

Orobanche cumana KAI2d2 mediates the perception of dehydrocostus lactone and strigolactones and is inhibited by triazole ureas

Dear Editor,

Orobanche cumana (Orobanchaceae), commonly known as broomrape, is an obligate root holoparasitic plant that infests sunflower (*Helianthus annuus*), causing substantial yield losses in many regions of Europe and Asia (Gagne et al., 1998; Westwood et al., 2010). Its seed germination is triggered by chemical signals exuded by sunflower roots, including strigolactones (SLs) and sesquiterpene lactones (STLs) such as dehydrocostus lactone (DCL) (Plakhine and Joel, 2010; Dor et al., 2020). In non-parasitic plants, SL perception by the α/β -hydrolase DWARF14 (D14) initiates a signaling cascade via the SCF^{MAX2} ubiquitin ligase complex. SL recognition and hydrolysis by D14 destabilize the D14 lid domain, which is required for the association of D14 with the MORE AXILLARY GROWTH 2 (MAX2) F-box protein (Yao et al., 2016; Vancea et al., 2025). In Orobanchaceae, perception of SL and STL signals is mediated by KARRIKIN INSENSITIVE2-divergent (KAI2d) receptors. KAI2d evolved from canonical KAI2 α/β -hydrolases, D14 homologs that respond to karrikins and unknown endogenous ligands (Galindo et al., 2002; Toh et al., 2015; Tsuchiya et al., 2015; Conn and Nelson, 2016; Takei et al., 2023). In *Striga hermonthica*, SL recognition by the KAI2d protein ShHTL7 also induces lid domain destabilization and MAX2 binding, suggesting that this mode of SL signaling is conserved in at least some KAI2ds (Vancea et al., 2025). However, the structural basis of STL recognition in *O. cumana* is unknown, and we lack selective inhibitors that block germination without interfering with SL signaling in the sunflower host.

We identified 11 *D14/KAI2* homologs in the *O. cumana* genome and classified them into four clades: *D14*, *D14-like*, *KAI2*, and *KAI2d* (Supplemental Figure 1). Whereas *Arabidopsis* and rice each have a single copy of *D14* and *KAI2*, *O. cumana* contains one *D14* (*OcD14*), one *KAI2c* (*OcKAI2c*), and nine *KAI2d* paralogs (*OcKAI2d1–OcKAI2d9*), indicating lineage-specific expansion and functional diversification of *KAI2d* genes akin to other *Striga* family members. Sequence alignment and AlphaFold modeling confirmed conservation of the Ser–His–Asp catalytic triad across all *OcKAI2d* proteins (Supplemental Figures 2 and 3), supporting a cleavage-based signaling mechanism. Ligand pocket analysis revealed two subgroups: *OcKAI2d1*, *OcKAI2d3*, and *OcKAI2d4* with smaller cavities (404–419 Å³), and the remaining receptors with larger pockets (704–1162 Å³). These different cavity volumes suggest diversification of ligand recognition.

We determined the crystal structures of *OcKAI2d2* and *OcKAI2d6* at resolutions of 2.19 and 1.85 Å, respectively (Figure 1A; Supplemental Table 1). Both proteins adopt the canonical α/β -hydrolase fold with a conserved Ser–His–Asp catalytic triad and open lid domain. Both are structurally similar to each other

(root-mean-square deviation = 0.62 Å) and to AtD14, AtKAI2, and ShHTL7 (root-mean-square deviations from 0.6 to 1.0 Å; Supplemental Figure 4). Their ligand pockets (*OcKAI2d2*: 863 Å³; *OcKAI2d6*: 704 Å³) suggest a capacity for SL binding and contain key residues involved in ligand recognition (Supplemental Figures 3 and 4). YLG competition assays confirmed SL sensitivity, with the SL analogs GR24 and MP3 (Jamil et al., 2018) yielding low micromolar IC₅₀ values for both receptors (Figure 1B; Supplemental Table 2). SL hydrolysis assays showed broad substrate specificity for *OcKAI2d2* (Figure 1C; Supplemental Figure 5). Differential scanning fluorimetry revealed SL-induced receptor destabilization, consistent with conformational changes required for binding to SCF^{MAX2} (Yao et al., 2016; Vancea et al., 2025) (Supplemental Figure 6A). These findings establish *OcKAI2d2* and *OcKAI2d6* as catalytically active SL receptors. Even *OcKAI2d1* and *OcKAI2d3* were able to hydrolyze GR24, despite their smaller ligand cavities (Supplemental Figure 6B). This observation is consistent with the GR24-hydrolyzing activity reported for AtD14 (Seto et al., 2019), whose cavity volume is 447 Å³ (Supplemental Figure 3), and demonstrates that *OcKAI2* paralogs with a range of pocket sizes can catalyze SL hydrolysis.

