# Supporting Information

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### SI Materials and Methods

**Plant Materials and Growth Conditions.** The *Lotus japonicus* Miyakojima MG20 mutants, *01–0017* and *01–1428*, were generated by ethylmethane sulfonate treatment. For the analysis of responses to different light quality, light-emitting diodes (LEDs) of blue (B), red (R), and far red (FR) light were used (B, MIL-B18; R, MIL-R18; FR, MIL-IR18; Sanyo). For observation of phenotypes during germination, the LEDs were used at intensities of 5, 17, and 20 μmol $m^{-2}s^{-1}$ for FR, B, and R light, respectively, as described (1). For analysis of the effect of jasmonic acid (JA) on root nodule formation under white light, 3-d-old plants were transplanted onto 1.5% agar-solidified Broughton & Dilworth (B&D) medium with or without JA and inoculated with *M. loti*; root nodule number was counted 28 days after inoculation (DAI).

**Sequencing *Lj phytochrome B*.** The *phytochrome B* (*PHYB*) gene of *L. japonicus* was identified through a homology search in DNA Data Bank of Japan using the *Arabidopsis PHYB* gene. For determination of nucleotide sequence, primers were designed (Table S1) from end to end covering the entire sequence of the *PHYB* gene. The amplified fragments were used as templates for sequencing the genes from MG20 and both mutants.

**Derived Cleaved Amplified Polymorphic Sequence Analysis.** Genomic DNA from (*i*) the $F_2$ population prepared by crossing each mutant (MG20 background) with MG20, (*ii*) the M3 generation of *phyB* mutants, (*iii*) *L. japonicus* MG20, and (*iv*) *L. japonicus* Gifu B129 was extracted by using a DNeasy Plant Mini Kit (Qiagen). Amplification of the *PHYB* fragment was conducted by PCR using derived cleaved amplified polymorphic sequence primers (Table S1). HindIII and HaeII for *01–0017* and *01–1428*, respectively, were used to digest the amplified fragments. When the amplified DNA contains a mutation (C to T), which is the case for *01–0017*, a 113-bp fragment will result after HindIII digestion. For *01–1428*, if the amplified DNA contains a deletion (C), the recognition sequence of HaeII will be altered. Digested fragments were separated on a 3.5% agarose gel, subjected to electrophoresis, and visualized by ethidium bromide staining.

**RNA Isolation and Real-Time RT-PCR.** To determine the identity of *L. japonicus* JA-responsive genes, 3-d-old uninoculated seedlings of MG20 were incubated in liquid B&D medium containing 50 μM methyl jasmonate (MeJA) for 24 h; the plants were then quick frozen in liquid $N_2$ and stored at −80 °C until use. For the analysis of JA-responsive gene expression under high or low R/FR light ratios, the same procedure as for the nodulation tests was carried out. At 7 DAI (day 22), the plant materials were sampled. To analyze JA-responsive gene expression in white light-grown MG20 and *phyB* mutant plants, 3-d-old seedlings were transplanted onto 1.5% agar-solidified B&D medium, and after 12 d (day 15), they were inoculated with *M. loti*. Plants were sampled at day 22. To analyze *LjNIN* gene expression, 3-d-old seedlings were transplanted onto 1.5% agar-solidified B&D medium, and 10 d later, the plants were moved to low R/FR light. After 2 d (day 15), plants were transferred onto 1.5% agar-solidified B&D medium with or without 0.1 μM JA and inoculated with *M. loti* MAFF303099 cells carrying plasmid pFAJPcycA. The plants were sampled 7 DAI. Total RNA was prepared using the RNeasy Plant Mini Kit (Qiagen), and a DNase I treatment was performed using DNase RT-Grade (Wako). The RNA was precipitated by ethanol and resuspended in RNase-free water. To quantify the relative amount of transcripts derived from JA-related genes, real-time RT-PCR was performed using the one-step SYBR Primescript RT-PCR Kit (TaKaRa) (2). The nucleotide sequences of the primers used are shown in Table S1.

