Supporting Information

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SI Methods

Microarray Analysis. Four biological replicates per treatment were tested for PD L*er* seed, and three were tested for *ga1-3* seed. Independent seed batches were used for each biological replicate. Array data were GC-RMA normalized, and the average KAR_1/water (K/W) fold change (FC) of a probeset was calculated for each genotype. Remarkably, maximum FC was ~6- to 7-fold, and only a few genes had FC > 3. In experiments with low numbers of replicates and small changes in expression, the application of false discovery rate (FDR) to reduce type 1 errors (false positives) from *P* value inferences results in substantial type 2 error (false negatives) [Nettleton D (2006) *Plant Cell* 18:2112–2121]. Consequently, we adopted an atypical approach to identify the most highly KAR_1-responsive genes.

Because our primary interest in this experiment was variation due to treatment rather than genotype, we combined the PD L*er* and *ga1-3* datasets and performed a paired Student's *t* test with the seven arrays between treatments to identify significant changes in expression for each gene ($P < 0.01$). This set was filtered by a FC > 1.5 cutoff to give the Criteria I list of highly KAR_1-responsive genes for each genotype (Table S1). Because we also anticipated a treatment–genotype interaction component to variation (i.e., some transcripts, such as *GA3ox1*, may have very low expression or altered response to KAR_1 as a result of GA abundance in one genotype), we tested the remaining probesets for each genotype with a paired Student's *t* test. Criteria II probesets with significant responses ($P < 0.05$ and FC > 1.5) to treatment were thus separately identified for PD L*er* and *ga1-3* (Table S2). It is notable that the majority of potential KAR_1-responsive genes were already captured by Criteria I selection (Table 1). This approach produced similar results to a linear model analysis without an interaction component. However, seed dormancy is notoriously variable from batch to batch, and therefore we considered paired testing to be more appropriate than a linear model.

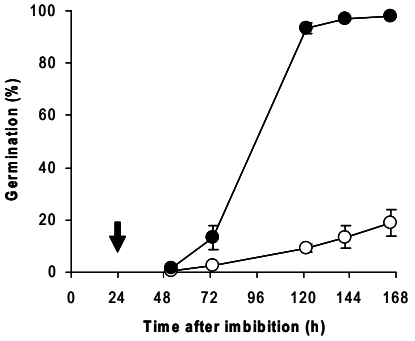

Fig. S1. Germination of PD L*er* seed on water agar with (filled circles) or without (open circles) 1 μM KAR_1 under continuous white light at 20 °C. Arrow indicates pregermination time point chosen for microarray analysis. Mean ± SD, *n* = 3, 100 seeds per sample.

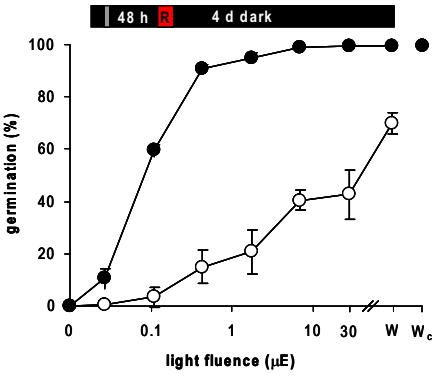

Fig. S2. Karrikins enhance light-dependent germination of *Arabidopsis* seed. Afterripened seed was imbibed on water-agar (open circles) or 1 μM KAR_1 (filled circles) in the dark for 1 h, exposed to 5 min of FR, incubated in the dark for 48 h, then given 1 h of R light of the indicated intensity. Germination was assessed after a further 4-d dark incubation. Seeds treated with a white light (100 μE) pulse (W) or continuous white light for 4 d (Wc) are shown for comparison. Mean ± SD, *n* = 3, ≥100 seeds per sample.

