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Karrikins versus strigolactones: A comparative analysis of their effects on seed germination in obligate and facultative Orobanchaceae parasites and fire-responsive species

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ABSTRACT:
The karrikin (KAR) and strigolactone (SL) signalling pathways are thought to be evolutionarily related, prompting extensive research on the similarities and differences between KAR- and SL-mediated plant developmental responses. Here, we investigated the effects of plant-derived smoke, KAR_1, *rac*-GR24 (a synthetic SL analogue), and gibberellic acid (GA_3) on seed germination in five Orobanchaceae species, including both obligate and facultative parasites, and in two non-parasitic species known to respond to smoke and KAR_1. Our results showed that seeds of the KAR_1-responsive *Sarcopoterium spinosum* and *Stachys cretica* were insensitive to *rac*-GR24. Although *rac*-GR24 promoted germination in all the obligate *Orobanche* parasites tested (*O. anatolica*, *O. minor*, and *O. pubescens*), it had no effect on the facultative parasites *Bellardia trixago* and *Parentucellia viscosa*. Smoke-water and KAR_1 did not induce germination in the three *Orobanche* species, yet significantly increased germination in *B. trixago*. Additionally, the seeds of *S. spinosum*, *S. cretica*, and *B. trixago* responded positively to GA_3, whereas GA_3 did not replace the requirement for *rac*-GR24 to stimulate germination in *Orobanche* species, except for *O. pubescens*. Overall, our findings indicate that (I) KARs and SLs are not interchangeable cues for germination in obligate Orobanchaceae parasites or the two fire-responsive non-parasitic species, and (II) KARs may confer an advantage to some facultative parasites in post-fire environments.

Keywords: facultative parasites, karrikins, obligate parasites, Orobanchaceae, smoke, strigolactones

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INTRODUCTION

Karrikins (KARs, KAR_1–KAR_6) and strigolactones (SLs) are important plant growth regulators containing a butenolide moiety. While KARs are present in smoke and charred plant material, SLs are β-carotene-derived phytohormones produced in extremely small amounts (Bouwmeester *et al.* 2021; Çatav *et al.* 2024a). SLs are known to be involved in the regulation of auxin transport, root architecture, shoot branching, leaf development, stomatal closure, and drought tolerance (Nelson 2021; Samynathan *et al.* 2024). In addition, SLs also serve as rhizospheric signals to induce root colonisation by arbuscular mycorrhizal fungi, shape soil microbiota, and detect neighbouring plants (Kee *et al.* 2023). SLs also stimulate the seed germination of obligate parasites within the Orobanchaceae family (Wang *et al.* 2024). Similar to SLs, KARs play critical roles in multiple physiological and developmental processes, including seed germination, seedling photomorphogenesis, cotyledon expan-

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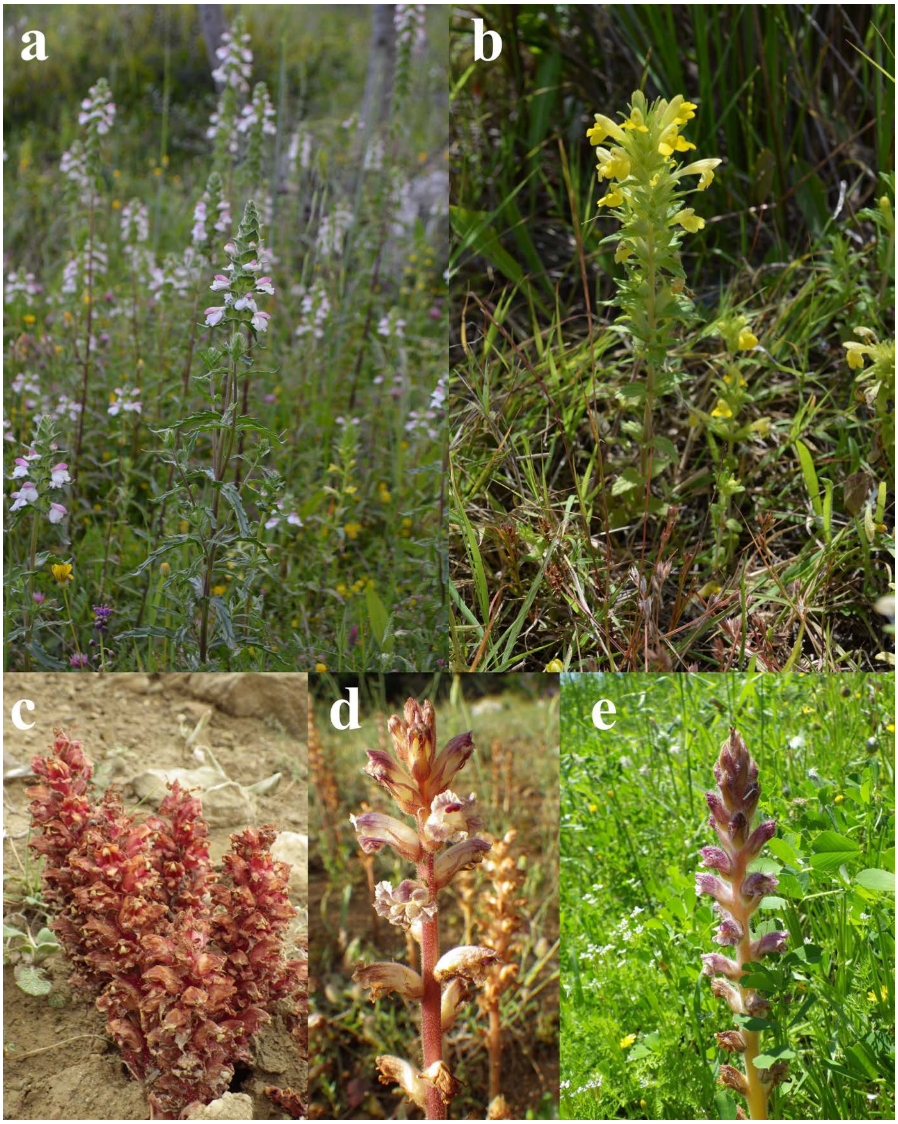