Previous studies identified DCL as a germination stimulant in *Orobanche* species (Takei et al., 2023). Accordingly, YLG competition assays revealed that *OcKAI2d2* binds DCL with an IC₅₀ comparable to those of the SLs GR24 and MP3 (Figure 1B; Supplemental Figure 6C). However, DCL binding stabilized *OcKAI2d2* (Supplemental Figure 6A), and *OcKAI2d2* did not hydrolyze DCL *in vitro* (Supplemental Figure 7). Both properties are characteristic of inhibitors of MAX2-mediated signaling (Shahul Hameed et al., 2022; Vancea et al., 2025) and appear incompatible with a mechanism in which SL hydrolysis induces receptor destabilization (Yao et al., 2016; Vancea et al., 2025). Nonetheless, DCL promoted the association of *OcKAI2d2* with rice MAX2 (also named D3) in yeast two-hybrid assays (Supplemental Figure 8). These observations indicate that DCL can engage the SCF^{MAX2} pathway via *OcKAI2d2* but may use a mechanism different from that of SLs.

Given the high similarity between the ligand-binding pockets of *OcKAI2d2* and *OcKAI2d6* (Figure 1A; Supplemental Figure 4), we hypothesized that *OcKAI2d6* might also interact with DCL. However, DCL failed to compete with YLG for *OcKAI2d6* (Supplemental Figure 6C), indicating that DCL has low affinity for *OcKAI2d6*.

To investigate DCL binding, we co-crystallized *OcKAI2d2* with DCL, yielding a 2.45-Å structure (Figure 1D). Electron density maps revealed that DCL bound adjacent to the catalytic