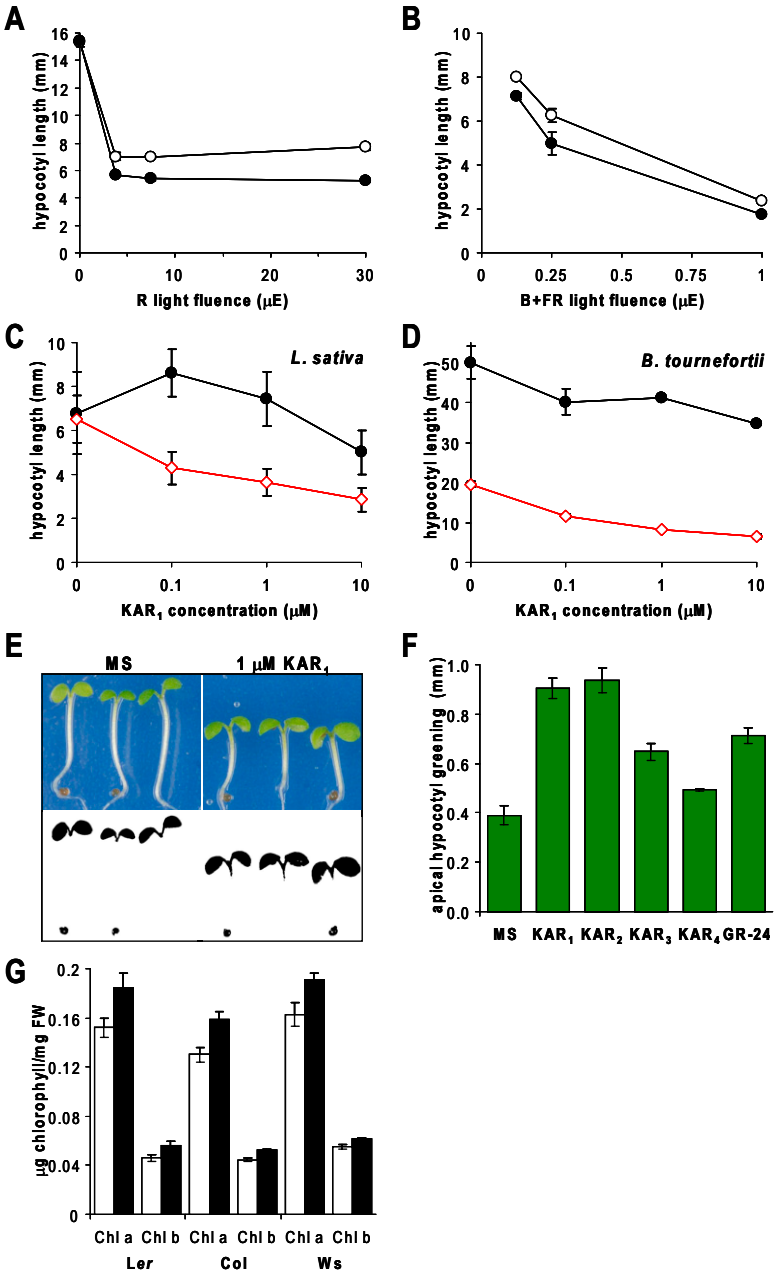

Fig. S3. Karrikins promote seedling responses to light. (*A*) *Ler* seedlings grown on 0.5× MS or 1 μM KAR_1 under the indicated R light fluence. (*B*) *Ler* seedlings grown on 0.5× MS or 1 μM KAR_1 under the indicated blue + far red (B+FR) light fluence. For *A* and *B*, mean ± SEM, $n = 3$, ≥25 seedlings per sample, except dark ≥13 seedlings per sample. (*C*) Lettuce seed (*Lactuca sativa* L. cv. Grand Rapids) was plated on 0.8% agar supplemented with indicated concentrations of KAR_1 under room light then wrapped in foil or put under Rc for 3 d growth. Mean ± SD, 20 seedlings per point except for dark, 13 seedlings. (*D*) A nondormant collection of *B. tournefortii* seed was plated on 0.8% agar under room light and then placed in darkness at 20 °C. After 45 h, germinated seeds (~2–5 mm radicle length but no cotyledon emergence) were transferred to 0.8% agar supplemented with indicated concentrations of KAR_1 and grown under Rc for 4 d. Mean ± SEM, $n = 3$, 9 seedlings per sample. (*E*) Green pigmentation of the apical hypocotyl of seedlings grown under Rc (as for hypocotyl elongation assays) was visualized through RGB splitting in ImageJ. (*F*) Maximum contiguous length of above threshold signal, as shown in (*E*), from shoot apex for seedlings grown on karrikins and GR-24 as in Fig. 4. Mean ± SEM, $n = 3$, ≥11 seedlings per sample. (*G*) Chlorophyll from 50 seedlings grown under Rc conditions on 0.5× MS (open bars) or 1 μM KAR_1 (filled bars) was extracted in buffered 80% acetone and assayed spectrophotometrically. Mean ± SEM, $n = 3$.

