Development 139, 1285-1295 (2012) doi:10.1242/dev.074567

Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in *Arabidopsis*

Mark T. Waters[1,*], David C. Nelson[2], Adrian Scaffidi[3], Gavin R. Flematti[3], Yueming K. Sun[3], Kingsley W. Dixon[4,5] and Steven M. Smith[1,3]

SUMMARY

Karrikins are butenolides derived from burnt vegetation that stimulate seed germination and enhance seedling responses to light. Strigolactones are endogenous butenolide hormones that regulate shoot and root architecture, and stimulate the branching of arbuscular mycorrhizal fungi. Thus, karrikins and strigolactones are structurally similar but physiologically distinct plant growth regulators. In *Arabidopsis thaliana*, responses to both classes of butenolides require the F-box protein MAX2, but it remains unclear how discrete responses to karrikins and strigolactones are achieved. In rice, the DWARF14 protein is required for strigolactone-dependent inhibition of shoot branching. Here, we show that the *Arabidopsis* DWARF14 orthologue, AtD14, is also necessary for normal strigolactone responses in seedlings and adult plants. However, the AtD14 paralogue KARRIKIN INSENSITIVE 2 (KAI2) is specifically required for responses to karrikins, and not to strigolactones. Phylogenetic analysis indicates that KAI2 is ancestral and that AtD14 functional specialisation has evolved subsequently. *Atd14* and *kai2* mutants exhibit distinct subsets of *max2* phenotypes, and expression patterns of *AtD14* and *KAI2* are consistent with the capacity to respond to either strigolactones or karrikins at different stages of plant development. We propose that AtD14 and KAI2 define a class of proteins that permit the separate regulation of karrikin and strigolactone signalling by MAX2. Our results support the existence of an endogenous, butenolide-based signalling mechanism that is distinct from the strigolactone pathway, providing a molecular basis for the adaptive response of plants to smoke.

KEY WORDS: Karrikin, Strigolactone, Butenolide, Plant growth regulator, Plant development, *Arabidopsis*

INTRODUCTION

Seed germination is a critical event in the plant life cycle. Many species exhibit physiological seed dormancy, which limits germination under favourable but transient environmental conditions that may not support long-term survival (Finkelstein et al., 2008). Wildfires present a brief opportunity for plants to exploit reduced competition for light, water and nutrients, and the dormant seed of a wide taxonomic range of species exhibit enhanced germination following smoke exposure (Roche et al., 1997; Chiwocha et al., 2009). The butenolide 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, or KAR_1, was identified in smoke as a bioactive compound that defines a family of related molecules known as karrikins (Flematti et al., 2004). Karrikins are potent germination stimulants, which are active in some species at concentrations as low as 1 nM (Flematti et al., 2004; Long et al., 2010). They also increase the sensitivity of seedlings to light, potentially enhancing seedling establishment and survival in the post-fire environment (Nelson et al., 2010). Elucidating the molecular genetic basis for responses to karrikins is key to exploiting their potential application in restoration ecology and agriculture (Daws et al., 2007; Stevens et al., 2007).

[1]ARC Centre of Excellence for Plant Energy Biology, [3]School of Biomedical, Biomolecular and Chemical Sciences, and [5]School of Plant Biology, The University of Western Australia, Crawley, Western Australia 6009, Australia. [2]Department of Genetics, University of Georgia, Athens, GA 30602, USA. [4]Kings Park and Botanic Garden, West Perth, Western Australia 6005, Australia.

*Author for correspondence (mark.waters@uwa.edu.au)

Accepted 18 January 2012

Germination of dormant *Arabidopsis thaliana* seed is generally promoted by karrikins, although karrikins cannot overcome the germination requirement for light and de novo synthesis of the phytohormone gibberellin (Nelson et al., 2009). To discover the molecular components of karrikin perception in *Arabidopsis*, we initiated a genetic screen for *karrikin insensitive* (*kai*) mutants. Two such mutants, exhibiting increased seed dormancy that could not be alleviated by karrikins, carried mutations in the *MAX2* gene. Additional *max2* alleles also conferred increased dormancy and insensitivity to karrikins, confirming that *MAX2* is required for karrikin responses (Nelson et al., 2011). *MAX2* is most renowned for its role in mediating responses to strigolactones, a class of plant-synthesized compounds that are exuded from roots, triggering the germination of parasitic weeds and promoting hyphal branching in arbuscular mycorrhizal fungi (Yoneyama et al., 2007; Dor et al., 2011a; Dor et al., 2011b). Recently, strigolactones were shown to be endogenous plant hormones that inhibit the outgrowth of axillary buds (Sorefan et al., 2003; Gomez-Roldan et al., 2008; Umehara et al., 2008) and influence root architecture (Kapulnik et al., 2011; Koltai, 2011; Ruyter-Spira et al., 2011). Two carotenoid-cleavage dioxygenases, CCD7/MAX3 and CCD8/MAX4, as well as a cytochrome P450, MAX1, are involved in strigolactone biosynthesis in *Arabidopsis*. Mutations in orthologous genes in rice, petunia and pea indicate that strigolactone control of shoot branching is conserved in angiosperms (Sorefan et al., 2003; Snowden et al., 2005; Arite et al., 2007). All of the *max* mutants share an increased shoot branching phenotype, but only *max2* is strigolactone insensitive, implying that MAX2 is involved in the strigolactone response. Both karrikins and strigolactones induce similar effects at the germination and seedling stages in a MAX2-

dependent manner (Nelson et al., 2011). Thus, despite their different origins, the karrikin and strigolactone signalling pathways both converge upon MAX2.

Consistent with a common signal transduction mechanism, karrikins and strigolactones share partial structural similarity. KAR_1 and KAR_2, the most active karrikins in *Arabidopsis*, have a butenolide moiety in common with the D-ring of strigolactones (supplementary material Fig. S1). The D-ring is necessary, but not sufficient, to stimulate the germination of parasitic weeds and to promote hyphal branching in arbuscular mycorrhizal fungi (Akiyama et al., 2005; Akiyama et al., 2010). Several simple butenolide variants are ineffective in seed germination stimulants of *Arabidopsis* (Nelson et al., 2011), indicating that the butenolide ring alone is not sufficient for bioactivity. Despite the molecular similarity, plant responses to karrikins and strigolactones differ in several respects. The synthetic strigolactone GR24 is highly effective in promoting germination of parasitic weeds (*Striga* and *Orobanche* spp.), but karrikins are not (Nelson et al., 2009). Both karrikins and GR24 are effective light-dependent inhibitors of hypocotyl elongation in *Arabidopsis*, but karrikins are much more effective than GR24 in promoting seed germination of *Arabidopsis* and *Brassica tournefortii* (Nelson et al., 2009; Nelson et al., 2010; Tsuchiya et al., 2010). Most strikingly, karrikins do not recover normal shoot branching in strigolactone-deficient mutants of *Arabidopsis* or pea (Nelson et al., 2011). Therefore, plants have the ability to distinguish between karrikins and strigolactones at various stages of development.

Given the similarities between karrikins and strigolactones, we reasoned that they might have related signalling components in addition to MAX2. More specifically, we hypothesized that plants must possess a mechanism to discriminate between the two classes of butenolides. Recently, it was shown that the rice *DWARF14* (*OsD14*) gene, a member of the α/β hydrolase superfamily, is required for strigolactone-dependent control of shoot branching and acts in the same pathway as other strigolactone-related genes (Arite et al., 2009; Gao et al., 2009; Liu et al., 2009). This raised the possibility that related proteins within the same family might be involved in karrikin responses. Here, we demonstrate that two members of the DWARF14 family provide a molecular basis for differentiation of strigolactone and karrikin signalling.

MATERIALS AND METHODS

Chemical synthesis

Synthesis of KAR_1, KAR_2 and GR24 was performed as described (Mangnus et al., 1992; Goddard-Borger et al., 2007).

Plant material

The *spt-1* mutant, which carried the *kai2-1* allele, was a gift from D. Smyth (Monash University). The *kai2-1* allele was isolated from the *spt-1* background by outcrossing to L*er* and selecting for KAR-insensitive F_2 individuals that lacked the stunted gynoecium phenotype of *spt* mutants. All other seeds were obtained from the European Arabidopsis Stock Centre (NASC). The *Atd14-1* mutant was isolated from the Wisconsin DsLox T-DNA insertion collection (NASC ID: N913109). The *kai2-2* allele was derived from the Institute of Molecular Agrobiology (IMA) *Ds* insertion lines collection (N100282). The *dlk2* mutants were isolated from the SALK T-DNA insertion collection: *dlk2-1* (N679066), *dlk2-2* (N657226) and *dlk2-3* (N665057). The *max2-8* mutant was described previously (Nelson et al., 2011). Genotyping primers are listed in supplementary material Table S1.

Plant growth conditions, germination tests and hypocotyl elongation assays

Plants were grown in peat, vermiculite and perlite mixture (6:1:1) under fluorescent lamps emitting 100-120 μmol photons s^{-1} m^{-2} with a 16-hour light/8-hour dark photoperiod and a 22°C light/16°C dark temperature cycle. Germination tests were performed under constant white light at 20°C (Nelson et al., 2009; Nelson et al., 2010). For hypocotyl elongation and cotyledon expansion assays, surface-sterilised seeds were sown on solidified 0.5× MS media (pH 5.9) and stratified in the dark at 4°C for 72 hours. At 20°C the seeds were exposed to white light for 3 hours, transferred to dark for 21 hours, and then exposed to continuous red light (LED, λ_{max}=652 nm, 20 μmol photons s^{-1} m^{-2}) for 4 days. Hypocotyls and cotyledons were measured using ImageJ (http://imagej.nih.gov/ij/). Karrikin and GR24 stock solutions (1000× or 2000×) were prepared in acetone; equivalent acetone volumes were added to untreated controls.

RNA isolation and transcript analysis

Total RNA was isolated from seedlings using the Qiagen RNeasy procedure. RNA isolation from seed, DNase treatment, cDNA synthesis and qRT-PCR were performed as described (Nelson et al., 2010). For primers, see supplementary material Table S2.

