| Pisum_sativum Lotus_japonicus UFO_A.thaliana A.majus O.sativa S.latifolia | 1 MEGFHPSMMMNSMMMNPS ISYTTPTTATASGATTMITTTTTTYN-TIISITPYMMSRIWSKLPYRLLDRITAFLPPAFFRARAVCKRYSLLFSNSFLELYLOVSPRH-WFTFFKHK 1 MEGFHPSMTMASPFPYTFPTSAGGIGVGVVGVGSSMLTTPTTTTYN-TIISITPYMMSRIWSKLPGKLLDRYTAFLPTPAFFRARAVCKRYSLLFSNTFLELYLOVSPRH 1 MEGFHPSMTMASPFPYTFPTSAGGIGVGVVGVGSSMLTTPTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT |
|--|---|
| | F box |
| Pisum_sativum Lotus_japonicus UFO_A thaliana A.majus O.sativa S.latifolia | 118 IRSKTHIYON |
| Pisum_sativum Lotus_japonicus UFO_A thaliana A.majus O.sativa S.latifolia | 229 YAVKNL SSESFHID/SGFYSINGTTSSLPRLCSLESGRWVSG — GKFYCM/CSPFSVLAYDIATNTNFKIDAPNRFLRSPLVECKG-KLLVAAVEKSKLNVPKSLRWSLOG 235 YAVKNLTSESFHID/GGFYSINGTTSDLPRLCSLESGRWVCAE — GKFYCM/CSPFSVLAYDIATTNFKIDAPNRFLRSPLVECKG-KLLVAAVEKSKLNVPKSLRWSLO 220 YAVKNLTSESFHID/GGFYSINGTTSDLPRLCSLESGRWVCAE — GKFYCM/YSPFSVLSYDTG/KMIDAPNRFLRSPLLESKG-KLILVAAVEKSKLNVPKSLRWSLO 215 YAVKNLTSESFHID/GGFYSINGTTSSLPRLCSLESGRWVCAE — GKFYCM/YSPFSVLSYDTG/KMIDAPNRFLRSPLLESKG-KLILVAAVEKSKLNVPKSLRWSLO 215 YAVKNLTSESFHID/GGFYSINGTTSSLPRLCSLESGRWVCAE — GKFYCM/YSPFSVLSYDTG/KMIDAPNRFLRSPLLESKG-KLILVAAVEKSKLNVPKSLRWSLO 215 YAVKNLTSESFHID/GGFYSINGTTSSLPRLCSLESGRWVCAE — GKFYCM/YSPFSVLSYDISJC/GXIDAPNRFLRSPLLVESKG-KLILVAAVEKSKLNVPKSLRWSLO 218 YAVKNLTSESFHID/GGFYSINGTTSSLPRLCSLESGRWVCAE — GKFYCM/YSPFSVLSYDISJC/GXIDAPNRFLRSPLVESKG-KLILVAAVEKSKLNVPKSLRWSLO 237 YAVKNLTTEFH/JSSGFYSINGTTSLPRLCSLESGRWVCAE — GKFYCM/YPFSVLYDISJC/GXIDAPNRFLRSPLVCAE (LVAAVEKSKLNVPKSLRWSLO) |
| | Probe for ISH |
| Pisum_sativum Lotus_japonicus UF0_A.thaliana A.majus O.sativa S.latifolia | 342 COSYWYETERNPOOLYNOFADNELONGFECVONGEFIVINIKG—SDKOLVYDTGRKRWONIPPCPYAG—YDGFELHGFAYDPRLATPVTALLDOLA PLPOT 346 Cothwyesernpoolytofaafeggggfecvorgefiviniko—tokallydlyrkrwonippcpyagYdgfelhgfayeprlatpvtalldola pfosina— 333 DNATWYETERNPOOLYTOFAAFEGGKGFECVONOEFNINKO—Tokallydlyrkrwonippcpyag |

Figure S1. SlUFO amino acid sequence and alignment of its homolog genes in other plant species. The highlighted square indicates a homologous amino acid conserved among UFOs. The F-box and probe region for in situ hybridization (ISH) are indicated with blue and red line, respectively.



Figure S2. In situ hybridization analysis of *SlUFO* mRNA in *S. latifolia* flowers at an early flower development stage. (a) Healthy male flower, (b) healthy female flower, and (c) *Microbotryum* infected female flower at stage 2; (d) Healthy male flower at stage 8 (e) healthy female flower and (f) *Microbotryum* infected female flower at stage 7. g, gynoecium; p, petal; s, stamen. Bars = 100 μm.