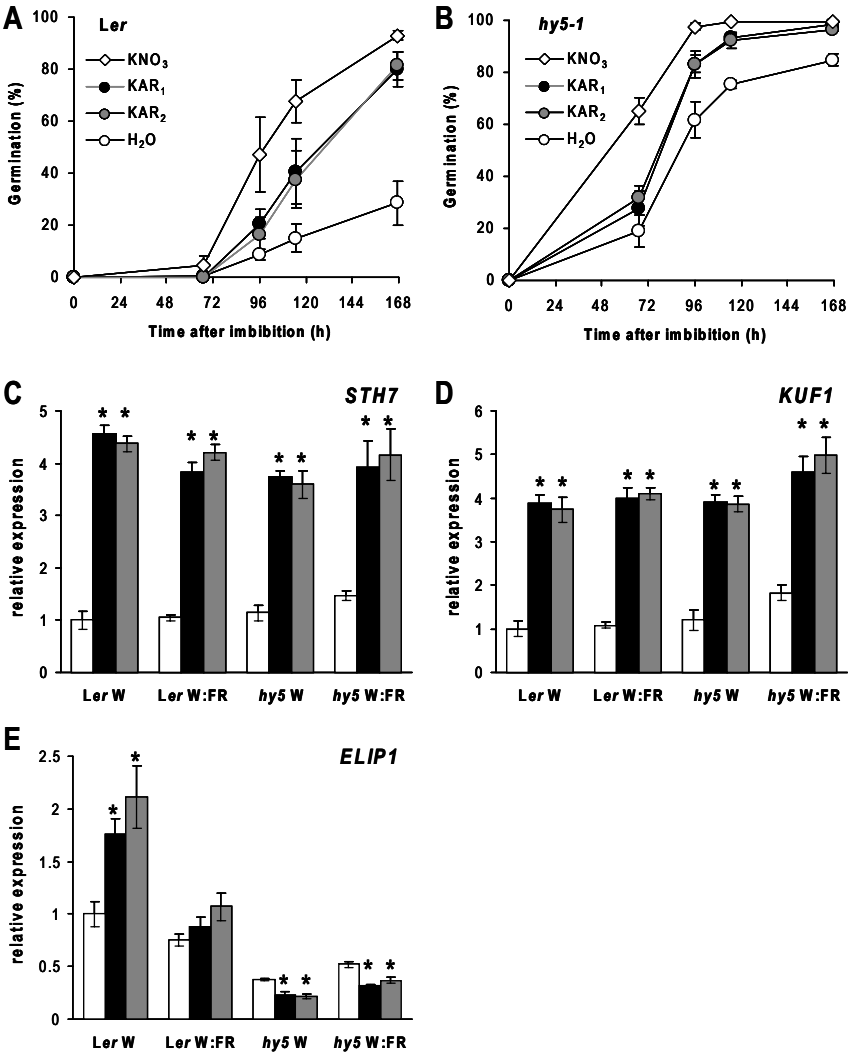

Fig. S4. *HY5* is not required for all karrikin responses in seed. Germination of primary dormant (*A*) L*er* and (*B*) *hy5-1* on water agar (open circles), 1 μM KAR_1 (black circles), 1 μM KAR_2 (gray circles), or 10 mM KNO_3 (open diamonds) under continuous white light at 20 °C. Mean± SEM, $n = 3$ independent seed batches, 65–100 seeds per sample. Relative expression of *STH7* (*C*), *KUF1* (*D*), and *ELIP1* (*E*) in PD L*er* and *hy5-1* seed. Seed was imbibed for 2 h under white light (W) on water-agar (open bars), 1 μM KAR_1 (black bars), or 1 μM KAR_2 (gray bars) and then placed into darkness for 22 h. A subset (W:FR) was treated with 5 min of FR (6 μE) light to deactivate Pfr before dark transfer. Relative expression was assayed by qRT-PCR after 24 h imbibition. L*er* W water control expression for each gene was set to 1, and other values scaled accordingly. Mean ± SEM, $n = 3$ independent seed batches. *, $P < 0.05$, two-tailed *t* test.

Table S1. Criteria I gene sets for PD L*er* and *ga1-3* seed imbibed for 24 h

Paired t test was performed against the seven (four PD Ler, three ga1-3 per treatment) ATH1 microarrays of samples treated with water or 1 mM KAR1. Probesets with $P < 0.01$ and FC of at least 1.5-fold magnitude are shown here for each genotype. Genes examined by qRT-PCR (Fig. 1) are highlighted in blue. The PD Ler up-regulated set are referred to as KAR-UP in the text.

Table S1 (XLS)

Table S2. Criteria II gene sets for PD L*er* and *ga1-3* seed imbibed for 24 h

Paired t test was performed against the four ATH1 microarrays per treatment of PD Ler samples treated with water or 1 mM KAR1. Probesets with $P < 0.05$ and FC of at least 1.5-fold magnitude are shown here. The same selection was performed against the three ATH1 microarrays per treatment of ga1-3 samples to generate the ga1-3 Criteria II list. Genes examined by qRT-PCR (Fig. 1) are highlighted in blue.

Table S2 (XLS)

Table S3. Summary of comparisons of KAR-UP genes to previously published genomic studies

Table S3 (XLS)

Table S4. Comparison of KAR-UP genes to previously published genomic studies

Table S4 (XLS)

Table S5. qRT-PCR primers

Table S5 (XLS)