Arabidopsis shoot branching assay

Seeds were sown on rock wool plugs held in black 1.5-ml microcentrifuge tubes (bottom end and cap removed). The tubes were placed in 24-well plastic boxes (I5100-43, Astral Scientific, NSW, Australia) containing Hoagland's nutrient solution (Heeg et al., 2008), and the boxes placed in the dark at 4°C for 3 days. Seeds were germinated under the growth conditions described above. Five days after germination, seedlings were thinned to one seedling per tube. One week later, individual plants were spaced out to four plants per box, with three boxes per genotype/treatment combination. Boxes were randomised with respect to position on the shelf. At this point, the media were supplemented with either 5 μM GR24 or 0.05% (v/v) acetone, and changed every 4-6 days as required. Rosette leaves were counted upon bolting, and secondary rosette branches (>5 mm in length) were counted when the primary inflorescence ceased growth.

Phylogenetic analysis

KAI2 homologues were identified with BLASTP searches of GenBank protein databases using the *Arabidopsis* KAI2 amino acid sequence as a query (http://www.ncbi.nlm.nih.gov). To identify DLK2 orthologues in monocots, the *Arabidopsis* DLK2 sequence was used in a separate BLASTP query. Additional sequences were obtained from the Plant Genome Database (http://www.plantgdb.org) and the incomplete JGI *Marchantia* genome sequencing effort (supplementary material Table S3). Sequences were sampled from a broad taxonomic spread and screened for duplication and truncation. Full-length sequences were then aligned using MAFFT (http://mafft.cbrc.jp/alignment/software) using the default settings, and the alignment was conservatively edited to remove regions of poor alignment using PFAAT (http://pfaat.sourceforge.net). MrBayes v3.1.2 (Ronquist and Huelsenbeck, 2003) was used to infer Bayesian trees (random start tree, eight chains of temperature 0.2, WAG substitution matrices, four discrete categories of gamma distribution substitution rate). Maximum likelihood phylogenies were produced using PHYML v2.4.4 (Guindon and Gascuel, 2003) (100 replicates, WAG matrix, four gamma categories, alpha parameter re-estimation for each replicate).

Statistical analysis

For comparisons of branch numbers per rosette leaf between different genotypes, one-way, two-sided ANOVA (Bonferroni *t*-test) was performed. For hypocotyl lengths, branching assays and transcript levels, the effects of genotype, treatment and genotype×treatment were analysed by two-sided ANOVA. *P*-values were derived from post-hoc tests using Tukey's correction for multiple pairwise comparisons. For germination data, seed germination percentages were arcsine transformed prior to analysis. Statistical analysis was performed with SAS Enterprise Guide 4.3 (SAS, Cary, NC; www.sas.com).

RESULTS

The *Arabidopsis* DWARF14 orthologue is required for strigolactone responses

In rice, *OsD14* is considered necessary for strigolactone signalling because the increased tillering phenotype of *Osd14* mutants cannot be reversed with exogenous GR24 (Arite et al., 2009). We searched

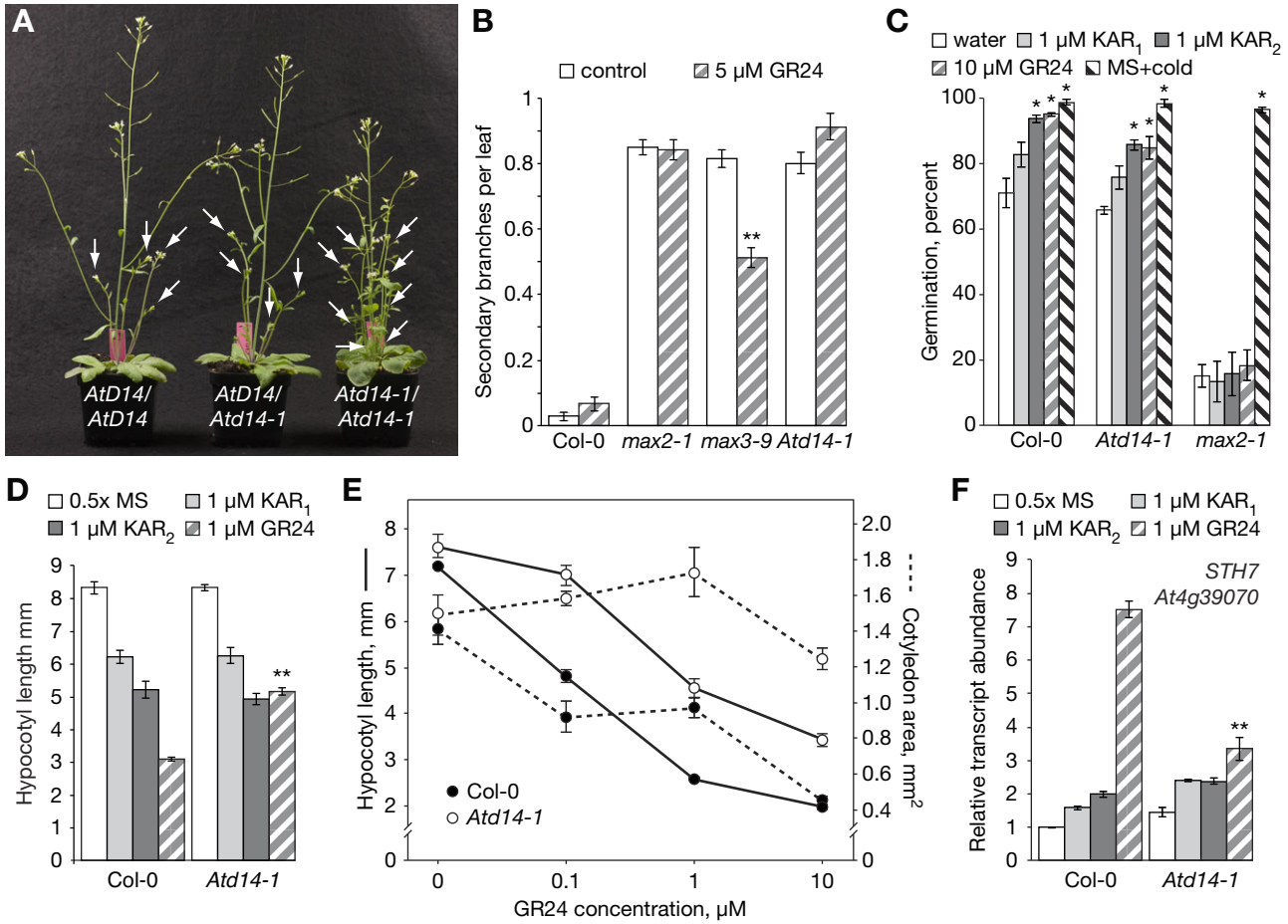

Fig. 1. DWARF14 is required for normal strigolactone responses in *Arabidopsis*. (**A**) Wild-type, heterozygous and homozygous *Atd14-1* siblings 32 days post-germination. Arrows indicate secondary rosette branches. (**B**) Secondary rosette branching in 46-day-old Col-0, *max2-1*, *max3-9* and *Atd14-1* plants grown hydroponically with either 0.05% (v/v) acetone or 5 μM GR24. *n*=12 plants. ***P*<0.001 (ANOVA) versus untreated controls. (**C**) Germination after 88 hours of Col-0, *max2-1* and *Atd14-1* mutants on water-agar plus 1 μM KAR_1, 1 μM KAR_2 or 10 μM GR24, or on 0.5× MS media after dark stratification (4°C, 3 days). *n*=3 independent seed batches, 100 seeds/sample. **P*<0.02 (ANOVA) versus water-agar control. (**D**) Hypocotyl elongation responses in Col-0 and *Atd14-1* seedlings grown for 4 days under continuous red light on 0.5× MS media plus 1 μM KAR_1, 1 μM KAR_2 or 1 μM GR24. *n*=3 biological replicates, 18-24 seedlings/sample. ***P*<0.001 (ANOVA) between genotypes under the same treatment. (**E**) Hypocotyl elongation and cotyledon expansion responses of Col-0 and *Atd14-1* seedlings grown for 4 days under continuous red light on 0.5× MS media plus GR24. *n*=3 biological replicates, 15-18 hypocotyls and 13-18 cotyledons per sample. (**F**) *STH7* transcript levels (normalised to *CACS* reference transcript and scaled to the value of Col-0 on 0.5× MS media) in Col-0 and *Atd14-1* seedlings grown as described in D. ***P*<0.001 (ANOVA) between genotypes under the same treatment. Data are means ± s.e.

for mutations in the closest *Arabidopsis* homologue of *OsD14*, *At3g03990*, which we term *AtD14*. We obtained one mutant allele, *Atd14-1*, which carries a T-DNA insertion within the first 100 bp of the *AtD14* coding region, rendering full-length transcripts undetectable (supplementary material Fig. S2A). Homozygous *Atd14-1* mutants have reduced stature and increased numbers of secondary rosette branches (Fig. 1A). Knockdown of *AtD14* transcripts by artificial microRNAs leads to a similar increased branching phenotype (supplementary material Fig. S2B,C).

Upon flowering, some axillary buds at the base of rosette leaves activate and grow out to form secondary branches. In strigolactone signalling mutants, inhibition of such outgrowth is lost, and more rosette branches develop. To test whether the *Atd14-1* branching phenotype is due to insensitivity to strigolactones or to a deficiency in strigolactone biosynthesis, we grew plants in hydroponic medium supplemented with GR24. The strigolactone biosynthetic mutant *max3* responded to GR24 application with a significant reduction in secondary bud outgrowth (*P*<0.001), but *Atd14-1* and *max2-1* were insensitive to GR24 (Fig. 1B). These data are consistent with the rice *Osd14* mutant phenotype and suggest that, like *MAX2*, *AtD14* is necessary for strigolactone response.

To test whether *Atd14-1* mutants are also defective in karrikin responses, we first examined the effects of karrikins on seed germination. In our hands, the *Arabidopsis* Col-0 ecotype exhibits little seed dormancy and consequently shows relatively little enhancement in germination by karrikins (Nelson et al., 2009; Nelson et al., 2011). The *Atd14-1* mutant exhibited a germination profile indistinguishable from wild type. By contrast, *max2-1* showed substantially delayed germination that was insensitive to karrikins or GR24 (Fig. 1C). Because of the weak dormancy of Col-0, a more sensitive assay for karrikin responses is the inhibition of hypocotyl elongation in seedlings grown under red light (Nelson et al., 2010; Nelson et al., 2011). Wild-type seedlings grown in the presence of KAR_1, KAR_2 and GR24 exhibited a shortened hypocotyl, with GR24 being the most potent compound (Fig. 1D). However, although *Atd14-1* hypocotyls responded normally to

karrikins they were substantially less sensitive to GR24: on average, wild-type seedlings grown on 1 μM GR24 were 37% of the height of seedlings grown without GR24, as compared with 62% for *Atd14-1* (Fig. 1D).

