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Germination response of three smoke- and karrikinolide-responsive species to gibberellic acid and reactive oxygen species

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There is a growing interest in understanding the mechanisms involved in seed germination stimulated by smoke and smoke-derived compounds. In this study, we aimed to determine the possible role of gibberellins (GAs) and reactive oxygen species (ROS) in the seed germination of three smoke-responsive and fire-adapted species, viz. *Onopordum caricum* (Asteraceae), *Sarcopoterium spinosum* (Rosaceae), and *Stachys cretica* (Lamiaceae). We conducted four germination experiments using smoke water, karrikinolide (KAR₁), cyanohydrin mandelonitrile, gibberellic acid (GA₃), paclobutrazol, hydrogen peroxide, and several agents generating ROS at different concentrations. The three species had a positive germination response to KAR₁. *Sarcopoterium spinosum* seeds were also sensitive to mandelonitrile. Exogenous GA₃ promoted the germination of all KAR₁-responsive species in a concentration-specific way. Furthermore, GA biosynthesis was found to be required for the induction of germination by smoke water and KAR₁. Finally, ROS treatments stimulated the germination of KAR₁- and cyanohydrin-sensitive *S. spinosum* seeds, but not only KAR₁-sensitive *O. caricum* and *S. cretica* seeds. In conclusion, we showed that smoke-promoted germination is far more complex than previously believed and that future studies should be carried out using species with different sensitivities to smoke chemicals.

Introduction

Burning of vegetation releases a broad range of compounds affecting numerous physiological and developmental processes in plants, including

seed germination and root growth (Wang *et al.* 2017, Cao *et al.* 2021, Pausas & Lamont 2022). Cell-wall derived karrikins (KARs) and a cyanide-containing compound glyconitrile are recognized as major smoke chemicals that promote

seed germination in many species (Flematti *et al.* 2015). The interplay among these chemicals can also produce synergistic effects on the germination of fire-adapted plants (Çatav *et al.* 2018, Çatav & Akbaş 2021). Moreover, lignin-derived compounds, such as syringaldehyde, benzaldehyde, and hydroquinone present in smoke have been suggested to be involved in germination enhancement in several plant species (Cao *et al.* 2023). Smoke also contains many other compounds from phenols, butenolides, and organic acids (e.g., resorcinol, 3,4,5-trimethylfuran-2(5*H*)-one, and 4-propoxybenzoic acid) that may inhibit germination or be antagonistic against karrikinolide (KAR₁) at certain concentrations (Baldwin *et al.* 1994, Burger *et al.* 2018). Taken together, the germination response to smoke is a multifaceted process, marked by the presence and interaction of various stimulatory and inhibitory chemicals.

The positive response to KARs of seed germination and seedling photomorphogenesis in some *Arabidopsis thaliana* ecotypes led to the discovery of the karrikin signalling pathway (Waters & Nelson 2023). The perception of KARs in *Arabidopsis* is achieved through an α/β hydrolase receptor protein (KARRIKIN INSENSITIVE2, KAI2), an F-box protein (MORE AXILLARY GROWTH2, MAX2), and repressor proteins, such as SMAX1 (SUPPRESSOR OF MAX2 1) and SMXL2 (SMAX1-LIKE2) (Yao & Waters 2020). The KAI2 gene present in streptophytes is thought to be acquired from proteobacteria via horizontal gene transfer about 1000 million years ago (Wang *et al.* 2022). Considering that the earliest evidence of fire has been dated to 425 million years ago (Wellman *et al.* 2003) and that KARs are related to burning plant material, KAI2 is assumed to be a receptor for an unidentified molecule (Kamran *et al.* 2023). Thus, KARs or their metabolites are suggested to mimic this molecule, also referred to as the KAI2 ligand (Yao *et al.* 2021).

There is a growing interest in understanding physiological and molecular changes in seeds in response to smoke during the germination process. It is postulated that KARs-mediated polyubiquitination and degradation of SMAX1 and SMXL2 proteins contribute to enhanced germination in *Arabidopsis* (Khosla *et al.* 2020).