Fig. 1. Images of the studied Orobanchaceae species in their natural habitats. (a) *Bellardia trixago*, (b) *Parentucellia viscosa*, (c) *Orobanche anatolica*, (d) *O. minor*, and (e) *O. pubescens*. All photos were taken by G. Zare in the field.

sion, root growth, and abiotic stress tolerance (Kamran *et al*. 2024). The fact that both KARs and SLs share a similar functional group has contributed to a better understanding of KAR/SL signalling in plants.

The perception and signalling of KARs and SLs are mediated by the F-box protein (MAX2), α/β-hydrolases (KAI2 and D14), and SMAX1- and D53-type SMXL repressor proteins (Waters & Nelson 2023). The *D14* genes evolved from the *KAI2* gene through gene duplication and subsequent neofunctionalisation (Barbier *et al*. 2023). Thus, the KAI2 and D14 receptor proteins are evolutionarily related and are involved in the sensing of KARs and SLs, respectively. In addition, KAI2 is thought to be a receptor for unidentified endogenous compounds, also known as KAI2 ligands (KLs). Furthermore, extensive duplication of *KAI2* in many Orobanchaceae species has led to the emergence of three clades: *KAI2c* (conserved clade), *KAI2i* (intermediate clade), and *KAI2d* (divergent clade), which are specific for KLs, KARs, and SLs, respectively (Conn & Nelson 2016). The activation of KAI2 and D14 by these molecules leads to the polyubiquitination and proteasomal degradation of specific SMXL proteins via the E3 ubiquitin ligase complex (Skp1-Cullin-F-box^{MAX2}), thereby triggering KL/KAR- and SL-specific plant developmental processes (Khosla *et al*. 2020).

Parasitism is estimated to have evolved independently 13 times in plants, and approximately 4750 species within angiosperms adopt a parasitic lifestyle. Of these, 60% are root parasites, with over 2000 species in the family Orobanchaceae belonging to this group (Westwood *et al*. 2010; Miyakawa *et al*. 2020; Brun *et al*. 2021). Parasitic Orobanchaceae species are known to exploit host resources by forming a specialised organ, the haustorium, causing substantial losses in agricultural production (Savov *et al*. 2024). In particular, holoparasitic broomrapes (*Orobanche* s.l.) pose a serious threat to the yields of legumes and many other important crops (potatoes, sunflowers, and tomatoes, etc.) in Mediterranean agricultural systems (Casadesús & Munné-Bosch 2021). Since *Orobanche* L. and *Striga* Lour. species are capable of producing hundreds of thousands of seeds which remain viable in the soil for prolonged periods, preventing infestations of these parasites is a major challenge (Yoneyama *et al*. 2010; Albanova *et al*. 2023). Common practices used to control parasitic Orobanchaceae weeds in agricultural cultivation include screening for resistant host varieties, reducing host SL biosynthesis, using trap crops, modifying host SL exudation profiles, and inducing suicidal germination in obligate parasites with stimulants (for a review, see Brun *et al*. 2018).