To examine this response further, we assayed hypocotyl length and cotyledon size over a range of GR24 concentrations. In wild-type seedlings, increasing concentrations of GR24 induced smaller cotyledons and a shorter hypocotyl (Fig. 1E). Although this trend was generally also true for *Atd14-1* seedlings, the response was much weaker, with inhibition of cotyledon expansion only detected at 10 μM GR24. Furthermore, *Atd14-1* hypocotyls were longer than wild-type hypocotyls at all concentrations tested (Fig. 1D,E). It is noteworthy that whereas KAR_1 and KAR_2 promote cotyledon expansion (Nelson et al., 2010), we find that GR24 has the opposite effect and inhibits cotyledon growth.

Further, we measured the accumulation of *STH7* transcripts, which increase in abundance in Landsberg *erecta* (L*er*) seedlings in response to KAR and GR24 (Nelson et al., 2010; Nelson et al., 2011). The *STH7* transcript responses to karrikins in *Atd14-1* and Col-0 seedlings were comparable (Fig. 1F). However, the induction of *STH7* transcripts by GR24 was significantly lower in *Atd14-1* mutants than wild type ($P<0.001$), consistent with the decreased hypocotyl response. Two additional transcripts showed a similar pattern to *STH7* (supplementary material Fig. S2D).

Together, these data indicate that AtD14 is dispensable for karrikin responses, but is essential for normal strigolactone responses in seedlings and mature plants.

KAI2, an *AtD14* paralogue, is required for responses to karrikins

The rice genome encodes an uncharacterised protein similar to DWARF14, termed D14-LIKE (Arite et al., 2009). We reasoned that DWARF14 homologues might have attained distinct functions during evolution, so we searched for mutations in the *Arabidopsis* orthologue of D14-LIKE (*At4g37470*) to ascertain whether this protein is important for karrikin or strigolactone signal transduction. We obtained two mutant alleles via two independent routes. First, we discovered that *spt-1*, a weak mutant allele of the bHLH transcription factor SPATULA (Alvarez and Smyth, 1999), was insensitive to karrikins whereas three additional null *spt* alleles responded normally (data not shown). Therefore, we suspected that a second mutation in the *spt-1* background was responsible for the karrikin-insensitive phenotype, and we renamed this unknown mutation *kai2-1*. Second, we used reverse genetics to identify a *Ds* transposon insertion allele of *At4g37470*, which we found was also karrikin insensitive. Given the genetic proximity of *SPATULA* (*At4g36930*) to *At4g37470* (~202 kb), we speculated that *kai2-1* might carry a mutation in *At4g37470*; upon sequencing we discovered a G-to-A transition, modifying a conserved glycine residue to glutamic acid (Fig. 2A, supplementary material Fig. S3). As *kai2-1* and the insertion mutant were allelic (supplementary material Fig. S4), we concluded that *At4g37470* was *KAI2* (Fig. 2A) and named the insertion allele *kai2-2*.

Both *kai2* alleles are in the L*er* background, which exhibits higher levels of seed dormancy than Col-0 (van Der Schaar et al., 1997; Nelson et al., 2009). We found that whereas 1 μM KAR_1, 1 μM KAR_2 and 10 μM GR24 strongly promoted the germination of primary dormant L*er* seed, both *kai2* alleles were fully insensitive to all three compounds (Fig. 2B). Like *max2*, the germination of fresh *kai2* seed on water-agar was negligible, but this enhanced dormancy could be overcome with cold stratification in the presence of nitrate (Fig. 2B). To examine additional karrikin responses, we performed hypocotyl elongation assays. We first noticed that *kai2* seedlings had longer

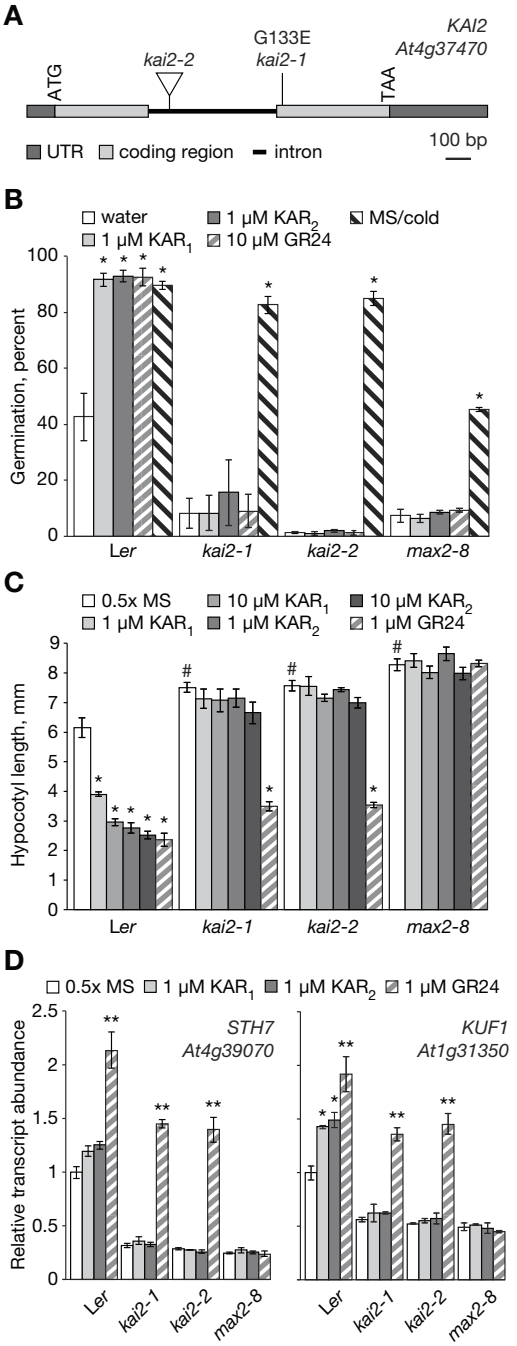

Fig. 2. KAI2 is required for germination and seedling responses to karrikins. (**A**) *KAI2* gene model. *kai2-1* has a missense mutation and *kai2-2* carries a *Ds* element insertion within the intron. (**B**) Germination after 116 hours of L*er*, *kai2-1*, *kai2-2* and *max2-8* mutants on water-agar plus 1 μM KAR_1, 1 μM KAR_2 or 10 μM GR24, or on 0.5× MS media after dark stratification (4°C, 3 days). $n=3$ independent seed batches, 100 seeds/sample. *$P<0.002$ (ANOVA) versus water-agar control. (**C**) Hypocotyl elongation responses of L*er*, *kai2-1*, *kai2-2* and *max2-8* seedlings. $n=3$ biological replicates, 16-27 seedlings/sample. *$P<0.001$ (ANOVA) versus control treatment within the same genotype; $^{\#}P<0.02$ between genotypes on 0.5× MS media. (**D**) Accumulation of *STH7* and *KUF1* transcripts (normalised to *CACS* reference transcript) in L*er*, *kai2-1*, *kai2-2* and *max2-8* seedlings grown for 4 days under continuous white light. $n=3$ biological replicates, greater than 40 seedlings/sample. Transcript abundance is scaled to the value of L*er* on 0.5× MS media. *$P<0.05$, **$P<0.001$ (ANOVA) between treatments within the same genotype. Data are means ± s.e.

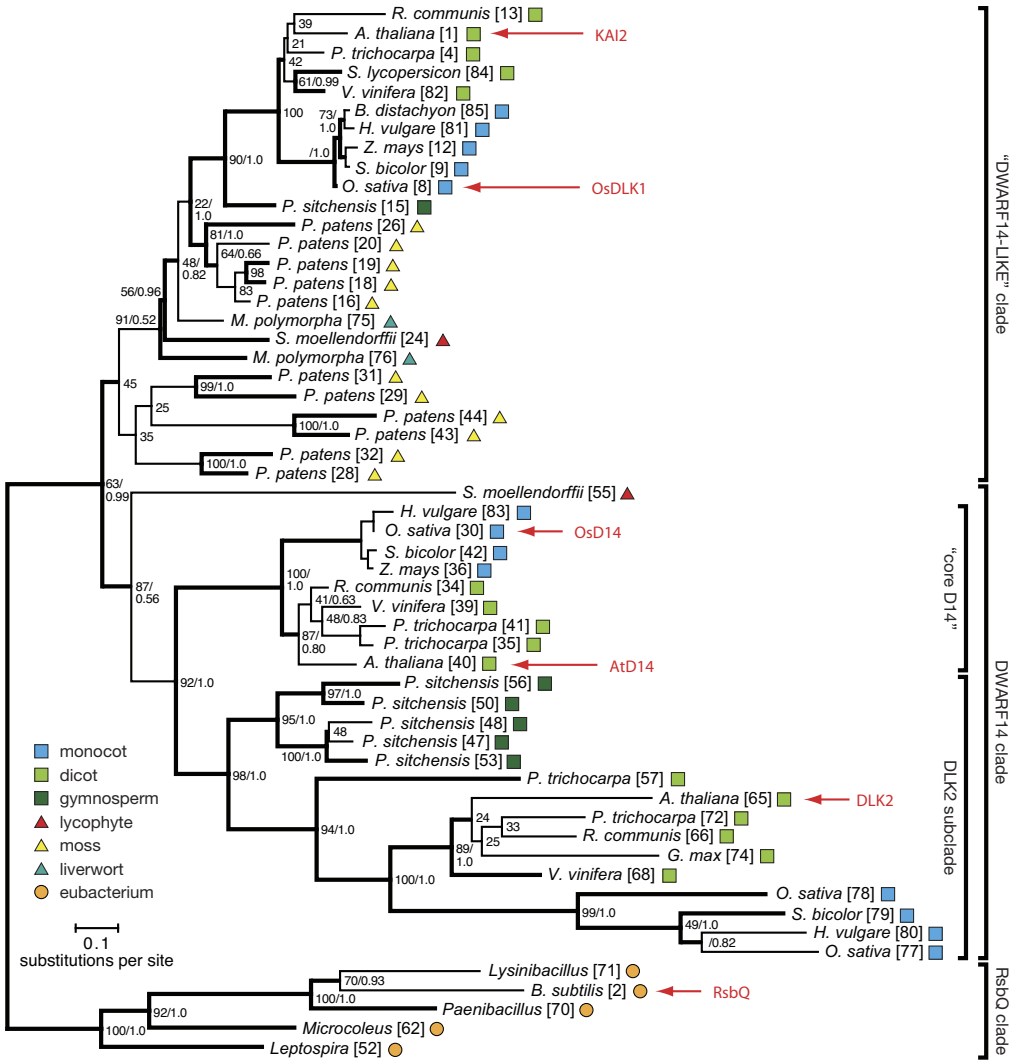

Fig. 3. Phylogenetic analysis of KAI2 and DWARF14 homologues. Maximum likelihood and Bayesian inference phylogenies were produced from an alignment of KAI2-like α/β hydrolases from land plants and bacteria. Topology support for nodes is indicated (maximum likelihood bootstrap values/posterior probability). Branches with strong support (>90 or 0.95) are highlighted in bold. Terminals are labelled with the species name followed by a sequence ID (see supplementary material Table S3). Note the three main clades: DWARF14, D14-LIKE and RsbQ. The DWARF14 clade is further subdivided into 'core D14' and D14-LIKE2 (DLK2) subclades. The tree is rooted on the RsbQ clade.