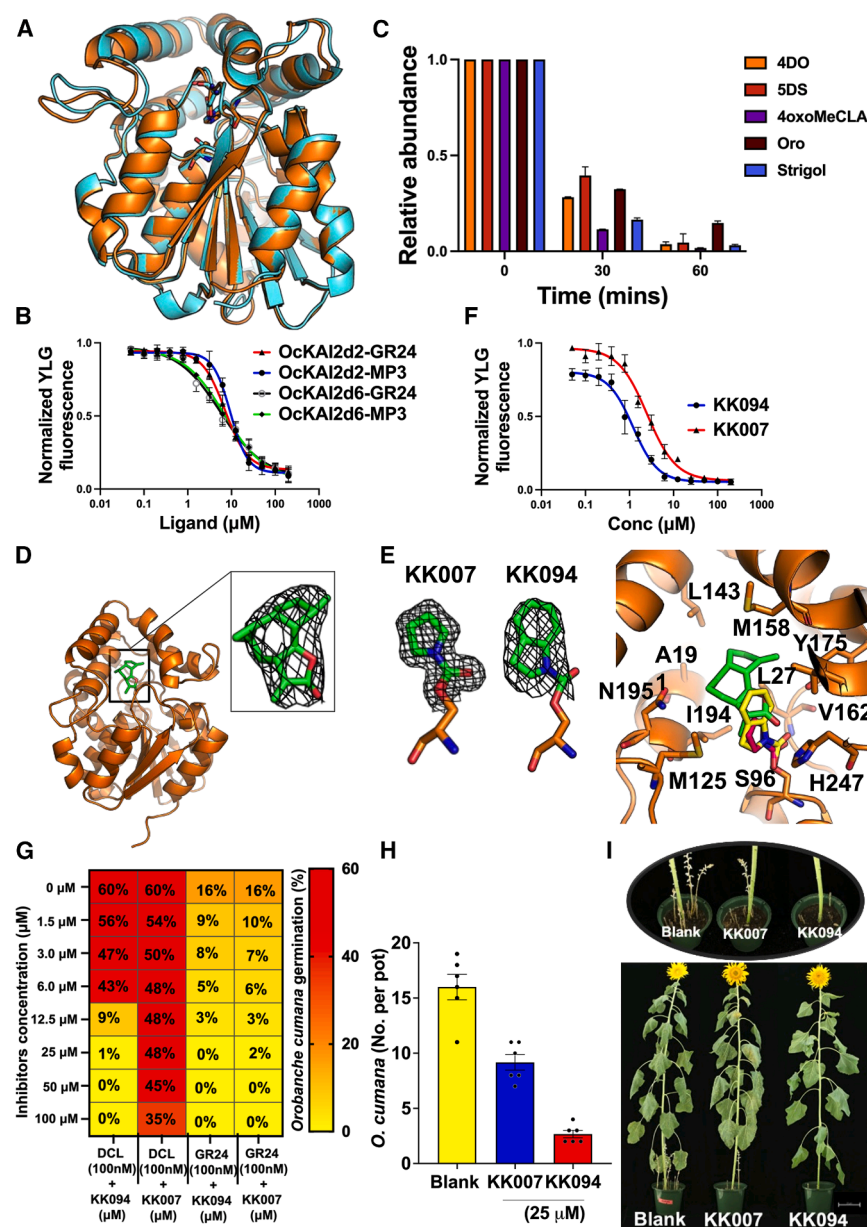


Figure 1. Structural basis of *OcKAI2d2*-ligand interactions and inhibition by triazole urea compounds.

(A) Superimposed crystal structures of *OcKAI2d2* (brownish orange) and *OcKAI2d6* (cyan), highlighting their canonical α/β -hydrolase folds.

(B) YLG competition assays with the synthetic SL analogs GR24 and MP3 (methyl phenylacetate 3) for *OcKAI2d2* and *OcKAI2d6*. All experiments were independently repeated three times, and the data are presented as the mean $\pm$ SEM.

(C) Enzymatic hydrolysis of strigolactones (SLs) by *OcKAI2d2*. 4DO, 4-deoxyorobanchol; 5DS, 5-deoxystrigol; 4oxoMeCLA, 4-oxo-methyl carlactonate; Oro, orobanchol. All experiments were independently repeated three times, and the data are presented as the mean $\pm$ SEM.

(D) *OcKAI2d2* structure (brownish orange) bound to dehydrocostus lactone (DCL; green sticks) with the 2Fo-Fc omit map contoured at 1σ (mesh).

(E) 2Fo-Fc omit maps contoured at 1σ (mesh) for KK007 and KK094 (green sticks), showing covalent linkage to the catalytic residue S96 (brownish orange stick) and adduct formation.

Superimposition of *OcKAI2d2*-DCL (green sticks) with KK007- (hot pink sticks) and KK094- (yellow sticks) bound structures; residues involved in the DCL interaction are shown as sticks (brownish orange).

(F) YLG competition assays with various concentrations (Conc) of KK007 and KK094 for *OcKAI2d2*. All experiments were independently repeated three times, and the data are presented as the mean $\pm$ SEM.

(G) Germination responses of *O. cumana* seeds to DCL and GR24 in the presence or absence of KK007 and KK094.

(H) Effects of KK007 and KK094 treatments (25 μ M each) on the number of emerged *O. cumana* plants per pot in the presence of host sunflower plants, compared with the blank control. The bar graph shows means $\pm$ SE ($n = 6$), with individual data points overlaid. Statistical significance was assessed by one-way ANOVA ($p < 0.05$). Different bar heights and non-overlapping error bars show differences between treatments. KK007 and KK094 reduced *O. cumana* emergence by 46% and 82%, respectively, relative to the blank control.