**Observation of Nodules, Nodule Primordia, and Infection Threads With or Without JA Treatment.** MG20 seeds were sown on 0.8% (W/V) agar medium and incubated at 24 °C in the dark. After 3 d, germinated seedlings were transplanted onto 1.5% agar-solidified B&D medium without nitrogen and incubated under continuous white light at a light intensity of 100 μmol $m^{-2}s^{-1}$. The root areas of the square plates were shaded using black paper. After 10 d (day 13), the plants were moved to light with a low R/FR ratio (R/FR = 0.1; R LED intensity = 10 μmol $m^{-2}s^{-1}$; FR LED intensity = 100 μmol $m^{-2}s^{-1}$). Two days later (day 15), the plants were transferred onto 1.5% agar-solidified B&D medium with or without 0.1 μM JA and inoculated with *M. loti* MAFF303099 carrying pFAJPcycA at a concentration of $1.0 \times 10^7$ cells per plant. β-glucuronidase (GUS) staining was performed 10 DAI, and nodules, nodule primordial, and infection threads were observed by microscopy.

**Measurement of Chlorophyll Content.** For the extraction of chlorophylls, the swollen seeds were sown on vermiculite-filled pots that were watered with B&D medium (3) and inoculated with *M. loti* ($1.0 \times 10^7$ cells $mL^{-1}$). The plants were grown at 24 °C under 16 h light and 8 h dark condition for 50 d. Chlorophylls *a* and *b* were extracted and measured by the method previously reported (4).

**Measurement of Sucrose Content.** Sucrose was extracted from the roots treated with low or high R/FR light by the method reported (5) and measured with a sucrose content kit (F-kit; Boehringer-Mannheim) according to the manufacturer's instructions.

**Measurement of Endogenous Concentration of JA and Jasmonoyl-Isoleucine (Day 10).** Three-day-old seedlings were transplanted onto B&D agar medium and were inoculated with *M. loti*. The plants were then incubated under continuous white light. After 7 d (7 DAI), plants were quick frozen in liquid $N_2$ (day 10). Endogenous concentrations of JA and jasmonoyl-isoleucine (JA-Ile) were measured by the method previously reported (6).


1. Fankhauser C, Casal JJ (2004) Phenotypic characterization of a photomorphogenic mutant. *Plant J* 39:747–760.
2. Tominaga A, et al. (2009) Enhanced nodulation and nitrogen fixation in the abscisic acid low-sensitive mutant *enhanced nitrogen fixation1* of *Lotus japonicus*. *Plant Physiol* 151:1965–1976.
3. Broughton WJ, Dilworth MJ (1971) Control of leghaemoglobin synthesis in snake beans. *Biochem J* 125:1075–1080.
4. Bruuinsma J (1963) The quantitative analysis of chlorophylls a and b in plant extracts. *Photochem Photobiol* 2:241–249.
5. Agarie S, et al. (2002) Overexpression of C4 PEPC caused $O_2$-insensitive photosynthesis in transgenic rice plants. *Plant Sci* 162:257–265.
6. Ohkama-Ohtsu N, et al. (2011) 12-oxo-phytodienoic acid-glutathione conjugate is transported into the vacuole in Arabidopsis. *Plant Cell Physiol* 52:205–209.