hypocotyls than wild type, a trait shared with *max2* (Fig. 2C). Crucially, although *kai2* hypocotyls were fully insensitive to karrikins they were still responsive to GR24 (Fig. 2C). This contrasts with *max2* seedlings, which were insensitive to both karrikins and GR24. To confirm this differential sensitivity on the transcript level, we measured the accumulation of two KAR- and GR24-responsive transcripts, *STH7* and *KUF1*, in seedlings. Both *kai2* alleles showed the same pattern of transcriptional marker response: no induction by karrikins but a strong positive response to GR24 (Fig. 2D). In addition, both *kai2* and *max2* exhibited decreased levels of *STH7* and *KUF1* transcripts relative to wild type, indicating that both MAX2 and KAI2 are required for normal expression of these two marker genes.

Together, these data suggest that KAI2 is required for responses to karrikins and is necessary for responses to exogenous strigolactone in seeds but not in seedlings. These results also indicate that KAI2 is necessary for normal seed germination and seedling development, whereas AtD14 is not.

KAI2 and AtD14 are members of distinct phylogenetic clades among land plants

KAI2 and DWARF14 belong to a family of poorly characterised proteins classified as α/β hydrolases (Pfam domain: Abhydrolase_6 PF12697). The only characterised member of this family is RsbQ from *Bacillus subtilis*. RsbQ is an energy-stress response regulator required for the activity of a protein phosphatase that ultimately regulates the transcription of a stress response operon (Brody et al., 2001). At the amino acid level, AtD14 and KAI2 are respectively 38% and 39% identical to RsbQ and both share the putative hydrolase catalytic triad of Ser96, Asp219 and His247 residues (supplementary material Fig. S3). To investigate the evolution of DWARF14 and KAI2, we carried out a phylogenetic analysis of related proteins. Beyond land plants, the most similar KAI2 homologues are found in eubacteria. Both Bayesian inference and maximum likelihood analyses produced consensus trees with highly similar topologies (Fig. 3), with the land plant sequences forming two sister clades. The DWARF14 clade contains AtD14 and OsD14, while the D14-LIKE clade contains the rice founding member OsDLK1 and its *Arabidopsis* orthologue KAI2.

The genome of the moss *Physcomitrella patens* encodes multiple KAI2 homologues, some of which are weakly assigned to the base of the D14-LIKE clade. However, none could be definitively assigned to the DWARF14 clade, which contains a sequence from just one non-seed plant, *Selaginella moellendorffii* (Fig. 3). By contrast, the D14-LIKE clade is taxonomically diverse, encompassing mosses, liverworts, lycophytes, gymnosperms and angiosperms. Such diversity within the D14-LIKE clade suggests that this protein is the ancestral form within the land plants, and that DWARF14 sequences arose from a gene

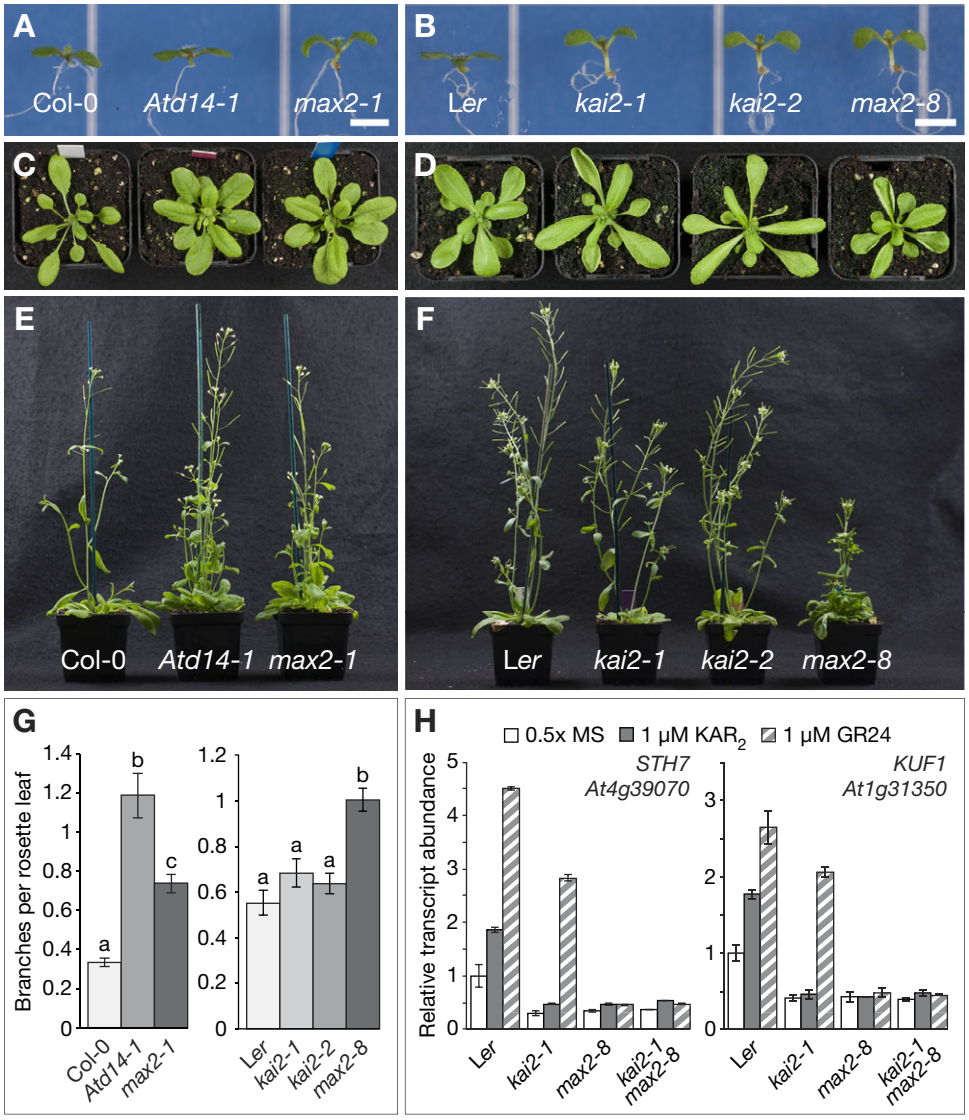

Fig. 4. *Atd14-1* and *kai2* mutants exhibit different component phenotypes of *max2*. (**A**,**B**) Morphology of 7-day-old seedlings grown in constant white light on 0.5× MS media. Scale bars: 2 mm. (**C**,**D**) Rosette morphology of 26-day-old plants. (**E**,**F**) Shoot branching phenotypes of 36-day-old plants. Note that *max2-8* flowers later than L*er* and *kai2*. (**G**) Quantification of shoot branching phenotypes of 41-day-old (left) and 44-day-old (right) plants grown on soil. *n*=7-9 plants. Significant differences in branch number are indicated with different letters (one-way ANOVA, α=0.01). (**H**) Accumulation of *STH7* and *KUF1* transcripts (normalised to *CACS* reference transcript) in L*er*, *kai2-1*, *max2-8* and *kai2-1 max2-8* seedlings grown as described in Fig. 1. *n*=2 biological replicates, greater than 30 seedlings/sample. Transcript abundance is scaled to the value of L*er* on 0.5× MS media. Data are means ± s.e.

duplication event within the vascular plant lineage. In addition, a subclade of the DWARF14 clade contains members from the angiosperms and at least one gymnosperm; we named this subclade DLK2, as it contains a second *Arabidopsis* protein with similarity to AtD14. Thus, there was another gene duplication event within the DWARF14 clade, which presumably occurred after the evolution of seed plants. The long branches within the DLK2 subclade suggest that there has been substantial sequence divergence within this group, while the 'core D14' sequences are relatively more conserved (Fig. 3). This distinction might reflect the acquisition of a novel function by DLK2 in angiosperms, while D14 function was constrained. Together, our phylogenetic analysis suggests that the DWARF14 clade contains more recently divergent homologues, whereas the D14-LIKE clade is evolutionarily more ancient.

To assess whether the *Arabidopsis* DLK2 protein has a role in karrikin or strigolactone signalling, we isolated three mutant T-DNA insertion alleles in the corresponding gene, *At3g24420* (supplementary material Fig. S5A,B). All three *dlk2* mutants exhibited normal hypocotyl elongation responses to KAR_1, KAR_2 and GR24 (supplementary material Fig. S5C). Furthermore, there was no discernible effect of *dlk2* mutations on seedling morphology or the number of rosette branches (supplementary material Fig. S5D,E). We conclude that DLK2 does not play a crucial role in karrikin or strigolactone signalling in *Arabidopsis*.