Given the evolutionary relationship between the KAR and SL signalling pathways (Conn *et al*. 2015), it has been proposed that these two groups of compounds may exert similar effects on seed germination. Accordingly, several studies have been conducted to determine whether karrikinolide (KAR_1) and plant-derived smoke can stimulate germination in holoparasitic Orobanchaceae species (Nun & Mayer 2005; Daws *et al*. 2008; Nelson *et al*. 2009; Brun *et al*. 2019). In addition, the effects of SL analogues on the germination of several KAR_1-responsive species have also been examined (Martinez *et al*. 2022; Takei *et al*. 2023). Overall, these studies suggest that KARs and SLs generally have little or no effect on seed germination in species responsive to other chemical cues. However, the paucity of comparative research in this area hinders our ability to make a more definitive assessment. Furthermore, the germination response of facultative Orobanchaceae parasites to KARs has not been extensively studied. To address these knowledge gaps, we investigated the effects of smoke, KAR_1 and *rac*-GR24 (a synthetic SL analogue), on the germination of five Orobanchaceae and two fire-responsive species. We also tested the effects of various gibberellic acid (GA_3) concentrations. This study was guided by the following hypotheses: (I) KAR_1 would not induce germination in obligate parasites to the same extent as *rac*-GR24, (II) KAR_1 and GA_3 might positively influence the germination of facultative parasites, and (III) *rac*-GR24 would have little or no effect on the germination of fire-responsive species.

MATERIALS AND METHODS

Study species and seed collection. In this study, we selected seven species, five of which belong to the family Orobanchaceae (Fig. 1), to evaluate their germination responses to plant-derived smoke, KAR_1, *rac*-GR24, and GA_3. Seeds of the obligate parasitic *Orobanche* species, *O. anatolica* Boiss. & Reut., *O. minor* Sm., and *O. pubescens* d'Urv., and the facultative parasitic species, *Bellardia trixago* (L.) All. and *Parentucellia viscosa* (L.) Caruel, were collected from natural populations growing in different regions of Türkiye (Table 1). *O. anatolica* is exclusively parasitic on species of *Salvia* (Lamiaceae), whereas *O. pubescens* and *O. minor* parasitise members of Apiaceae, Asteraceae, Fabaceae, and Lamiaceae. The facultative parasites in our study (*B. trixago* and *P. viscosa*) have no specific host preference and are thus considered generalists. After field collection, the seeds were kept in a dry, dark environment at 20°C. Since the collection dates differed among species, the seed storage duration

Table 1. The main characteristics and seed-collection information for the Orobanchaceae species studied. FP and OP are facultative and obligate parasites, respectively. MSW is the mean seed weight (mg). "Collection site" refers to the province names in Türkiye where the seeds were collected. "Seed age" denoted the time lapse between field collection and the start of the experiments (years). SCP (seed conditioning period) indicates the number of days of conditioning.

Species	Lifestyle	MSW	Collection site	Collection year	Seed age	SCP
Bellardia trixago (L.) All.*	FP	0.027	Muğla	2014	4	7
Orobanche anatolica Boiss. & Reut.	OP	0.012	Ankara	2012	6	14
Orobanche minor Sm.	OP	0.006	Alanya	2014	4	14
Orobanche pubescens d'Urv.	OP	0.005	Muğla	2013	5	14
Parentucellia viscosa (L.) Caruel	FP	0.015	Muğla	2014	4	14

*In the preliminary experiment, a small fraction of *B. trixago* seeds began to germinate after 7 days of seed conditioning. Therefore, a 7-day period was chosen for the seed conditioning of this species in the germination assay.

prior to the start of the germination experiments also varied (Table 1). This extended storage period did not affect the germination experiments because Orobanchaceae seeds can remain viable in the soil seed bank for several years (Albanova *et al.* 2023), as also demonstrated by our results showing significant levels of dormancy in all the tested species.

We also collected fruits of *Sarcopoterium spinosum* (L.) Spach (Rosaceae) and *Stachys cretica* subsp. *smyrnaea* Rech.f. (Lamiaceae) in Muğla, Türkiye on July 15, 2018, since our previous studies have shown that different populations of these two species are responsive to smoke and KAR_1 (Çatav *et al.* 2014, 2024b). In addition to testing our karrikins-versus-strigolactones hypothesis, these two species also served as controls for the solutions (especially smoke and smoke-derived chemicals) and compounds used with the Orobanchaceae species. Their seeds were stored in Eppendorf tubes or plastic containers at room temperature for three months before the experiments were initiated.

