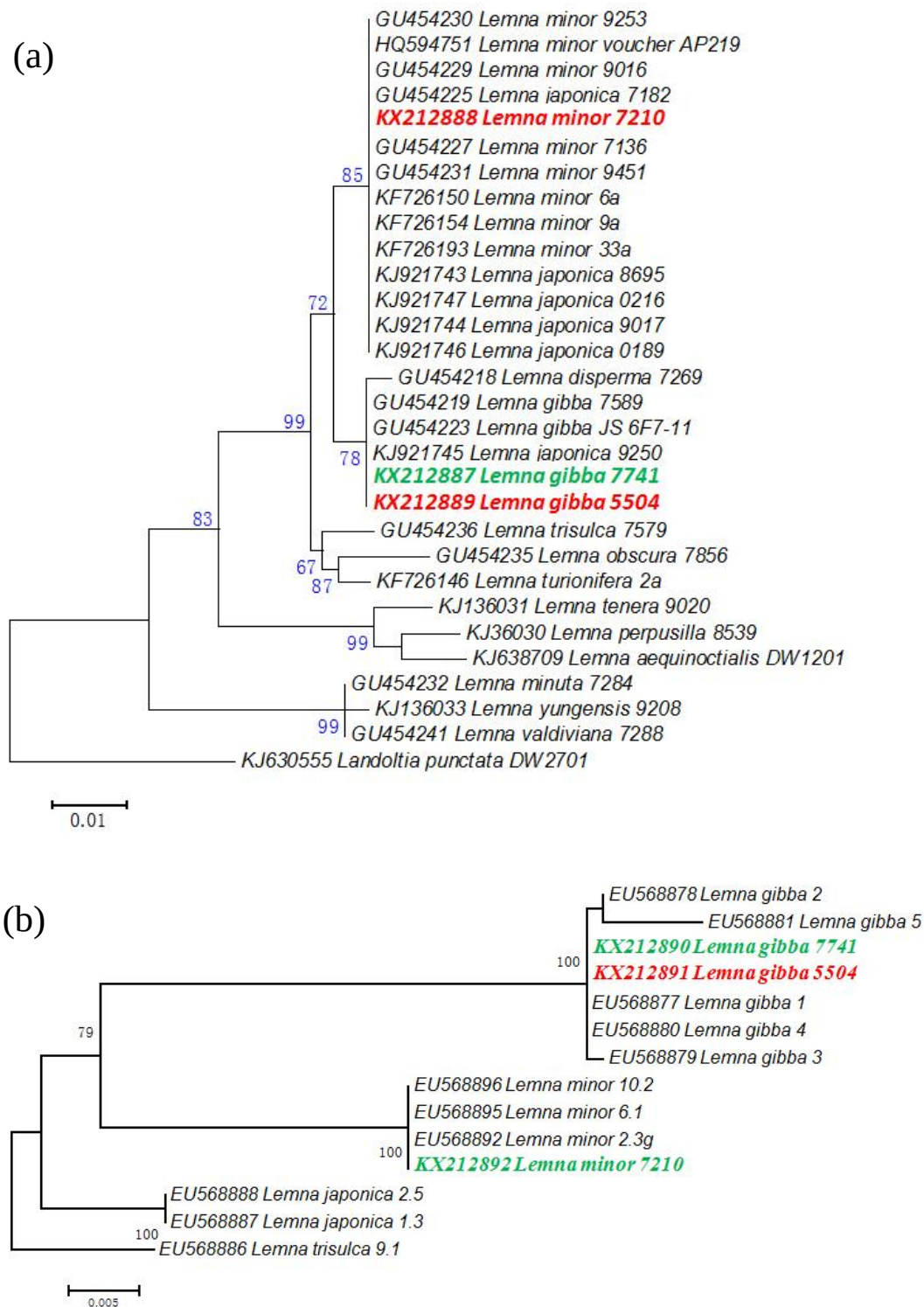


Figure S1. Phylogenies inferred with the chloroplast *atpF-atpH* intergenic spacer (a) and *rps16* intron (b) sequences in *Lemna* species. Evolutionary analyses were conducted in MEGA⁷. The evolutionary history was inferred by using the Maximum Likelihood method based on the Tamura-Nei model. The trees with the highest log likelihood are shown. The bootstrap values are shown next to the branches. The trees are drawn to scale and rooted with *Landoltia punctata* DW2701 (a) or *Lemna trisulca* 9.1 (b). The scale bars represent 0.01 (a) or 0.005 (b) substitutions per site.