Atd14 and *kai2* mutants exhibit different component phenotypes of *max2*

The increased shoot branching pattern of *Atd14-1* and the increased seed dormancy of *kai2* are both common to *max2* mutants. To analyse the shared phenotypes more closely, we compared each mutant at different stages of development. At the 7-day seedling stage, *Atd14-1* resembles wild type, but *max2-1* seedlings have an elongated hypocotyl and hooked, epinastic cotyledons (Fig. 4A). By contrast, *kai2* seedlings are indistinguishable from *max2-8* (Fig. 4B), a similarity that mirrors the germination phenotypes (Fig. 2B). Later during vegetative development, *Atd14-1* closely resembles *max2-1*, with shortened leaf petioles and broader, more highly lobed leaves (Fig. 4C). By contrast, the leaves of *kai2* are frequently elongated with curled margins, a phenotype that is most severe in the *kai2-2* allele (Fig. 4D). Although *max2* also exhibits some leaf curling, the overall leaf shape and rosette size of *max2* are distinct from *kai2* (Fig. 4D). Upon flowering, *Atd14-1* has an increased branching phenotype similar to *max2* (Fig. 4E,G), but

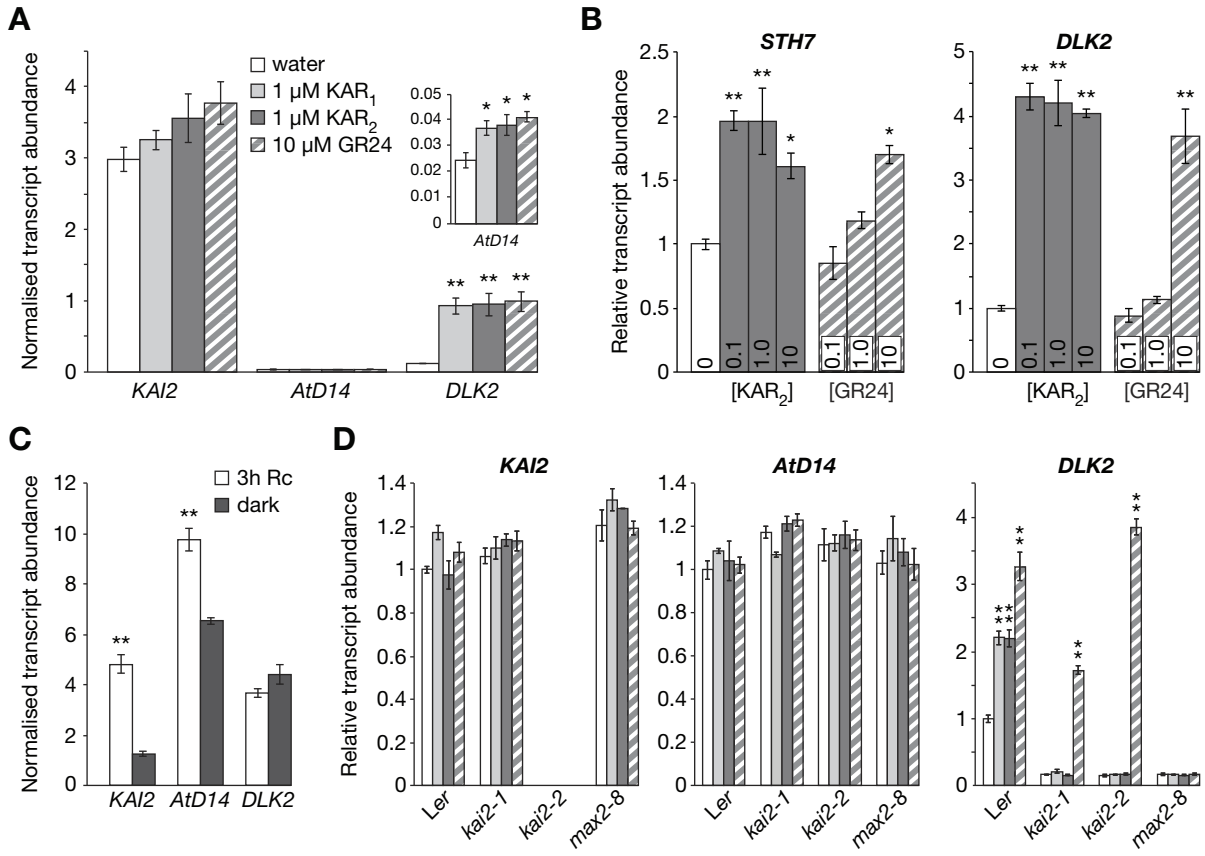

Fig. 5. *KAI2*, *AtD14* and *DLK2* transcripts are differentially expressed in seed and seedlings. (**A**) Abundance of *KAI2*, *AtD14* and *DLK2* transcripts, expressed as fold change over *CACS* reference transcript levels, in L*er* seed imbibed for 24 hours in continuous white light at 20°C on water-agar supplemented with 1 μM KAR_1, 1 μM KAR_2 or 10 μM GR24. (**B**) *STH7* and *DLK2* transcript levels in L*er* seed imbibed for 24 hours in continuous white light at 20°C on water-agar with varying concentrations of KAR_2 or GR24 (0.1-10 μM). Transcript abundance is normalised to *CACS* reference transcript and scaled to the value for the water-agar control. (**C**) Abundance of *KAI2*, *AtD14* and *DLK2* transcripts, expressed as a fold change over *CACS* reference transcript levels, in L*er* seedlings grown in darkness for 96 hours followed by 3 hours darkness or continuous red light (Rc). (**D**) *KAI2*, *AtD14* and *DLK2* transcript levels in L*er*, *kai2-1*, *kai2-2* and *max2-8* seedlings grown as described in Fig. 2. *KAI2* transcripts were undetectable in *kai2-2* mutants. Transcript abundance is scaled to the level of L*er* on 0.5× MS media. **P*<0.05, ***P*<0.01 (ANOVA) relative to the untreated/dark control (A-C) or to the untreated control within the same genotype (D). Data are means ± s.e.; A,B, *n*=3-4 biological replicates, 20 mg dry seed/sample; C,D, *n*=3 biological replicates, greater than 50 seedlings/sample.

kai2 has a branching pattern that is indistinguishable from wild type, indicating that strigolactone signalling is unperturbed (Fig. 4F,G). Thus, KAI2 is not required for branching control, consistent with KAI2 being necessary for karrikin responses and our previous finding that karrikins cannot substitute for strigolactones in the control of axillary bud outgrowth (Nelson et al., 2011). Together, these data show that *Atd14-1* resembles *max2* during the adult growth phase and that *kai2* is similar to *max2* only during the seed and juvenile phases. These broad phenotypic similarities suggest that MAX2 functions together with AtD14 and KAI2, but at distinct stages of plant development.

To test for genetic interaction between *KAI2* and *MAX2*, we generated a *kai2-1 max2-8* double mutant and analysed levels of *STH7* and *KUF1* transcripts in seedlings. As expected, the GR24 responses observed in *kai2-1* were abolished in the double mutant, but otherwise the transcript profiles were indistinguishable from those of either single mutant (Fig. 4H). Furthermore, *kai2-2 max2-8* hypocotyl responses resemble those of *max2* (supplementary material Fig. S6), consistent with KAI2 and MAX2 operating in the same signalling pathway.

AtD14, *KAI2* and *DLK2* are differentially expressed during seed germination and seedling de-etiolation

Compared with wild type, *kai2* exhibits reduced germination on water-agar, but *Atd14-1* does not (Figs 2, 3), showing that KAI2, but not AtD14, has an important role in seed germination. Previously, we discovered that primary dormant L*er* seed is substantially more sensitive to karrikin than to GR24 in a germination assay, requiring 10-fold more GR24 than karrikin to stimulate germination (Nelson et al., 2009). We reasoned that spatiotemporal regulation of *KAI2* and *AtD14* expression might be responsible for the differential sensitivity of seed to karrikins and GR24. We measured transcript levels in L*er* seed that had been water imbibed for 24 hours, and found that *KAI2* transcripts were ~100-fold more abundant than *AtD14* transcripts (Fig. 5A), suggesting that KAI2 dominates in seeds. Simultaneously, we discovered that *KAI2* and *AtD14* transcripts respond minimally to KAR or GR24, but that *DLK2* transcripts increase in abundance several fold (Fig. 5A), making *DLK2* a serendipitously good marker for KAR and GR24 responses. To confirm the differential

sensitivity of L*er* seed to KAR and GR24 on the transcript level, we exposed seed for 24 hours to different concentrations of KAR_2 and GR24. Based on *STH7* and *DLK2* transcript abundance, L*er* seeds are 10- to 100-fold less sensitive to GR24 than to KAR_2 (Fig. 5B). Taken together, these results suggest that KAI2 predominately mediates the stimulation of germination by butenolides, and that KAI2-dependent signalling is promoted by KAR in preference to GR24. Notably, these findings are consistent with the insensitivity of *kai2* seed to GR24 (Fig. 2B).

Whereas *kai2* seeds do not respond to GR24, *kai2* seedlings evidently do (Fig. 2C). We hypothesized that AtD14 might mediate the GR24 responses in *kai2* seedlings, and found that in seedlings *AtD14* transcripts were slightly more abundant than *KAI2* transcripts, in contrast to imbibed seed (Fig. 5C). Although *AtD14* transcripts responded positively to light, the relative induction was considerably lower than for *KAI2* transcripts, which increased 5-fold in light-grown seedlings over dark-grown controls (Fig. 5C). *DLK2* transcripts, however, were unresponsive to light. Together, these results show that *KAI2* and *AtD14* transcripts are similarly expressed in seedlings, and the particular sensitivity of *KAI2* to light might explain the known light requirement for karrikin effects on germination and seedling development (Nelson et al., 2009; Nelson et al., 2010).

To further investigate the regulation of *KAI2*, *AtD14* and *DLK2* transcripts in seedlings, we assessed the response of each to KAR and GR24. As in seed, neither *KAI2* nor *AtD14* transcript levels varied in response to KAR or GR24 in wild-type seedlings, nor was there any evidence of KAI2- or MAX2-dependent regulation of *KAI2* or *AtD14* expression (Fig. 5D). By contrast, and as observed in imbibed seed, *DLK2* transcripts responded positively to both KAR and GR24 and were ~6-fold downregulated in *kai2* and *max2* (Fig. 5D). Thus, whereas *DLK2* is among those genes (such as *STH7* and *KUF1*) that respond to KAR and GR24 and may enact physiological responses, *KAI2* and *AtD14* are more uniformly expressed, performing early in the signal transduction process. Importantly, both KAR- and GR24-mediated regulation of *DLK2*, *STH7* and *KUF1* require MAX2, but only KAR-mediated regulation requires KAI2. These findings provide additional evidence for KAI2 and AtD14 specificity and for both proteins acting upstream of MAX2.