Preparation of the stock solutions of smoke-water and tested compounds. To prepare the smoke-water (SW), 80 g of wheat straw was burned in a bee smoker. The resulting smoke was then bubbled through 500 mL of distilled water (dH_2O) for 13 minutes. The working concentrations of SW were prepared by diluting them with dH_2O after filtering the stock solution through a 0.22-micron filter (Downes *et al.* 2013). Stock solutions of *rac*-GR24 (33.52 mM) and KAR_1 (1.67 mM) were prepared by dissolving 5 mg of each compound in 0.5 mL of acetone and 20 mL of ethanol, respectively. Both stock solutions were stored at -20°C until required. For the highest working concentrations of KAR_1 and *rac*-GR24, the solvent concentrations were found to be 0.006% and 0.03%, respectively. Mandelonitrile (a cyanohydrin used instead of smoke-derived glyceronitrile, hereafter MAN) and GA_3 stock solutions were freshly prepared before the germination assay using dH_2O and ethanol, respectively. The highest working concentration of GA_3 contained a solvent concentration of 0.05%. The chemicals used in this study were all purchased from Carbosynth, ChemPep Inc., Merck, and Sigma-Aldrich.

Seed conditioning. The seeds from the Orobanchaceae species were conditioned prior to the germination experiment. Briefly, the seeds were placed in sterile Petri dishes (Ø 55 mm) containing two sheets of Whatman filter paper (No. 1) and 2 mL of dH_2O, using a paintbrush. An additional filter paper was then placed on top of the seeds. Finally, the Petri dishes were wrapped in parafilm and incubated in the dark at 23°C for 7 or 14 days (Mangnus *et al.* 1992; Nun & Mayer 2005).

Germination protocol. Seeds of each species were placed in Petri dishes (Ø 55 mm) containing a pair of Whatman #1 filter papers saturated with 2 mL of the test solution. Sterile dH_2O served as the control in all the assays. The Petri dishes were sealed with parafilm and incubated at 20°C in the dark (Daws *et al.* 2008; Çatav *et al.* 2024b). Each treatment consisted of four replicates of 25 to 40 seeds. Germination counts were made twice weekly under dim white light (15 µmol m^{-2} s^{-1}) using an Olympus SZX7 stereomicroscope, with the emergence of the radicle used as the criterion for germination. The germinated seeds were counted and removed from the Petri dishes at each observation. The experiments were terminated after seven weeks of incubation.

Germination assays. To clarify how SLs and GAs affect the germination of smoke- and KAR-responsive seeds, we used *S. spinosum* and *S. cretica* as test species. In this experiment, the seeds were treated with different concentrations of *rac*-GR24 (0.1, 1, and 10 µM), GA_3 (0.01, 0.1, and 1 mM), and smoke-water (1, 5, and 10%). We also tested the effects of KAR_1 (0.1 µM), MAN (50 µM), and their combination on seed germination in both species. The aim of the second experiment was to evaluate the germination response of root parasitic plants to smoke, KARs and GAs. Accordingly, conditioned seeds of five Orobanchaceae species were treated with various concentrations of smoke-water (1 and 5%), KAR_1 (0.1 and 1 µM), and GA_3 (0.1 and 1 mM). Two *rac*-GR24 concentrations (1 and 10 µM) were also included to assess the response of obligate and facultative parasites to SLs. The working concentrations of the smoke-water and the tested compounds were selected based on previous studies (Nelson *et al.* 2009; Brun *et al.* 2019; Çatav *et al.* 2024b). The experiments began on December 5 and 19, 2018, respectively.

Data analysis. For germination analysis, each seed was scored as either germinated (1) or non-germinated (0) according to its species and treatment combination within each Petri dish. Analyses were conducted separately for each species. To test whether germination differed among treatments, generalised linear models (GLM) assuming binomial error distribution and logit link function were fitted, using germination status as the response variable and treatment as the explanatory variable. In each model, the control treatment was specified as the reference level, and the germination response of each treatment was compared directly with the control. Given that multiple treatment-versus-control comparisons were performed for each species, the significance level was assessed at $\alpha = 0.01$, following Moreira *et al.* (2010).

RESULTS

Experiment 1. Under controlled conditions, the germination percentages of *S. cretica* and *S. spinosum* were 0% and 43.8%, respectively (Fig. 2). As expected, smoke-water treatments, especially at 1 and 5%, markedly increased germination in both species ($p < 0.01$). While both species were responsive to KAR_1, only *S. spinosum* showed a positive germination response to MAN. The interaction between KAR_1 and MAN did not produce any additive or synergistic effects. The *rac*-GR24 treatments failed to promote germination in either species compared to the control (Fig. 2), although a negligible increase was noted in *S. cretica* at 10 µM *rac*-GR24 (Fig. 2b). GA_3 significantly enhanced germination in both species ($p < 0.01$), but the sensitivity of *S. cretica* and *S. spinosum* to GA_3 varied by concentration (Fig. 2).