Figure S3. In situ hybridization analysis of *SlUFO* mRNA in *S. latifolia* flowers at a late flower development stage. (a) Female flower at stage 12 and (b) higher magnification. (c) Hermaphroditic flower resulted from *Microbotryum* infection at stage 12, and (d) higher magnification. g, gynoecium; p, petal; ds, developing stamen; ss, suppressed stamen. Bars = 100 µm.

| Number | Name | Sequence (5'-3') | Reference |
|--------|----------------------|--|----------------------|
| 1 | R2ALS1363(+) | GCGAGGCACTCCTTA | ALSV manual |
| 2 | R2ALS1551(-) | GCAAGGTGGTCGTGA | ALSV manual |
| 3 | ALSV-2600F | AGTGCTTCCACTCGTTTTAC | This study |
| 4 | ALSV-3000R | GTAGCATGACCACTCATGAT | This study |
| 5 | ALSR2-1213(+) | ATACCACCTCATACAGGTACAC | Kasajima et al. 2017 |
| 6 | ALSR2-1484(-) | CGTTCCACGACCGTGTGGCCAGA | Kasajima et al. 2017 |
| 7 | SIPDSF1 | ACCCGAGGAATGGAAAGAGA | This study |
| 8 | SIPDSF2 | TGCTGAAAAGTAGATGGTCGTATGT | This study |
| 9 | SIUFO_F2 | CAATGCACACATGGCAAAAT | This study |
| 10 | SIUFO_R2 | CAAACCCTCTTCCTCCTTCA | This study |
| 11 | SISUPF4 | CCG ACG ATC ATG GAA TCA TCA AGA | Kazama et al. 2009 |
| 12 | SISUPR3 | CCA AGA CGA AGC TCC AAA TCC A | Kazama et al. 2009 |
| 13 | Sl18SF1 | GGC AAC GGA TAT CTC GGC TCT C | Kazama et al. 2009 |
| 14 | SI18SR1 | TGA CGC CCA GGC AGA CGT GC | Kazama et al. 2009 |
| 15 | SL_UBQ F | AATTTTCGCCTTCCTCATCC | Zemp et al. 2014 |
| 16 | SL_UBQ R | GCTTGCCAGCGAAAATAAGT | Zemp et al, 2014 |
| 17 | atPDS_1070F | ACCGACGAGGTGTTTATTGG | This study |
| 18 | atPDS_1809R | CGACATGGTTCACAGTTTGG | This study |
| 19 | SIPDS-100F | GGCCCTGATTTCACAgtcgacCAGGAGAAGCATGGTTCC | This study |
| 20 | SIPDS-200R | CCCTGACCTTCTAGCAGggatccACGAGAGTTAAGCTTCACTTCACC | This study |
| 21 | SIPDS-250R | CCCTGACCTTCTAGCAGggatccCACACTTCCATCCTCGTTCAACTC | This study |
| 22 | SIPDS-150F | GGCCCTGATTTCACAgtcgacAGGCTGTGCATGCCCATTG | This study |
| 23 | SIPDS-300R | CCCTGACCTTCTAGCAGggatccATAAGCATCTCCTCTTATCTCTTTC | This study |
| 24 | SIPDS-200F | GGCCCTGATTTCACAgtcgacGGTGAAGTGAAGCTTAACTCTCGT | This study |
| 25 | ALSV-sal-SlUFO_1029F | GGCCCTGATTTCACAgtcgacACATGGTGGAAAATCCAAGC | This study |
| 26 | ALSV-sal-SlUFO_1159F | GGCCCTGATTTCACAgtcgacGATTGTGGGTCCTACAAGGATG | This study |
| 27 | ALSV-sal-SlUFO_1245F | GGCCCTGATTTCACAgtcgacGAAGGAGGAAGAGGGTTTG | This study |
| 28 | ALSV-bam-SlUFO_1190R | CCCTGACCTTCTAGCAGggatccTTGGGTCCCACATCCTTGTAG | This study |
| 29 | ALSV-bam-SlUFO_1290R | CCCTGACCTTCTAGCAGggatcc TAATAAACTCACCATTTCCAACAC | This study |

Table 1. List of oligonucleotide primers used in this study.

| 30 | ALSV-bam-SlUFO_1418R | CCCTGACCTTCTAGCAGggatccTGCAAATCCATGCAAATCA | This study |
|----|----------------------|---|------------|
| 31 | ALSV-sal-SISUP_310F | GGCCCTGATTTCACAgtcgacCCAAACCCTAACCCTAACCCT | This study |
| 32 | ALSV-sal-SISUP_331F | GGCCCTGATTTCACAgtcgacAATCCAAACCCTAGCTCAAATCTT | This study |
| 33 | ALSV-sal-SISUP_406F | GGCCCTGATTTCACAgtcgacTCACTCTCACTTTCCTCTCTCCA | This study |
| 34 | ALSV-bam-SISUP_441R | CCCTGACCTTCTAGCAGggatccCAATGGAGGTGGTGGAAGAGA | This study |
| 35 | ALSV-bam-SISUP_507R | CCCTGACCTTCTAGCAGggatccTTTCCATTTCCCTATCCCATC | This study |

Materials and Methods

Isolation of SlUFO

SlUFO partial sequence was obtained from cDNA prepared from male and female total RNA flower buds. A genomic DNA fragment and the complete coding region was obtained using above partial sequence.

In Situ Hybrdidization

Paraffin-sections of flower buds were prepared as described previously (Kazama et al. 2009). In situ hybridizations were performed using the probe shown in Figure 1. The procedure was applied accordingly to the previous report (Kazama et al. 2009).

Reference

1 Kazama, Y.; Fujiwara, M.T.; Koizumi, A.; Nishihara, K.; Nishiyama, R.; Kifune, E.; Abe, T.; Kawano, S. A SUPERMAN-like gene is exclusively expressed in female flowers of the dioecious plant *Silene latifolia*. *Plant Cell Physiol*. **2009**, *50*, 1127–1141.