(I) Representative images of sunflower plants grown in pots under three treatments: blank control, KK007 (25 μ M), and KK094 (25 μ M). Top row: close-up views of pots showing the emergence of *O. cumana* around sunflower stems, corresponding to the data shown in (H). Bottom row: overall sunflower growth under the same treatment conditions, showing minimal impact of the treatments on the host plant. Scale bar: 20 cm.

residues S96 and H247 within a hydrophobic pocket formed primarily by lid-domain helices (Supplemental Figure 9). Key interactions included hydrogen bonds with Ser96, His247, and Tyr175, which stabilized the intact DCL molecule noncovalently. Structural comparison showed minimal conformational changes upon binding, which were limited to rotamer shifts and a minor inward displacement (0.28 Å) of the $\alpha 2$ lid helix (Supplemental Figure 9C). Unlike SLs, which undergo hydrolysis, DCL remained intact in its complex with *OcKAI2d2*, confirming the ability of the receptor to accommodate non-hydrolyzable ligands and supporting its role in DCL-induced germination. Compared with that of *OcKAI2d2*, the lid helix position of apo-*OcKAI2d6* is slightly more closed (Figure 1A) and would produce minor side-

chain clashes with DCL, potentially limiting DCL interaction (Supplemental Figures 6C and 10).

Triazole urea compounds have been shown to irreversibly inhibit SL receptors by covalently modifying the catalytic serine. To test their effects on *O. cumana*, we co-crystallized *OcKAI2d2* with two triazole urea inhibitors, KK007 and KK094, and resolved structures at 1.39 and 2.35 Å (Figure 1E; Supplemental Figure 8; Supplemental Table 1). Electron density confirmed a covalent linkage to Ser96, with both inhibitors occupying the active site and sterically blocking SL and DCL binding (Figure 1E; Supplemental Figures 11A–11D). YLG hydrolysis assays showed potent inhibition, with IC_{50} values of 2.5 ± 0.4 (KK007)

and 1.2 ± 0.2 (KK094) μM , outperforming SLs and DCL (Figure 1F; Supplemental Table 2). Differential scanning fluorimetry revealed increased thermal stability, consistent with covalent binding (Supplemental Figure 11E). These results establish KK007 and KK094 as potent covalent OckAI2d2 inhibitors that target the catalytic serine, thereby preventing ligand binding and SL hydrolysis.

We next investigated the inhibitory activity of triazole urea compounds for *O. cumana* seed germination. In the absence of inhibitors, germination was unexpectedly ~five-fold higher with 100 nM DCL than with an equivalent concentration of GR24. This enhanced response may reflect either greater intrinsic sensitivity of *O. cumana* seeds to DCL or greater stability of DCL during the 1-week incubation period. KK007 and KK094 showed dose-dependent inhibition of DCL-induced germination, with KK094 achieving complete suppression at 100 μM and KK007 reducing germination by 41% at the same concentration (Figure 1G; Supplemental Figure 12). When GR24 was used as the stimulant, KK094 fully inhibited germination at 25 μM , whereas KK007 required 50 μM (Figure 1G; Supplemental Figure 13). Both compounds also significantly reduced radicle elongation (Supplemental Figure 11). In greenhouse trials, KK094 (25 μM) reduced *O. cumana* emergence by 83%, compared with 43% for KK007 (Supplemental Figure 14). These findings confirm that OckAI2d-mediated germination and emergence were effectively blocked by the triazole urea inhibitors KK007 and KK094, consistent with their relative *in vitro* affinities and thermal stabilization profiles, supporting their potential as chemical control agents.

To evaluate potential host toxicity, sunflower seeds were treated with KK007 and KK094 at various concentrations. Neither compound affected germination or seedling development (Supplemental Figure 15). Treated seedlings showed normal shoot growth and morphology, and sunflower plants grown in pots maintained typical growth at all developmental stages. These observations indicate that, under the conditions tested, the inhibitors did not adversely affect sunflower viability (Figure 1I; Supplemental Figure 16).