Experiment 2. The effects of smoke, KAR_1, *rac*-GR24, and GA_3 on germination in the studied Orobanchaceae species are shown in Table 2 and Fig. 3. With the exception of *B. trixago*, the germination percentages of the parasitic

Table 2. Mean (± SE) germination percentages in the control and treatment groups of the studied Orobanchaceae species.

Species	Control	Smoke-water (%)		KAR_1 (μM)		GR24 (μM)		GA_3 (mM)	
		1	5	0.1	1	1	10	0.1	1
F. parasites									
B. trixago	23 ± 4	**83 ± 4**	**92 ± 4**	**96 ± 1**	**97 ± 1**	26 ± 5	37 ± 4	**41 ± 4**	**9 ± 1**
P. viscosa	3 ± 2	7 ± 2	2 ± 1	0 ± 0	14 ± 4	1 ± 1	0 ± 0	6 ± 2	0 ± 0
O. parasites									
O. anatolica	0 ± 0	0 ± 0	1 ± 1	0 ± 0	0 ± 0	**22 ± 5**	**23 ± 4**	0 ± 0	0 ± 0
O. minor	1 ± 1	1 ± 1	0 ± 0	0 ± 0	0 ± 0	**16 ± 1**	**94 ± 4**	0 ± 0	0 ± 0
O. pubescens	3 ± 2	0 ± 0	1 ± 1	4 ± 2	2 ± 1	**59 ± 2**	**51 ± 4**	**17 ± 4**	**34 ± 1**

The pairwise comparison of each treatment with its respective control was conducted using a generalised linear model. Statistically significant ($p < 0.01$) values are shown in bold.

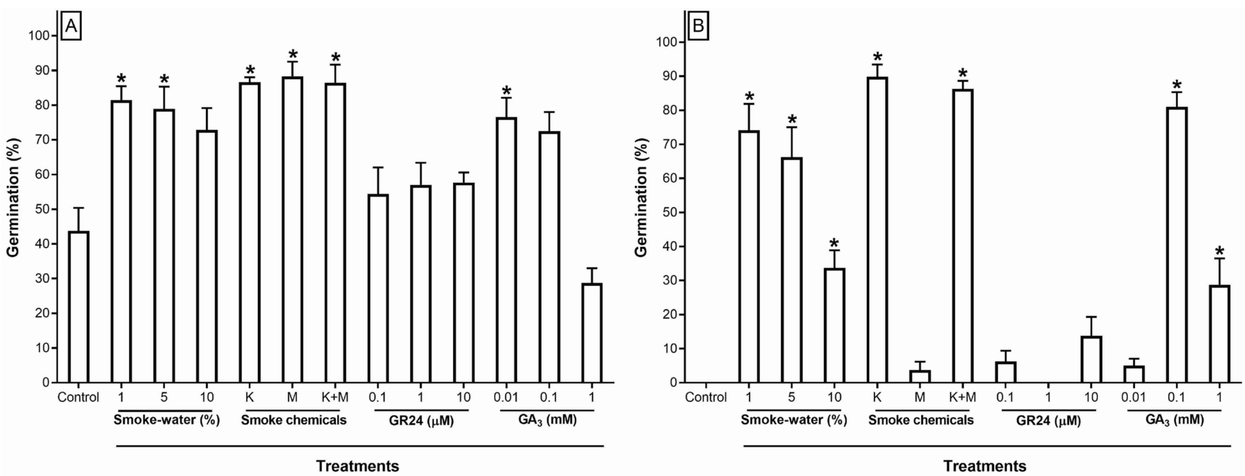

Fig. 2. Germination response of *Sarcopoterium spinosum* (A) and *Stachys cretica* (B) to smoke-water, smoke chemicals (K: KAR_1, M: Mandelonitrile, and K+M: KAR_1+Mandelonitrile combined), *rac*-GR24, and GA_3. Asterisks (*) denote significant differences compared to the control at $p < 0.01$.

plants under control conditions ranged from 0 to 3%, indicating a high degree of seed dormancy. Germination in *B. trixago* was also quite low, with only 23% of seeds germinating without treatment. Despite the high dormancy, robust germination occurred in response to smoke or *rac*-GR24 treatments in all the tested Orobanchaceae species except *P. viscosa* (Table 2). The smoke-water and KAR_1 treatments significantly stimulated germination in *B. trixago* compared to the control ($p < 0.01$). In addition, a marginal yet statistically non-significant improvement in germination was observed in *P. viscosa* at 1 μM KAR_1 ($p = 0.030$). Conversely, the obligate parasitic plants tested were unresponsive to smoke and KAR_1 ($p > 0.01$; Table 2). *Rac*-GR24 markedly increased germination in all *Orobanche* species at both concentrations ($p < 0.01$), with 94% of *O. minor* seeds germinating at 10 μM *rac*-GR24. However, the seeds of the facultative parasites (*B. trixago* and *P. viscosa*) did not significantly respond to *rac*-GR24 ($p > 0.01$). Finally, both GA_3 treatments promoted germination in *O. pubescens* ($p < 0.01$; Table 2), whereas the *B. trixago* seeds exhibited a positive response only at 0.1 mM GA_3 ($p < 0.01$).