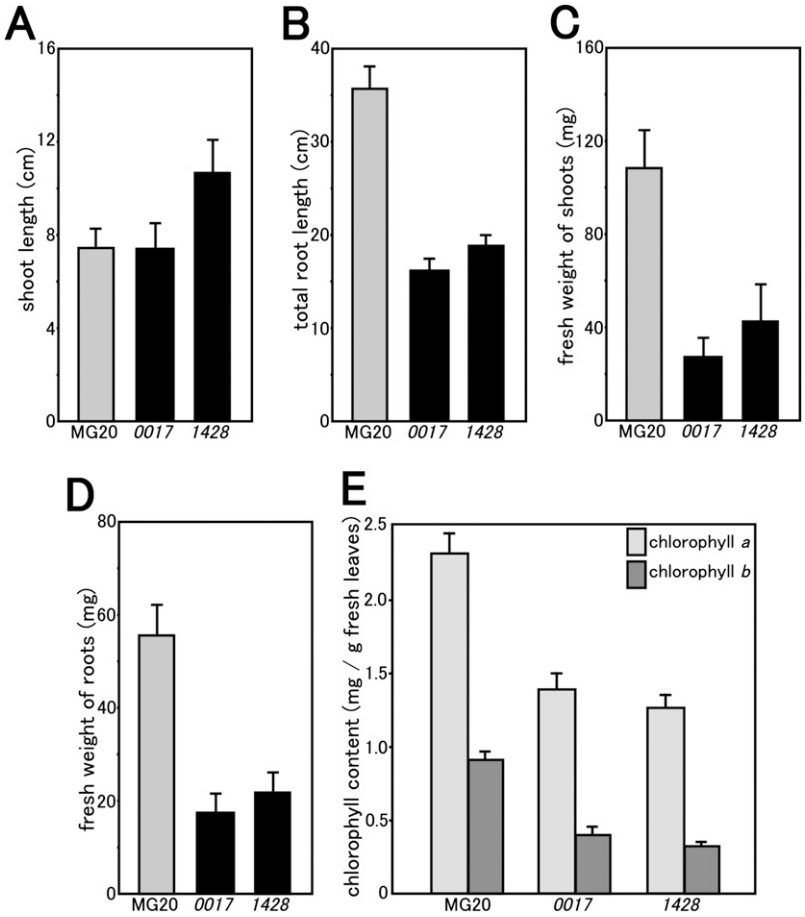


**Fig. S1.** Growth phenotypes of *L. japonicus phytochrome B* (*phyB*) mutants (*01–0017* and *01–1428*) compared with WT MG20 plants; 3-d-old plants were inoculated with *M. loti* and grown in pots containing vermiculite. After 28 d, shoot length (*A*), total root length (*B*), shoot fresh weight (*C*), and root fresh weight (*D*) were measured. (*A–D*) Values are means of 29 (MG20), 28 (*01–0017*), and 25 (*01–1428*) plants. Error bars represent SE. (*E*) Chlorophyll *a* and *b* contents were measured from leaves of MG20 and both *phyB* mutants (50 DAI). (*E*) Values are means of 10 (MG20), 9 (*01–0017*), and 8 (*01–1428*) plants.


**Fig. S2.** Phenotypes under different light conditions of seedlings of MG20 and *phyB* mutant plants. Seeds were sown on 0.8% (W/V) agar medium and incubated at 24 °C in the dark. After 24 h, different light treatments were given for three continuous days. (Scale bar: 1 cm.)

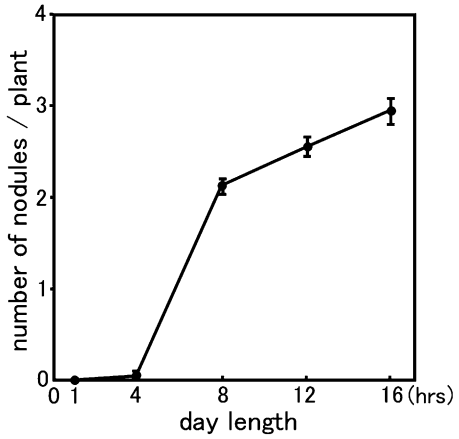


**Fig. S3.** Root nodule formation in response to day length; 3-d-old seedlings of MG20 were inoculated with *M. loti* and grown under 1 h light and 23 h dark ($n = 25$), 4 h light and 20 h dark ($n = 30$), 8 h light and 16 h dark ($n = 26$), 12 h light and 12 h dark ($n = 28$), or 16 h light and 8 h dark ($n = 46$) conditions for 28 d. Error bars represent SE.