In conclusion, we identified nine KAI2d homologs in the *O. cumana* genome and resolved the crystal structures of two representative receptors, OckAI2d2 and OckAI2d6. Both receptors exhibit the canonical α/β -hydrolase fold with intact catalytic triads and large ligand-binding pockets. Their SL-induced destabilization and broad SL hydrolysis activity support their role in perceiving a wide range of SLs via the canonical SCF^{MAX2}-dependent signaling pathway, as established for D14 and ShHTL7.

Importantly, our structural data show that OckAI2d2 also binds DCL, a non-hydrolyzable STL, within its SL-binding pocket. Although DCL is positioned near the catalytic Ser96 and His247, it is not cleaved, and its binding stabilizes the receptor rather than destabilizing it, in contrast to the response triggered by SLs. Structural modeling indicated that uncleaved DCL cannot be accommodated in the closed MAX2-bound conformation of OckAI2d2. Nevertheless, DCL still promoted the association of OckAI2d2 with a MAX2 protein in yeast two-hybrid assays. These findings establish OckAI2d2 as a bifunctional receptor capable of activating SCF^{MAX2}-mediated germination through both canonical SL hydrolysis and a distinct mode of engagement by non-hy-

drolyzable STLs, whose mechanistic basis remains to be characterized.

We also demonstrate that the triazole urea inhibitors KK007 and KK094 effectively block both signaling routes by covalently modifying the catalytic serine of OckAI2d proteins. These inhibitors suppress *O. cumana* germination and emergence *in vitro* and under greenhouse conditions. Importantly, they do not impair sunflower growth, underscoring their potential as selective agents for broomrape control. Further studies are needed to evaluate their environmental impacts, particularly on non-target organisms such as arbuscular mycorrhizal fungi, to ensure safe, sustainable application.

DATA AND CODE AVAILABILITY

Coordinates for the reported structures have been deposited in the Protein Data Bank (PDB) under accession codes 9WGQ, 9WGO, 9WGN, 9WGM, and 9WVK.

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AUTHOR CONTRIBUTIONS

S.A.-B., S.T.A., U.F.S.H., and M.J. conceived and designed the experiments. U.F.S.H. and R.Z. performed the protein purification and biochemical assays, and U.F.S.H. performed the protein crystallization. U.F.S.H. and S.T.A. collected the crystallographic data and conducted the structural analysis. M.J. and R.Z. performed the *in planta* experiments and germination inhibitor bioassays. K.A. performed the SL hydrolysis experiments. T.A. synthesized the chemical compounds used in this study. Y.S. conducted the sequence analysis. M.J. analyzed the plant experiment and bioassay data and prepared the plots. A.B. performed the yeast two-hybrid assay. S.T.A., S.A.-B., U.F.S.H., and M.J. wrote the manuscript.

SUPPLEMENTAL INFORMATION

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Umar F. Shahul Hameed^{2,8},
Muhammad Jamil^{1,8}, Randa A. Zarban^{1,5},
Yoshimoto Saito⁴, Kawthar F. Alashoor¹,
Aparna Balakrishna¹, Tadao Asami³,
Stefan T. Arold^{2,*} and Salim Al-Babili^{1,6,7,*}

¹The Bioactives Laboratory, KAUST Center of Excellence for Food Security, King Abdullah University of Science and Technology, Thuwal 23955-6900, Saudi Arabia

²KAUST Center of Excellence for Smart Health, Division of Biomedical Sciences, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

³Graduate School of Agricultural and Life Sciences, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

⁴Marine Open Innovation (MaOI) Institute, 9-25 Hinodecho, Shimizu-ku, Shizuoka 424-0922, Japan

⁵Department of Agricultural Biotechnology, King Faisal University, Hofuf 36362, Saudi Arabia

⁶The Plant Science Program, Biological and Environmental Science and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

⁷KAUST Center of Excellence for Sustainable Food Security, King Abdullah University of Science and Technology (KAUST), Thuwal 23955-6900, Saudi Arabia

⁸These authors contributed equally to this work

*Correspondence: Stefan T. Arold (stefan.arold@kaust.edu.sa), Salim Al-Babili (salim.babili@kaust.edu.sa)
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REFERENCES