DISCUSSION

Strigolactones (SLs) are β-carotene-derived phytohormones synthesised from a precursor called carlactone (Wang *et al.* 2024). GR24 is an SL analogue often used in a racemic form (*rac*-GR24) in germination experiments. *Rac*-GR24

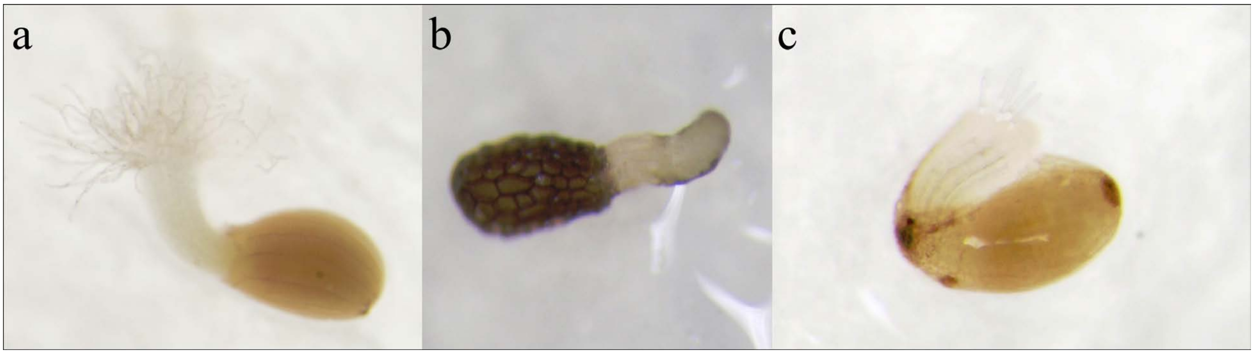

Fig. 3. Stereomicroscopic images of germinated seeds in some of the studied parasitic species. (a) *Bellardia trixago*, (b) *Orobanche anatolica*, and (c) *Parentucellia viscosa*. The magnification of the stereomicroscope was adjusted to a range of 28× to 56×.

contains the enantiomers $GR24^{5DS}$ and $GR24^{ent\text{-}5DS}$, which signal through the D14 and KAI2 receptor proteins, respectively. Therefore, only $GR24^{5DS}$ exhibits its bioactivity similar to that of natural SLs (Waters *et al.* 2017; Nelson 2021). The effect of *rac*-GR24 on the germination of KAR-responsive species, such as *Arabidopsis thaliana* (L.) Heynh., *Brassica tournefortii* Gouan, and *Lactuca sativa* L. has been examined in several studies (Nelson *et al.* 2009; Martinez *et al.* 2022; Takei *et al.* 2023). The results of these studies indicate that *rac*-GR24 has minimal or no effect on the germination of KAR-responsive species, depending on the concentration. In the present study, the germination responses of KAR_1-responsive *Sarcopoterium spinosum* and *Stachys cretica* to *rac*-GR24 were investigated across a concentration range of 0.1 to 10 µM. Our findings revealed that none of the tested *rac*-GR24 concentrations affected the germination of *S. spinosum* compared to the control, although a negligible increase was observed in *S. cretica* at 10 µM *rac*-GR24. Overall, *rac*-GR24 may have a minor effect on the germination of some KAR-responsive species at higher concentrations, likely due to the $GR24^{ent\text{-}5DS}$ enantiomer which activates the KAI2 receptor protein.