Nelson *et al.* (2009) further revealed that both gibberellin (GA) biosynthesis and light are essential for KAR₁-stimulated germination in *A. thaliana*. In addition, endogenous levels of phytohormones (e.g., abscisic acid, ethylene, and GAs) associated with germination and dormancy were found to be changed in KAR₁-treated seeds of several species (Schwachtje & Baldwin 2004, Ruduś *et al.* 2019, Sami *et al.* 2021). Furthermore, KAR₁ and smoke were shown to enhance dehydrogenase, α -amylase and β -amylase activities in *Avena fatua* and *Lactuca sativa* seeds (Kępczyński *et al.* 2013, Gupta *et al.* 2019). Compared with KARs, almost no information is available on glyconitrile-related biochemical changes in seeds. However, cyanide has been identified as the active compound responsible for cyanohydrin-promoted germination (Flematti *et al.* 2011). In this regard, reactive oxygen species (ROS) are assumed to play a role in the dormancy release caused by cyanide-containing glyconitrile (Nelson *et al.* 2012, Yu *et al.* 2022).

Although existing research has provided insights into how smoke-related chemicals affect seed germination, a complete understanding is still lacking. In particular, there are gaps in knowledge about how different species respond and the exact changes triggered by specific compounds. These gaps need to be addressed to fully comprehend the mechanisms underlying smoke-induced germination. For instance, most of the studies mentioned above utilized seeds from a few species only (e.g., *Arabidopsis thaliana*, *Avena fatua* and *Lactuca sativa*). In addition, *A. thaliana* and *A. fatua* seeds are known to be sensitive to both KAR₁ and cyanide, and this makes the assessment of the physiological basis of KAR₁-triggered germination more complicated (Tilsner & Upadhyaya 1987, Bethke *et al.* 2006, Nelson *et al.* 2009, Ruduś *et al.* 2019). Moreover, our understanding of the germination response of fire-adapted species to GAs and ROS, believed to contribute to smoke-enhanced germination, remains limited. To partly fill these gaps, we studied the effects of GA₃, paclobutrazol, H₂O₂, and several ROS-generating compounds on the seed germination of three fire-adapted species (KAR₁-sensitive: *Onopordum caricum* and *Stachys cretica*; both KAR₁- and cyanohydrin-sensitive: *Sarcopoterium spin-*

sum). We hypothesized that (i) KAR₁-sensitive seeds have positive germination responses to exogenous GA₃ treatments, (ii) GA biosynthesis might be a prerequisite for the stimulatory effect of KAR₁ on germination, and (iii) H₂O₂ and ROS-generating compounds improve the germination of cyanohydrin-sensitive species.

Material and methods

Study species, study area, and seed collection

Onopordum caricum (Asteraceae), *Stachys cretica* subsp. *smyrnaea* (Lamiaceae) and *Sarcopoterium spinosum* (Rosaceae) were selected for this study as different populations of these species have shown a positive germination response to plant-derived smoke in our earlier works (Çatav et al. 2012, 2014, 2015, 2018). Fruits were harvested from at least 12 healthy individuals growing in their natural environment in Muğla Province, Turkey, in July and August 2018. The study site has a Mediterranean climate, with an average annual total precipitation of 1208.3 mm and annual mean temperature of 15.1 °C (data from the Turkish State Meteorological Service). Seeds were removed from intact fruits and stored in moisture-free bags at room temperature until the germination assays began in September 2018. The mean (± SE) seed masses of the studied species (*O. caricum* = 10.54 ± 0.25 mg, *S. spinosum* = 2.40 ± 0.04 mg, *S. cretica* = 2.76 ± 0.07 mg) were evaluated by weighing four samples of 100 seeds and dividing the results by 100.

Preparation of smoke-water and test solutions

Smoke water (hereafter, SW) was prepared using wheat straw as plant material, following the procedure outlined in Çatav et al. (2018). After filtration through a Whatman grade 42 filter paper, SW (pH 4.27) was stored at 4 °C until required. The compounds used in this work are listed in Table 1. The stock solution of each compound was prepared using an appropriate solvent (dH₂O, ethanol, and dimethyl sulfoxide) and diluted just before use.