AtD14 and KAI2 both mediate seedling responses to GR24

As the residual response of *Atd14-1* seedlings to GR24 might reflect the redundant activity of KAI2, we generated *kai2-2 Atd14-1* double mutants. Such seedlings were insensitive to both karrikins and GR24 in a hypocotyl elongation assay (Fig. 6A). In addition, the expression patterns of *STH7*, *KUF1* and *DLK2* transcripts in *kai2-2 Atd14-1* seedlings resembled those of untreated *kai2-2* seedlings, even in the presence of GR24 (Fig. 6B). *IAA1* transcripts, which are positively auxin responsive and are upregulated in *max2* (Hayward et al., 2009; Nelson et al., 2011), were also upregulated in *kai2-2*, but not in *Atd14-1*. Significantly, GR24 was able to suppress *IAA1* transcript levels in all genotypes except the double mutant (Fig. 6B). These data indicate that both KAI2 and AtD14 can mediate GR24 responses, but that KAI2 is functionally dominant in seedlings, as exemplified by the long hypocotyls and aberrant transcript levels of untreated *kai2* seedlings. We suggest that the stronger responses of wild-type seedlings to GR24 over karrikins result from the combined activity of both AtD14 and KAI2: whereas GR24 signals via both KAI2 and AtD14, karrikins only act via KAI2. However, this potential dual signalling role of KAI2 in seedlings might be physiologically unimportant: it is possible that KAI2 does not mediate responses to endogenous strigolactones given that the strigolactone-deficient mutants *max1*, *max3* and *max4* do not share any phenotypes with *kai2* mutants.

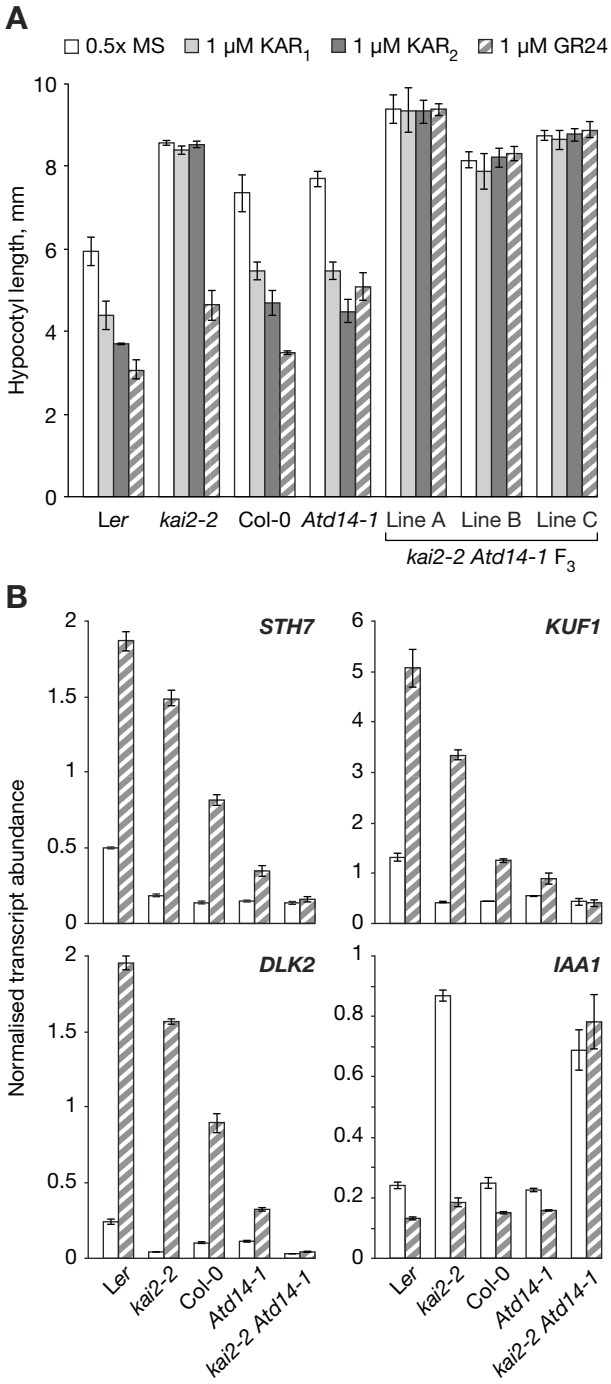

Fig. 6. KAI2 and AtD14 act redundantly in seedling responses to GR24. (**A**) Hypocotyl elongation responses of L*er*, *kai2-2*, Col-0, *Atd14-1* and *kai2-2 Atd14-1* seedlings. F_3 progeny from three independent F_2 double mutants were analysed in parallel. *n*=2 independent replicates, 21-31 seedlings/sample. (**B**) Transcript abundance (expressed as a fold change over *CACS* reference transcripts) in seedlings grown as described in Fig. 1. *n*=3 biological replicates, greater than 50 seedlings/sample. For *kai2-2 Atd14-1*, each replicate was based on F_3 progeny from an independent F_2 individual. Data are means ± s.e.

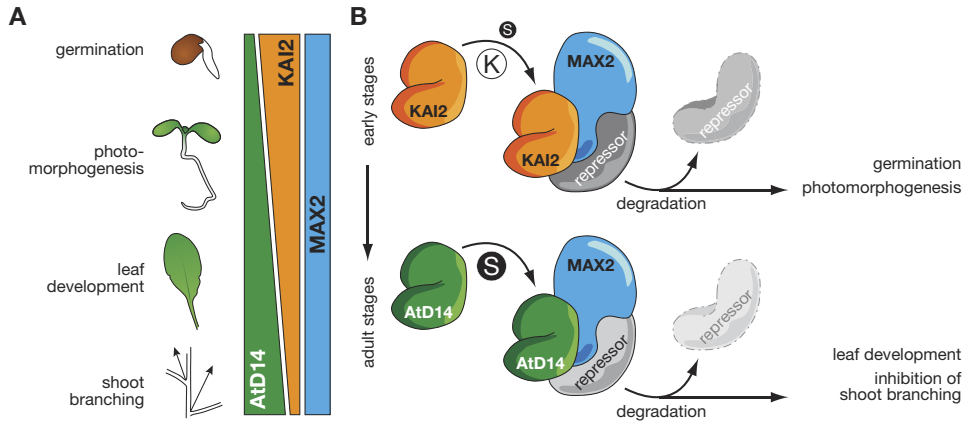

Fig. 7. Model for butenolide signal discrimination by KAI2, AtD14 and MAX2 during plant development. (**A**) The relative expression level of AtD14 and KAI2 varies at different developmental stages and thus each protein influences different growth processes. During seed germination and seedling establishment, KAI2 and karrikin-related signalling play a dominant role, but in later vegetative development AtD14 and strigolactone signalling prevail. MAX2, a likely signalling partner in all of these events, maintains an even expression profile throughout (see supplementary material Fig. S7). (**B**) MAX2, an F-box component of the proteasomal SCF complex, is postulated to degrade various repressor proteins that suppress karrikin or strigolactone responses. KAI2 and AtD14 are potential modulators of MAX2 activity; in this model, they are shown as karrikin and strigolactone receptors. In early development, KAI2 could bind MAX2 in a manner dependent on karrikin or a karrikin-like molecule (K), promoting the degradation of MAX2-bound repressor proteins. To a lesser extent, this process could also be mediated by strigolactones (S). In later development, AtD14 could increase the sensitivity of the SCF^{MAX2} complex to strigolactones, ultimately leading to the repression of shoot branching.

DISCUSSION

With the discovery that karrikin and strigolactone signalling pathways both require MAX2 (Nelson et al., 2011), it remained unclear how plants discriminate between these two structurally similar but physiologically distinct growth regulators. Here, we show that the independent perception of karrikins and strigolactones results from evolutionary specialisation within a divergent family of α/β hydrolase-like proteins.

Karrikins and strigolactones are two known types of plant signalling compounds that share a butenolide ring. Karrikins have only been identified in smoke and have not been reported as endogenous plant compounds. Our findings provide further support for the existence of an endogenous, non-strigolactone signal that acts through the butenolide signalling system to influence seed germination and early seedling development. First, karrikin response is broadly conserved among angiosperms (Flematti et al., 2004; Stevens et al., 2007; Long et al., 2011), but many such species (including *Arabidopsis*) are not associated with fire-prone environments and thus are not under an obvious selective pressure to retain a capacity to respond to smoke signals per se. We now know that at least two loci that facilitate this signalling mechanism, *KAI2* and *MAX2*, have been maintained in the *Arabidopsis* genome. Second, both *max2* and *kai2* mutants have similar phenotypes in terms of seed germination, seedling morphology and *STH7*, *KUF1*, *DLK2* and *IAA1* transcript levels. These mutant phenotypes are opposite to the effects of karrikin or strigolactone application on wild type, consistent with the failure of a signalling system to respond to a putative endogenous butenolide. Third, the germination and seedling phenotypes of *max2* and *kai2* are unlikely to be due to a defect in strigolactone signalling because the strigolactone-deficient *max1*, *max3* and *max4* mutants are normal in these respects (Nelson et al., 2011). Moreover, *kai2* seedlings still retain the capacity for strigolactone response through AtD14, yet show a reduced photomorphogenic phenotype not seen in strigolactone-deficient mutants or in *Atd14* seedlings. We propose that plants from fire-prone regions might have recruited an endogenous signalling mechanism, which is involved in seed germination and seedling development and still present in plants more broadly (including *Arabidopsis*), to detect and respond to karrikins found in smoke.

The normal strigolactone responses of *kai2* mutants suggest that, whereas MAX2 has a diverse role throughout plant development, the function of KAI2 is more specialised. Recently, KAI2 was independently identified in a screen for light signalling mutants (Sun and Ni, 2011). The *hyposensitive to light* (*htl*) mutants exhibited reduced sensitivity to light of all wavelengths, indicating a general lesion in seedling photomorphogenesis; however, no germination phenotype was reported, nor was any similarity to *max2*, which also exhibits the same broad photomorphogenesis phenotypes (Shen et al., 2007). Interestingly, *KAI2/HTL* appears to be a target of the transcriptional regulator HY5, a central component of light signalling that acts downstream of multiple photoreceptors (Oyama et al., 1997; Ulm et al., 2004; Sun and Ni, 2011). The MAX2 and HY5 signalling pathways are likely to be separate with respect to hypocotyl development (Tsuchiya et al., 2010), suggesting that KAI2 might operate in the MAX2 pathway and HY5 in another, but with the capacity to regulate *KAI2* transcriptionally. Although the precise relationship between KAI2 and HY5 is unclear, it is consistent with the demonstrated crosstalk between light and karrikin signalling and the observation that *hy5-1* seedlings have weak karrikin responses (Nelson et al., 2010).