Depending on their structure and stereochemistry, SLs stimulate the germination of obligate Orobanchaceae parasites at picomolar levels (Xie *et al.* 2007; Miura *et al.* 2022; Yap & Tsuchiya 2023). Conversely, facultative Orobanchaceae parasites are not considered to require SL signals for germination induction (Kabiri *et al.* 2016; Ogawa *et al.* 2022). Consistent with these observations, our findings indicate that *rac*-GR24 promotes germination only in obligate parasites (*Orobanche anatolica*, *O. minor*, and *O. pubescens*), with no effect on facultative parasites (*Bellardia trixago* and *Parentucellia viscosa*). The germination response of obligate Orobanchaceae parasites to KAR_1 has also been investigated by various research groups (Daws *et al.* 2008; Chiwocha *et al.* 2009; Nelson *et al.* 2009; Brun *et al.* 2019). Daws *et al.* (2008) first reported that KAR_1 exhibited activity comparable to GR24 on 13 root parasites of the genera *Cistanche* Hoffmanns. & Link, *Conopholis* Wallr., *Lathraea* L., *Orobanche*, and *Striga*. However, the purity of the KAR_1 solution used in Daws *et al.* (2008) was questioned by Chiwocha *et al.* (2009). Subsequent investigations by Nelson *et al.* (2009) and Brun *et al.* (2019) demonstrated that KAR_1 failed to induce germination in *Orobanche minor*, *O. cumana* Wallr., *Phelipanche ramosa* (L.) Pomel, and *Striga hermonthica* (Delile) Benth. In line with these findings, we observed no significant effect of KAR_1 (Carbosynth, purity ≥ 98%) on the germination of three *Orobanche* species. In the present study, we also tested the effect of KAR_1 on germination in the facultative parasites *B. trixago* and *P. viscosa*. The results revealed that KAR_1 dramatically increased germination in *B. trixago*, and produced a modest effect in *P. viscosa*. Differences in the number, type, and functionality of the *KAI2* genes may underlie the varying germination responses of obligate and facultative Orobanchaceae parasites to KARs (Nelson 2021; Kee *et al.* 2023).

Plant-derived smoke, which contains a wide variety of compounds (including KARs and cyanohydrins), stimulates germination in many plant families (Kępczyński & Kępczyńska 2023; Çatav *et al.* 2024a; Monemizadeh *et al.* 2025). However, little is known about how smoke affects Orobanchaceae species. From an ecological standpoint, a positive response to smoke and smoke chemicals is unlikely to be advantageous for obligate Orobanchaceae parasites, as germination in a post-fire environment lacking mature host plants would be suicidal (Nelson 2021). Facultative parasites, in contrast, can survive independently. To our knowledge, only two studies have addressed the smoke response of Orobanchaceae seeds (Nun & Mayer 2005; Ergan 2017). Nun & Mayer (2005) found that cellulose-derived smoke-water and coumarin (a benzopyrone) stimulated the germination of *Orobanche aegyptiaca* Pers. Despite using a conditioning protocol very similar to that implemented by Nun and Mayer, we observed no pronounced effect of smoke-water on the germination of the obligate parasites *O. anatolica*, *O. minor*, and *O. pubescens*. This discrepancy may stem from species-specific responses or the differing compositions of smoke-waters utilised in the two studies. Moreover, Chiwocha *et al.* (2009) noted that coumarin shares structural similarities with a compound called pyrone, which can be isolated from the same fraction as KAR_1. It is also noteworthy that pyrone and KAR_1 have equivalent molecular weights and very similar UV absorption properties. Therefore, the positive germination response of *O. aegyptiaca* to smoke-water could be related to pyrone but not to KAR_1. However, Chiwocha *et al.* (2009) also stated that the fraction containing KAR_1 and pyrone, as well as coumarin alone, had no effect on the germination of *O. minor*. Further research is therefore needed to clarify the role of smoke in the germination of obligate Orobanchaceae parasites, using diverse smoke-water samples and their fractions. In the present study, we also tested the effect of smoke on the germination of two facultative parasitic species. Our results showed that similar to KAR_1, smoke-water significantly enhanced germination in *Bellardia trixago*, a facultative parasite. This result is consistent with the findings of Ergan (2017), who provided evidence of smoke-stimulated seed germination in *B. trixago*, and the presence of this species in recently burned forest areas in southwestern Türkiye. Such findings suggest wildfires may benefit some facultative Orobanchaceae parasites by promoting germination and establishment.