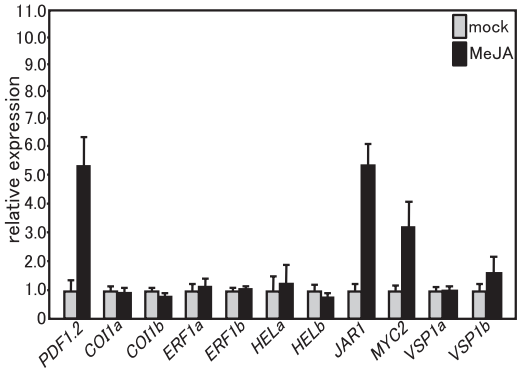


**Fig. S4.** Expression analysis of *L. japonicus* genes with similarity to *Arabidopsis thaliana* JA-related genes *PDF1.2*, *COI1*, *ERF1*, *HEL*, *JAR1*, *MYC2*, and *VSP1*. Similarity of amino acid sequence of PDF1.2, COI1a, COI1b, ERF1a, ERF1b, HELa, HELb, JAR1, MYC2, VSP1a, and VSP1b to the homologs of *A. thaliana* is about 33% (51 aa in length), 65% (586 aa in length), 70% (569 aa in length), 44% (167 aa in length), 43% (164 aa in length), 64% (117 aa in length), 84% (62 aa in length), 62% (584 aa in length), 50% (675 amino acid in length), 38% (247 aa in length), and 33% (215 aa in length), respectively. For real-time RT-PCR analysis, total RNA was prepared from MG20 plants after 50 μM MeJA or no (null) treatment. Transcript amounts were normalized against *ATP synthase* (internal control) transcripts (1). The mean value of expression in MG20 without MeJA treatment was set as one. The data represent averages ± SE of three independent experiments from roots derived from six different plants.

1. Nakagawa T, Kawaguchi M (2006) Shoot-applied MeJA suppresses root nodulation in *Lotus japonicus*. *Plant Cell Physiol* 47:176–180.

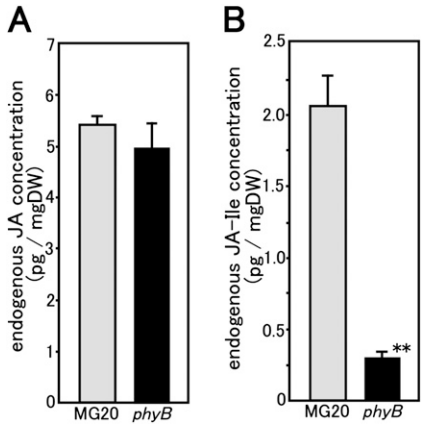


**Fig. S5.** Endogenous concentration of JA and JA-Ile in white light-grown MG20 vs. *phyB* (*01–0017*) mutant plants. (*A*) Comparison of endogenous JA concentrations. (*B*) Comparison of endogenous JA-Ile concentrations; 3-d-old seedlings were inoculated with *M. loti*. After 7 d, the endogenous concentrations were measured. The data represent the averages ± SE of three independent experiments using roots derived from six different plants.

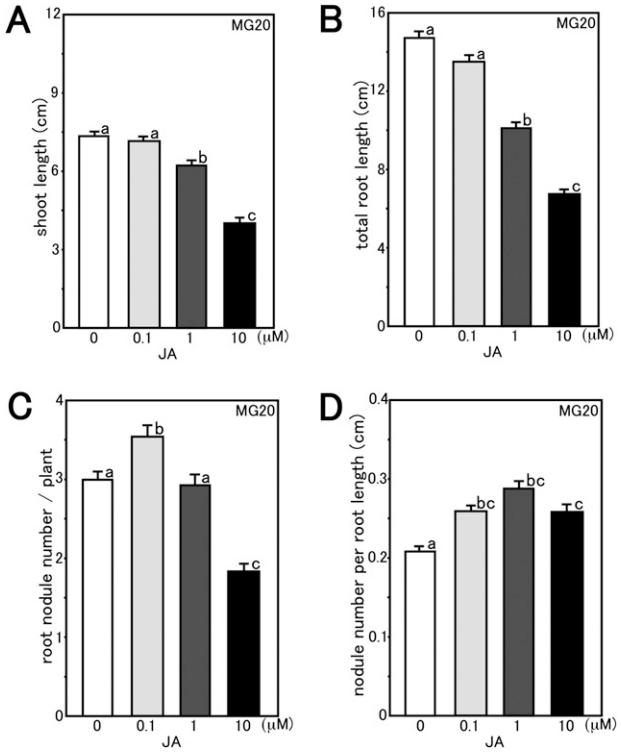


**Fig. S6.** Effect of JA treatment on symbiotic phenotype and growth in white light-grown MG20 28 DAI. (*A*) Shoot growth, (*B*) root growth, (*C*) nodule number per plant, and (*D*) nodule number per unit root length are shown. Values are means of 48 (0 μM JA), 48 (0.1 μM JA), 48 (1 μM JA), and 47 (10 μM JA) plants. Error bars represent SE, and the significance of differences among the four groups was determined by the two-tailed multiple *t* test with Bonferroni correction after ANOVA (six comparisons in four groups). Means denoted by the same letter did not differ significantly at $P < 0.05$.