Recently, it was demonstrated that the moss *Physcomitrella patens* responds to GR24 and has the genetic machinery for strigolactone biosynthesis (Proust et al., 2011). As the *P. patens* genome also encodes a MAX2 orthologue (Waters et al., 2011) and several candidate D14-LIKE homologues (Fig. 3), it appears that the strigolactone signalling system is evolutionarily ancient, arising before the divergence of the bryophytes and the vascular plants some 420 million years ago (Kenrick and Crane, 1997). However, given that the genome of the liverwort *Marchantia polymorpha* encodes two KAI2 orthologues but no DWARF14 orthologues, it is plausible that strigolactone signalling was not the original function of this

protein family in the first land plants. In *Arabidopsis*, it is clear that KAI2 mediates the activity of both karrikins and GR24, whereas AtD14 is specific for GR24 and, presumably, endogenous strigolactones. If the more relaxed chemical specificity of KAI2 is common to its orthologues in other species, then the ancestral protein might have had a more diverse role. Elucidating the most likely ancestral function of the DWARF14 family of α/β hydrolases will require a close examination of the responses of mosses and liverworts to butenolides and the effects of gene-specific knockouts. We propose that, in angiosperms such as rice and *Arabidopsis*, gene duplication and specialisation within the DWARF14 family has led to distinct signalling functions for KAI2 and D14 orthologues. In basal land plants, the division between karrikins, strigolactones and potentially other butenolide-containing compounds might be less distinct.

The overlapping phenotypes of *max2*, *kai2* and *Atd14-1* suggest that KAI2 and AtD14 act together with MAX2. MAX2 is an F-box protein that interacts with other core components of the SCF complex, a class of E3 ligases that ubiquitylates specific proteins and so marks them for degradation (Woo et al., 2001; Stirnberg et al., 2007). In the SCF complex, the F-box protein confers substrate specificity, acting as an adapter between the core subunits and the target protein (Somers and Fujiwara, 2009). Previously, we postulated that a D14 homologue might regulate MAX2 activity in a similar manner to the gibberellin (GA) signalling system (Nelson et al., 2011). The GA receptor GID1, which is also an α/β hydrolase superfamily member, promotes the binding of growth-repressing DELLA proteins to the F-box protein SLY1 in a GA-dependent manner, thus regulating DELLA degradation by the SCF complex (Harberd et al., 2009; Sun, 2010). We now extend this model to include AtD14 and KAI2 as central signalling components for strigolactones and karrikins, respectively (Fig. 7). Although direct protein interactions between MAX2 and either KAI2 or AtD14 have not yet been demonstrated, both MAX2 and KAI2 are localised to the nucleus (Shen et al., 2007; Stirnberg et al., 2007; Sun and Ni, 2011). In this GA signalling-like scenario, both AtD14 and KAI2 can function with MAX2, but the relative response of the SCF^{MAX2} complex to karrikins and strigolactones is dependent, in part, on the relative availability of AtD14 and KAI2 proteins and their respective signalling butenolides. Notably, *KAI2* and *AtD14* are differentially expressed during development, whereas *MAX2* is constitutively expressed at a steady level (supplementary material Fig. S7). With regard to karrikin and strigolactone signalling, there is evidence for a 'division of labour' between KAI2 and AtD14 in modulating aspects of MAX2 activity. Specifically, our data suggest that KAI2 is responsible for the strigolactone-independent functions of MAX2. We propose that during seed germination and seedling establishment, KAR (or an endogenous butenolide) promotes KAI2-MAX2 interaction, leading to degradation of growth repressor proteins. At later stages, most notably during flowering in *Arabidopsis* when secondary bud outgrowth is initiated, increased AtD14 activity would allow strigolactone-dependent functions of MAX2 to dominate.

As hypothetical hydrolases, AtD14 and KAI2 might generate an active signal from strigolactones and karrikins. We note that the serine residue of the catalytic triad could accommodate a receptor-ligand interaction by means of a nucleophilic attack upon an enol-ether functionality within the ligand, a mechanism proposed for both strigolactones and karrikins (Chiwocha et al., 2009; Zwanenburg et al., 2009). However, no catalytic activity has been demonstrated for either KAI2 or RsbQ (Brody et al., 2001; Sun and Ni, 2011), and, in our hands, purified KAI2 does not exhibit any catalytic activity towards KAR_1 in vitro. Other lines of evidence are consistent with a hypothesis that KAI2 and AtD14 are ligand-binding proteins. First, the *Bacillus subtilis* RsbQ protein has been shown to bind the serine protease inhibitor PMSF, and thus the postulated active site of RsbQ is thought to bind 'a hydrophobic small compound', the identity of which is unknown (Kaneko et al., 2005). Additionally, although GID1 and DWARF14-like proteins are not related on the amino acid sequence level, both contain the topologically similar α/β hydrolase fold (Kaneko et al., 2005; Shimada et al., 2008). In GID1, valine replaces histidine in the catalytic triad, which compromises its hydrolytic activity while providing a binding site for gibberellins (Ueguchi-Tanaka et al., 2005). Thus, KAI2 and AtD14 might perform a hybrid role in which the substrate is hydrolysed, followed by a conformational change in protein structure that could modulate MAX2 activity. KAI2 and AtD14 could bind their ligand directly or in partnership with MAX2 and/or specific repressor proteins. To date, we have not been able to detect any binding of KAR_1 with KAI2 in vitro, implying that the interaction might be highly transient or that other factors (such as protein partners) are involved. Although the details of the molecular interactions require further investigation, it is now clear that D14 and KAI2 proteins allow plants to discriminate between strigolactones and karrikins to trigger appropriate environmental and developmental responses.

Acknowledgements
We thank Matthew Hodges (University of Oxford, UK) for assistance with phylogenetic analysis; John Bowman and David Smyth (Monash University, Australia) for providing sequence data, *spt* mutants and valuable discussion; and Winslow Briggs (Carnegie Institution, Stanford, USA), Rowena Long, Emilio Ghisalberti (UWA, Australia), Christine Beveridge, Elizabeth Dun and Philip Brewer (UQ, Australia) for valuable discussion.

Funding
This project was supported by the Australian Research Council [DP0880484, LP0882775 and DP1096717].

Competing interests statement
The authors declare no competing financial interests.

Supplementary material
Supplementary material available online at
http://dev.biologists.org/lookup/suppl/doi:10.1242/dev.074567/-/DC1

References
Akiyama, K., Matsuzaki, K.-i. and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**, 824-827.
Akiyama, K., Ogasawara, S., Ito, S. and Hayashi, H. (2010). Structural requirements of strigolactones for hyphal branching in AM fungi. *Plant Cell Physiol.* **51**, 1104-1117.
Alvarez, J. and Smyth, D. R. (1999). CRABS CLAW and SPATULA, two Arabidopsis genes that control carpel development in parallel with AGAMOUS. *Development* **126**, 2377-2386.
Arite, T., Iwata, H., Ohshima, K., Maekawa, M., Nakajima, M., Kojima, M., Sakakibara, H. and Kyozuka, J. (2007). DWARF10, an RMS1/MAX4/DAD1 ortholog, controls lateral bud outgrowth in rice. *Plant J.* **51**, 1019-1029.
Arite, T., Umehara, M., Ishikawa, S., Hanada, A., Maekawa, M., Yamaguchi, S. and Kyozuka, J. (2009). d14, a strigolactone-insensitive mutant of rice, shows an accelerated outgrowth of tillers. *Plant Cell Physiol.* **50**, 1416-1424.
Brody, M. S., Vijay, K. and Price, C. W. (2001). Catalytic function of an alpha/beta hydrolase is required for energy stress activation of the sigma(B) transcription factor in Bacillus subtilis. *J. Bacteriol.* **183**, 6422-6428.
Chiwocha, S. D. S., Dixon, K. W., Flematti, G. R., Ghisalberti, E. L., Merritt, D. J., Nelson, D. C., Riseborough, J. A. M., Smith, S. M. and Stevens, J. C. (2009). Karrikins: a new family of plant growth regulators in smoke. *Plant Sci.* **177**, 252-256.
Daws, M. I., Davies, J., Pritchard, H. W., Brown, N. A. C. and Staden, J. (2007). Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. *Plant Growth Regul.* **51**, 73-82.
Dor, E., Joel, D. M., Kapulnik, Y., Koltai, H. and Hershenhorn, J. (2011a). The synthetic strigolactone GR24 influences the growth pattern of phytopathogenic fungi. *Planta* **234**, 419-427.