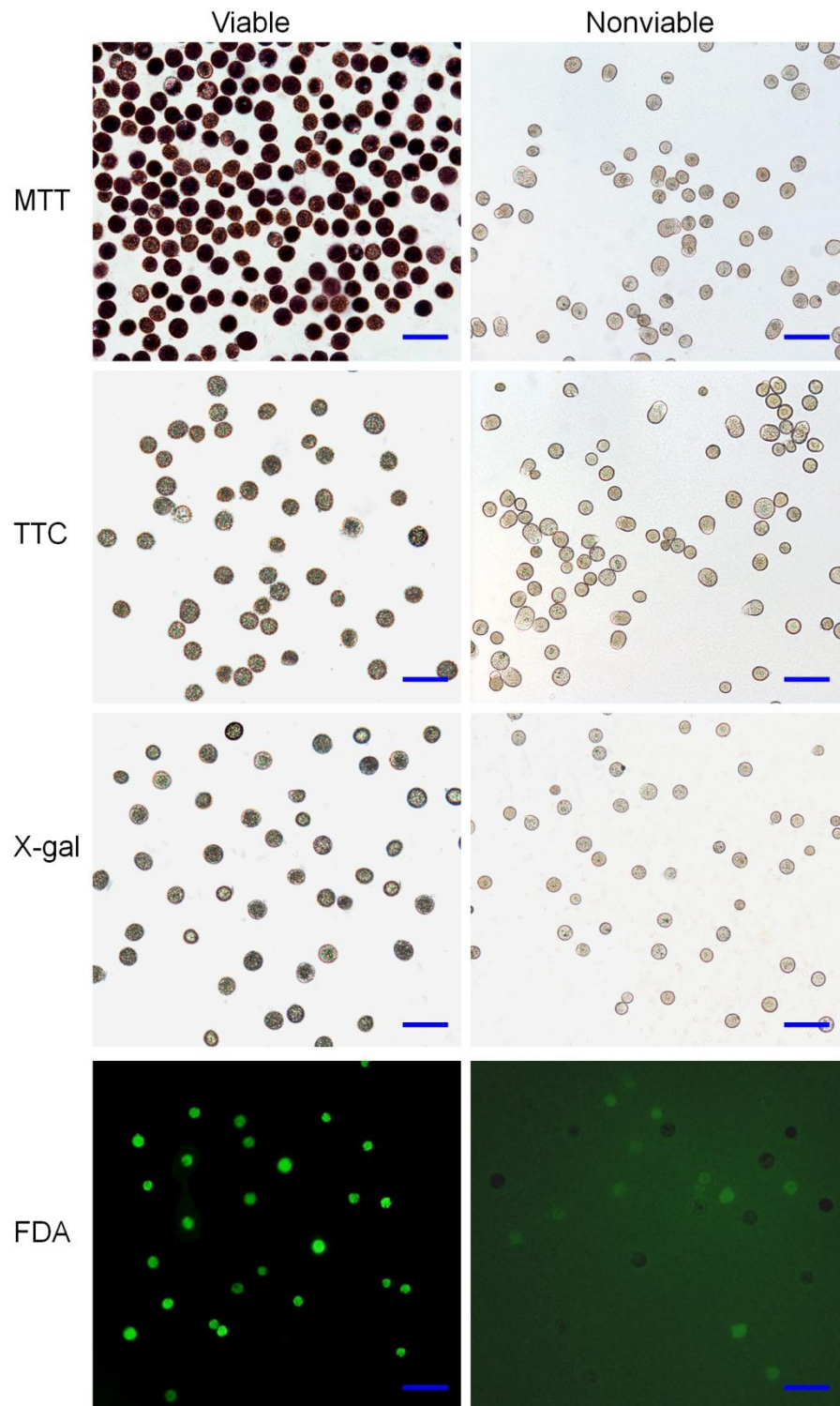


Figure S2. Different chemical staining of fresh (left side) and dead (heat inactivated; right side) pollen grains from clone 7741. The staining methods were listed to the left of each row. Scale bars represent 50 μm .

Table S1. Basal salts in the media used in this study

Chemicals	H (mmol/L)	MH (mmol /L)	E (mmol /L)
NH ₄ NO ₃		80	
KNO ₃	5	5	15
KH ₂ PO ₄	1	1	5
MgSO ₄ .7H ₂ O	2	2	2
Ca(NO ₃) ₂ .4H ₂ O	5	4	5
H ₃ BO ₃	0.046	0.1	0.046
MnCl ₂ .4H ₂ O	0.009		0.018
MnSO ₄ .H ₂ O		0.1	
ZnSO ₄ .7H ₂ O	0.0008	0.03	0.0008
CuSO ₄ .5H ₂ O	0.0003	0.0001	0.0003
H ₂ MoSO ₄ .H ₂ O	0.0001		
Na ₂ MoSO ₄ .2H ₂ O		0.001	0.0005
KI		0.005	
CoCl ₂ .6H ₂ O		0.0001	
FeSO ₄ .7H ₂ O	0.1	0.1	
FeCl ₃ .6H ₂ O			0.02
Na ₂ EDTA	0.1	0.1	
EDTA			0.03
tartaric acid			0.02
pH	5.7	5.7	4.6

Note. H—Hoagland medium ²

MH—Modified Hoagland medium

E—E-medium ³

References:

- 1 Kumar, S., Stecher, G. & Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Molecular Biology and Evolution* **33**, 1870-1874 (2016).
- 2 Hoagland, D. R. & Arnon, D. I. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular* **347**, 1-32 (1950).
- 3 Cleland, C. F. & Briggs, W. R. Flowering responses of the long-day plant *Lemna gibba* G3. *Plant Physiology* **42**, 1553-1561 (1967).