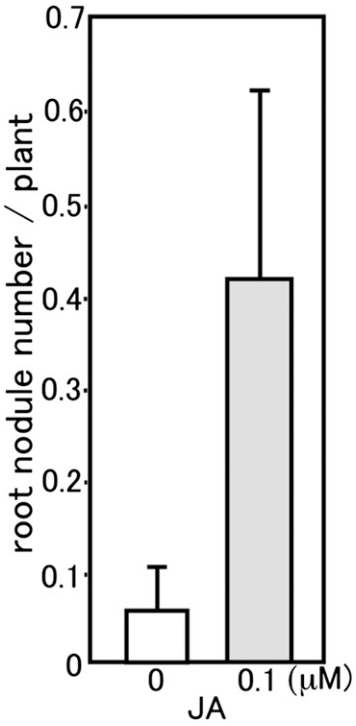


**Fig. S7.** Effects of JA on root nodule formation. Effect of JA treatment on root nodule formation in inoculated low R/FR light-grown MG20. GUS stained nodules and nodule primordia of 25-d-old (10 DAI) plants were observed. Values are the means of 17 (0 μM JA) and 14 (0.1 μM JA) plants. Error bars represent SE ($P = 0.099$ by Student *t* test).

**Table S1. Primers used in this study**

| Primer name | Sequence (5′ → 3′) |
|---|---|
| *LjPHYB* sequencing | |
| LjPHYB F | CTTCTCCTATCACACTTC |
| LjPHYB R | GGCTAGCTTTCACCAAGT |
| dCAPs analysis | |
| 0017Hind F | CTCCTCACGGTTGCCAAGCT |
| 0017 R | ACAGCCTCATCGAGCTCCG |
| 1428Hae F | AGATGGTCAGATCATTGGCG |
| 1428 R | GCTAACTCTTTCATCCTAGC |
| Real-time RT-PCR | |
| LjPDF1.2 F | GTGATCAGAGGTGTAAAGCC |
| LjPDF1.2 R | AGTTATCACTGCACTGGAAG |
| LjCOI1a F | AGAGGCTAAAAAGACTTAGGATTGAA |
| LjCOI1a R | AAGCAATTAATCCTCTATGGGAAA |
| LjCOI1b F | AGCTAAGAAGATTCGCCTTGTATC |
| LjCOI1b R | TGTAACCAAGGCCTACATCAGTT |
| LjERF1a F | GGCAATGTGGTTGTCTTTGA |
| LjERF1a R | TGATGACATTAACAACTGTTCCAAA |
| LjERF1b F | AGAATGGCTGCGGCAGTA |
| LjERF1b R | GCCGCCTCCTTACACCTC |
| LjHELa F | GAGAATGAGGATAAGTAGCAGCATC |
| LjHELa R | CCACCACACCGCCACTAT |
| LjHELb F | TGCCACCAATGTGAGAGCTA |
| LjHELb R | TCACTGCATTCAAGTCCCAAT |
| LjJAR1 F | GGTGAAGAGTATGAAATTGTTATGACC |
| LjJAR1 R | CCACATCTCCTAGCCTATACCG |
| LjMYC2 F | CGAAGAACGGCAGCAGTAAC |
| LjMYC2 R | AGCTCAGAATCTCACCGGATT |
| LjNIN F | CCAGCTCCAACAAGACGAA |
| LjNIN R | AGCTGGTCCAATCCACCAT |
| LjATPsyn F | ACATGCTTGCACCATACCAA |
| LjATPsyn R | TCCCCAACTCCAGCAAATAC |
| LjVSP1a F | CATCCTTCAATGAATGGGTCA |
| LjVSP1a R | AACTCAAGCTAGCAGGCAATG |
| LjVSP1b F | TCAAGGCATGGATCATGAAG |
| LjVSP1b R | CTTAAACAATCCTAATATGGCCTGA |

dCAPS, derived cleaved amplified polymorphic sequence.