Dor, E., Yoneyama, K., Wininger, S., Kapulnik, Y., Yoneyama, K., Koltai, H., Xie, X. and Hershenhorn, J. (2011b). Strigolactone deficiency confers resistance in tomato line SL-ORT1 to the parasitic weeds Phelipanche and Orobanche spp. *Phytopathology* **101**, 213-222.
Finkelstein, R., Reeves, W., Ariizumi, T. and Steber, C. (2008). Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.* **59**, 387-415.
Flematti, G. R., Ghisalberti, E. L., Dixon, K. W. and Trengove, R. D. (2004). A compound from smoke that promotes seed germination. *Science* **305**, 977.
Gao, Z., Qian, Q., Liu, X., Yan, M., Feng, Q., Dong, G., Liu, J. and Han, B. (2009). Dwarf 88, a novel putative esterase gene affecting architecture of rice plant. *Plant Mol. Biol.* **71**, 265-276.
Goddard-Borger, E. D., Ghisalberti, E. L. and Stick, R. V. (2007). Synthesis of the germination stimulant 3-methyl-2H-furo[2,3-c]pyran-2-one and analogous compounds from carbohydrates. *Eur. J. Org. Chem.* **2007**, 3925-3934.
Gomez-Roldan, V., Fermas, S., Brewer, P. B., Puech-Pagès, V., Dun, E. A., Pillot, J.-P., Letisse, F., Matusova, R., Danoun, S., Portais, J.-C. et al. (2008). Strigolactone inhibition of shoot branching. *Nature* **455**, 189-194.
Guindon, S. and Gascuel, O. (2003). A simple, fast, and accurate algorithm to estimate large phylogenies by maximum likelihood. *Syst. Biol.* **52**, 696-704.
Harberd, N., Belfield, E. and Yasumura, Y. (2009). The angiosperm Gibberellin-GID1-DELLA growth regulatory mechanism: how an "inhibitor of an inhibitor" enables flexible response to fluctuating environments. *Plant Cell* **21**, 1328.
Hayward, A., Stirnberg, P., Beveridge, C. and Leyser, O. (2009). Interactions between auxin and strigolactone in shoot branching control. *Plant Physiol.* **151**, 400-412.
Heeg, C., Kruse, C., Jost, R., Gutensohn, M., Ruppert, T., Wirtz, M. and Hell, R. (2008). Analysis of the Arabidopsis O-acetylserine(thiol)lyase gene family demonstrates compartment-specific differences in the regulation of cysteine synthesis. *Plant Cell* **20**, 168-185.
Hruz, T., Laule, O., Szabo, G., Wessendorp, F., Bleuler, S., Oertle, L., Widmayer, P., Gruissem, W. and Zimmermann, P. (2008). Genevestigator v3: a reference expression database for the meta-analysis of transcriptomes. *Adv. Bioinformatics* **2008**, 420747.
Kaneko, T., Tanaka, N. and Kumasaka, T. (2005). Crystal structures of RsbQ, a stress-response regulator in Bacillus subtilis. *Protein Sci.* **14**, 558-565.
Kapulnik, Y., Delaux, P.-M., Resnick, N., Mayzlish-Gati, E., Wininger, S., Bhattacharya, C., Séjalon-Delmas, N., Combier, J.-P., Bécard, G., Belausov, E. et al. (2011). Strigolactones affect lateral root formation and root-hair elongation in Arabidopsis. *Planta* **233**, 209-216.
Kenrick, P. and Crane, P. (1997). The origin and early evolution of plants on land. *Nature* **389**, 33-39.
Koltai, H. (2011). Strigolactones are regulators of root development. *New Phytol.* **190**, 545-549.
Liu, W., Wu, C., Fu, Y., Hu, G., Si, H., Zhu, L., Luan, W., He, Z. and Sun, Z. (2009). Identification and characterization of HTD2: a novel gene negatively regulating tiller bud outgrowth in rice. *Planta* **230**, 649-658.
Long, R. L., Williams, K., Griffiths, E. M., Flematti, G. R., Merritt, D. J., Stevens, J. C., Turner, S. R., Powles, S. B. and Dixon, K. W. (2010). Prior hydration of Brassica tournefortii seeds reduces the stimulatory effect of karrikinolide on germination and increases seed sensitivity to abscisic acid. *Ann. Bot.* **105**, 1063-1070.
Long, R. L., Stevens, J. C., Griffiths, E. M., Adamek, M., Gorecki, M. J., Powles, S. B. and Merritt, D. J. (2011). Seeds of Brassicaceae weeds have an inherent or inducible response to the germination stimulant karrikinolide. *Ann. Bot.* **108**, 933-944.
Mangnus, E., Dommerholt, F., De Jong, R. and Zwanenburg, B. (1992). Improved synthesis of strigol analog GR24 and evaluation of the biological-activity of its diastereomers. *J. Agric. Food Chem.* **40**, 1230-1235.
Nelson, D. C., Riseborough, J.-A., Flematti, G. R., Stevens, J., Ghisalberti, E. L., Dixon, K. W. and Smith, S. M. (2009). Karrikins discovered in smoke trigger Arabidopsis seed germination by a mechanism requiring gibberellic acid synthesis and light. *Plant Physiol.* **149**, 863-873.
Nelson, D. C., Flematti, G. R., Riseborough, J.-A., Ghisalberti, E. L., Dixon, K. W. and Smith, S. M. (2010). Karrikins enhance light responses during germination and seedling development in Arabidopsis thaliana. *Proc. Natl. Acad. Sci. USA* **107**, 7095-7100.
Nelson, D. C., Scaffidi, A., Dun, E. A., Waters, M. T., Flematti, G. R., Dixon, K. W., Beveridge, C. A., Ghisalberti, E. L. and Smith, S. M. (2011). F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in Arabidopsis thaliana. *Proc. Natl. Acad. Sci. USA* **108**, 8897-8902.
Oyama, T., Shimura, Y. and Okada, K. (1997). The Arabidopsis HY5 gene encodes a bZIP protein that regulates stimulus-induced development of root and hypocotyl. *Genes Dev.* **11**, 2983-2995.
Proust, H., Hoffmann, B., Xie, X., Yoneyama, K., Schaefer, D. G., Yoneyama, K., Nogué, F. and Rameau, C. (2011). Strigolactones regulate protonema branching and act as a quorum sensing-like signal in the moss Physcomitrella patens. *Development* **138**, 1531-1539.
Roche, S., Koch, J. and Dixon, K. (1997). Smoke enhanced seed germination for mine rehabilitation in the southwest of Western Australia. *Restor. Ecol.* **5**, 191-203.
Ronquist, F. and Huelsenbeck, J. P. (2003). MRBAYES 3, Bayesian phylogenetic inference under mixed models. *Bioinformatics* **19**, 1572-1574.
Ruyter-Spira, C., Kohlen, W., Charnikhova, T., van Zeijl, A., van Bezouwen, L., de Ruijter, N., Cardoso, C., Lopez-Raez, J. A., Matusova, R., Bours, R. et al. (2011). Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in Arabidopsis: another belowground role for strigolactones? *Plant Physiol.* **155**, 721-734.
Shen, H., Luong, P. and Huq, E. (2007). The F-box protein MAX2 functions as a positive regulator of photomorphogenesis in Arabidopsis. *Plant Physiol.* **145**, 1471-1483.
Shimada, A., Ueguchi-Tanaka, M., Nakatsu, T., Nakajima, M., Naoe, Y., Ohmiya, H., Kato, H. and Matsuoka, M. (2008). Structural basis for gibberellin recognition by its receptor GID1. *Nature* **456**, 520-523.
Snowden, K. C., Simkin, A. J., Janssen, B. J., Templeton, K. R., Loucas, H. M., Simons, J. L., Karunairetnam, S., Gleave, A. P., Clark, D. G. and Klee, H. J. (2005). The Decreased apical dominance1/Petunia hybrida CAROTENOID CLEAVAGE DIOXYGENASE8 gene affects branch production and plays a role in leaf senescence, root growth, and flower development. *Plant Cell* **17**, 746-759.
Somers, D. E. and Fujiwara, S. (2009). Thinking outside the F-box: novel ligands for novel receptors. *Trends Plant Sci.* **14**, 206-213.
Sorefan, K., Booker, J., Haurogné, K., Goussot, M., Bainbridge, K., Foo, E., Chatfield, S., Ward, S., Beveridge, C., Rameau, C. et al. (2003). MAX4 and RMS1 are orthologous dioxygenase-like genes that regulate shoot branching in Arabidopsis and pea. *Genes Dev.* **17**, 1469-1474.
Stevens, J. C., Merritt, D. J., Flematti, G. R., Ghisalberti, E. L. and Dixon, K. W. (2007). Seed germination of agricultural weeds is promoted by the butenolide 3-methyl-2*H*-furo[2,3-c]pyran-2-one under laboratory and field conditions. *Plant Soil* **298**, 113-124.
Stirnberg, P., Furner, I. J. and Leyser, O. (2007). MAX2 participates in an SCF complex which acts locally at the node to suppress shoot branching. *Plant J.* **50**, 80-94.
Sun, T.-p. (2010). Gibberellin-GID1-DELLA: a pivotal regulatory module for plant growth and development. *Plant Physiol.* **154**, 567-570.
Sun, X.-D. and Ni, M. (2011). HYPOSENSITIVE TO LIGHT, an alpha/beta fold protein, acts downstream of ELONGATED HYPOCOTYL 5 to regulate seedling de-etiolation. *Mol. Plant* **4**, 116-126.
Tsuchiya, Y., Vidaurre, D., Toh, S., Hanada, A., Nambara, E., Kamiya, Y., Yamaguchi, S. and McCourt, P. (2010). A small-molecule screen identifies new functions for the plant hormone strigolactone. *Nat. Chem. Biol.* **6**, 741-749.
Ueguchi-Tanaka, M., Ashikari, M., Nakajima, M., Itoh, H., Katoh, E., Kobayashi, M., Chow, T.-y., Hsing, Y.-i. C., Kitano, H., Yamaguchi, I. et al. (2005). GIBBERELLIN INSENSITIVE DWARF1 encodes a soluble receptor for gibberellin. *Nature* **437**, 693-698.
Ulm, R., Baumann, A., Oravecz, A., Máté, Z., Adám, E., Oakeley, E. J., Schäfer, E. and Nagy, F. (2004). Genome-wide analysis of gene expression reveals function of the bZIP transcription factor HY5 in the UV-B response of Arabidopsis. *Proc. Natl. Acad. Sci. USA* **101**, 1397-1402.
Umehara, M., Hanada, A., Yoshida, S., Akiyama, K., Arite, T., Takeda-Kamiya, N., Magome, H., Kamiya, Y., Shirasu, K., Yoneyama, K. et al. (2008). Inhibition of shoot branching by new terpenoid plant hormones. *Nature* **455**, 195-200.
van Der Schaar, W., Alonso-Blanco, C., Léon-Kloosterziel, K. M., Jansen, R. C., van Ooijen, J. W. and Koornneef, M. (1997). QTL analysis of seed dormancy in Arabidopsis using recombinant inbred lines and MQM mapping. *Heredity* **79**, 190-200.
Waters, M., Smith, S. and Nelson, D. (2011). Smoke signals and seed dormancy: where next for MAX2? *Plant Signal. Behav.* **6**, 1418-1422.
Woo, H. R., Chung, K. M., Park, J. H., Oh, S. A., Ahn, T., Hong, S. H., Jang, S. K. and Nam, H. G. (2001). ORE9, an F-box protein that regulates leaf senescence in Arabidopsis. *Plant Cell* **13**, 1779-1790.
Yoneyama, K., Yoneyama, K., Takeuchi, Y. and Sekimoto, H. (2007). Phosphorus deficiency in red clover promotes exudation of orobanchol, the signal for mycorrhizal symbionts and germination stimulant for root parasites. *Planta* **225**, 1031-1038.
Zwanenburg, B., Mwakaboko, A. S., Reizelman, A., Anilkumar, G. and Sethumadhavan, D. (2009). Structure and function of natural and synthetic signalling molecules in parasitic weed germination. *Pest. Manag. Sci.* **65**, 478-491.