Abscisic acid (ABA) and gibberellins (GAs) are key phytohormones regulating seed dormancy and germination. ABA is the major determinant in the induction and maintenance of dormancy, while GAs play a role in the synthesis and secretion of hydrolytic enzymes and the initiation of germination (Kathpalia & Bhatla 2018; Jhanji *et al.* 2024). Several species, including *Avena fatua* L. and *Brassica oleracea* L., exhibit significantly reduced seed ABA levels following KAR_1 treatment (Sami *et al.* 2021; Kępczyński *et al.* 2024). Moreover, GA biosynthesis has been shown to be essential for KAR_1-stimulated germination, and KAR_1-responsive species typically respond to exogenous GA_3 (Nelson *et al.* 2009; Çatav *et al.* 2024b). In accordance with these findings, the GA_3 treatments enhanced the germination in all the KAR_1-responsive species studied, including the facultative parasite *B. trixago*. The upregulation of genes related to ABA catabolism and the reduction of seed ABA levels have also been observed in SL-treated broomrape and witchweed species (Bao *et al.* 2017; Brun *et al.* 2019). In addition, the exposure of *Orobanche* and *Striga* seeds to GA biosynthesis inhibitors (e.g. ancymidol, paclobutrazol, and uniconazole) has been found to significantly reduce the stimulatory effect of GR24 on germination, particularly during the conditioning period (Zehhar *et al.* 2002; Song *et al.* 2005; Yap & Tsuchiya 2023). These results suggest that the inhibition of GA biosynthesis may decrease the sensitivity of broomrape and witchweed seeds to SLs. On the other hand, exogenous GA_3 had little or no effect on the germination of the conditioned seeds of several *Orobanche*, *Phelipanche*, and *Striga* species (Pereira *et al.* 2017; Bunsick *et al.* 2020). In our study, 0.1 and 1 mM GA_3 markedly increased the germination of *O. pubescens*, whereas *O. anatolica* and *O. minor* did not respond to any GA_3 concentration. Overall, GA signalling likely contributes to KAR-triggered germination, but further work is required to clarify the precise function of GAs in the SL-dependent germination of obligate Orobanchaceae parasites during and after conditioning.

CONCLUSION

In this study, we conducted a comparative evaluation of the germination responses of obligate and facultative Orobanchaceae parasites and two fire-responsive species to KAR_1, *rac*-GR24, plant-derived smoke, and GA_3. Our results demonstrated that *rac*-GR24 specifically induced germination in obligate *Orobanche* parasites, while having no effect on facultative parasites or fire-responsive species, thereby supporting our first and third hypotheses. In contrast, KAR_1 and smoke-water significantly stimulated germination in the facultative hemiparasite *B. trixago* and in both fire-responsive species, aligning with our second hypothesis. Notably, GA_3 enhanced germination across all KAR_1-responsive species, but failed to substitute for *rac*-GR24 in most obligate parasites, with the exception of *O. pubescens*. Together, these findings reveal a clear divergence in chemical sensitivity among parasitic and non-parasitic species and suggest that KARs and SLs are not functionally interchangeable cues. Moreover, fire signals may afford certain facultative parasites a significant advantage by promoting germination under post-fire conditions.

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COMPREHENSIVE PLANT BIOLOGY

REZIME

Karikini naspram strigolaktona: komparativna analiza efekata na klijanje semena obligatnih i fakultativnih parazita iz porodice Orobanchaceae i vrsta koje reaguju na požar

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Signalni putevi karikina (KAR) i strigolaktona (SL) smatraju se evolutivno povezanim, što je podstaklo brojna istraživanja sličnosti i razlika između odgovora u procesima razvića biljaka posredovanih ovim jedinjenjima. U ovom radu ispitivani su efekti biljnog dima, KAR_1, rac-GR24 (sintetičkog analoga strigolaktona) i giberelinske kiseline (GA_3) na klijanje semena pet vrsta iz porodice Orobanchaceae, uključujući obligatne i fakultativne parazite, kao i dve neparazitske vrste poznate po odgovoru na dim i KAR_1. Rezultati su pokazali da semena vrsta *Sarcopoterium spinosum* i *Stachys cretica*, koje pozitivno reaguju na KAR_1, nisu bila osetljiva na rac-GR24. Iako je rac-GR24 stimulisao klijanje kod svih ispitivanih obligatnih parazita roda Orobanche (*O. anatolica*, *O. minor* i *O. pubescens*), nije imao efekat na fakultativne parazite *Bellardia trixago* i *Parentucellia viscosa*. Dimna voda i KAR_1 nisu indukovali klijanje kod tri vrste roda Orobanche, ali su značajno povećali klijanje kod *B. trixago*. Takođe, semena vrsta *S. spinosum*, *S. cretica* i *B. trixago* pozitivno su reagovala na GA_3, dok GA_3 nije mogao da zameni potrebu za rac-GR24 u stimulaciji klijanja kod vrsta roda Orobanche, osim kod *O. pubescens*. Sveukupno, rezultati ukazuju da: (I) KAR i SL jedinjenja nisu međusobno zamenljivi signali za klijanje kod obligatnih parazita iz porodice Orobanchaceae niti kod dve neparazitske vrste koje reaguju na požar; (II) karikini mogu predstavljati adaptivnu prednost za pojedine fakultativne parazite u postpožarnim uslovima sredine.

Ključne reči: fakultativni paraziti, karikini, obligatni paraziti, Orobanchaceae, dim, strigolaktoni