- Conn, C.E., and Nelson, D.C. (2015). Evidence that KARRIKIN-INSENSITIVE2 (KAI2) Receptors may Perceive an Unknown Signal that is not Karrikin or Strigolactone. *Front. Plant Sci.* **6**:1219.
- Dor, E., Plakhine, D., Joel, D.M., Larose, H., Westwood, J.H., Smirnov, E., Ziadna, H., and Hershenhorn, J. (2020). A new race of sunflower broomrape (*Orobancha cumana*) with a wider host range due to changes in seed response to strigolactones. *Weed Sci.* **68**:134–142.
- Gagne, G., Roeckel-Drevet, P., Grezes-Besset, B., Shindrova, P., Ivanov, P., Grand-Ravel, C., Vear, F., Tourvieille De Labrouhe, D., Charmet, G., and Nicolas, P. (1998). Study of the variability and evolution of *Orobancha cumana* populations infesting sunflower in different European countries. *Theor. Appl. Genet.* **96**:1216–1222.
- Galindo, J.C.G., De Luque, A.P., Jorrín, J., and Macías, F.A. (2002). SAR Studies of Sesquiterpene Lactones as *Orobancha cumana* Seed Germination Stimulants. *J. Agric. Food Chem.* **50**:1911–1917.
- Jamil, M., Kountche, B.A., Haider, I., Guo, X., Ntui, V.O., Jia, K.-P., Ali, S., Hameed, U.S., Nakamura, H., Lyu, Y., et al. (2018). Methyl phenlactonoates are efficient strigolactone analogs with simple structure. *J. Exp. Bot.* **69**:2319–2331.
- Plakhine, D., and Joel, D.M. (2010). Ecophysiological consideration of *Orobancha cumana* germination. *Helia* **33**:13–18.
- Seto, Y., Yasui, R., Kameoka, H., Tamiru, M., Cao, M., Terauchi, R., Sakurada, A., Hirano, R., Kisugi, T., Hanada, A., et al. (2019). Strigolactone perception and deactivation by a hydrolase receptor DWARF14. *Nat. Commun.* **10**:191.
- Shahul Hameed, U., Haider, I., Jamil, M., Guo, X., Zarban, R.A., Kim, D., Al-Babili, S., and Arold, S.T. (2022). Structural basis for specific inhibition of the highly sensitive ShHTL7 receptor. *EMBO Rep.* **23**:e54145.
- Takei, S., Uchiyama, Y., Bürger, M., Suzuki, T., Okabe, S., Chory, J., and Seto, Y. (2023). A Divergent Clade KAI2 Protein in the Root Parasitic Plant *Orobancha minor* Is a Highly Sensitive Strigolactone Receptor and Is Involved in the Perception of Sesquiterpene Lactones. *Plant Cell Physiol.* **64**:996–1007.
- Toh, S., Holbrook-Smith, D., Stogios, P.J., Onoprienko, O., Lumba, S., Tsuchiya, Y., Savchenko, A., and McCourt, P. (2015). Structure-function analysis identifies highly sensitive strigolactone receptors in *Striga*. *Science* **350**:203–207.
- Tsuchiya, Y., Yoshimura, M., Sato, Y., Kuwata, K., Toh, S., Holbrook-Smith, D., Zhang, H., McCourt, P., Itami, K., Kinoshita, T., and Hagihara, S. (2015). Probing strigolactone receptors in *Striga hermonthica* with fluorescence. *Science* **349**:864–868.
- Vancea, A.I., Huntington, B., Steinchen, W., Savva, C.G., Shahul Hameed, U.F., and Arold, S.T. (2025). Mechanism of cooperative strigolactone perception by the MAX2 ubiquitin ligase–receptor–substrate complex. *Nat. Commun.* **16**:10291.
- Westwood, J.H., Yoder, J.I., Timko, M.P., and dePamphilis, C.W. (2010). The evolution of parasitism in plants. *Trends Plant Sci.* **15**:227–235.
- Yao, R., Ming, Z., Yan, L., Li, S., Wang, F., Ma, S., Yu, C., Yang, M., Chen, L., Chen, L., et al. (2016). DWARF14 is a non-canonical hormone receptor for strigolactone. *Nature* **536**:469–473.