Germination protocol

Four germination experiments were carried out to examine the mechanisms underlying smoke-induced seed germination more closely (for details, see below). Seeds of the studied species were placed in Petri dishes (Ø 90 mm) containing two Whatman grade 1 round (Ø 85 mm) filter papers with 10 ml of dH₂O or test solutions unless stated otherwise. Three samples of 25 seeds were used per treatment group. Petri dishes were wrapped with parafilm to avoid water loss and transferred to an incubator adjusted at 20 °C in darkness. Germination counts were conducted weekly throughout a 35-day experimental period in dim-light conditions. A seed was considered germinated when the radicle protruded more than 2 mm from the testa. After completing the experiments, the viability of ungerminated seeds was assessed using the cut test.

Table 1. Compounds used in this study; CAS no. refers to the registration number of a chemical substance in the Chemical Abstracts Service (<https://www.cas.org/>), ROS = reactive oxygen species.

Compound	Abbreviation	CAS no.	Type
Karrikinolide	KAR ₁	857054-02-5	smoke-derived compound
Mandelonitrile	MAN	532-28-5	cyanohydrin
Menadione	MD	58-27-5	compound generating ROS
Methyl viologen	MV	75365-73-0	compound generating ROS
Hydrogen peroxide	H ₂ O ₂	7722-84-1	reactive oxygen species
3-amino-1,2,4-triazole	AT	61-82-5	inhibitor of catalase
Gibberellic acid	GA ₃	77-06-5	phytohormone
Paclitaxel	PAC	76738-62-0	inhibitor of gibberellin biosynthesis

Experiments

In the first experiment, we aimed to verify whether *O. caricum*, *S. cretica* and *S. spinosum* seeds respond to smoke and, if so, ascertain the smoke-derived compounds responsible for the stimulation of germination. To do this, seeds were subjected to various concentrations of SW (2.5% and 5%), KAR₁ (0.01 and 0.1 μM), and MAN (10 and 50 μM). In the second experiment, our goal was to determine whether GA₃ would trigger germination in KAR₁-responsive species. To accomplish this, we used four concentrations of GA₃ (10⁻⁶, 10⁻⁵, 10⁻⁴ and 10⁻³ M). In the third experiment, we aimed to clarify ROS involvement in KAR₁-stimulated germination. For this, seeds of KAR₁-responsive species were exposed to various concentrations of 3-amino-1,2,4-triazole (AT; 0.5 and 1 mM), H₂O₂ (0.1, 0.5, 1 and 5 mM), menadione (MD; 1 mM), and methyl viologen (MV; 0.1 mM). In the MD and MV treatments, seeds were pre-incubated in these solutions for 3 or 6 h and then transferred into Petri dishes containing dH₂O. In the fourth experiment, our goal was to evaluate whether gibberellin biosynthesis is necessary for KAR₁-induced germination. To this end, *O. caricum* and *S. cretica* seeds were treated with SW and KAR₁ in the presence and absence of paclobutrazol (PAC) (10⁻⁴ and 3 × 10⁻⁴ M; PAC₁ and PAC₂, respectively). The final concentrations of the tested compounds were selected based on previous studies (Gardner *et al.* 2001, Schwachtje & Baldwin 2004, Oracz *et al.* 2009, Cembrowska-Lech *et al.* 2015, Çatav *et al.* 2018) and preliminary data.

Data analyses

Prior to statistical analyses, germination percentages were converted to values between 0 and 1 and subjected to arcsine-square-root transformation (Downes *et al.* 2013). Bartlett's test was used for the evaluation of homogeneity of variance. Finally, one-way ANOVA followed by Tukey's post-hoc test were used to identify differences between treatment groups.

Results

The smoke water (SW) treatments markedly increased the germination percentages of the three studied species as compared with those of the control (Table 2). For instance, application of 2.5% SW increased the germination percentage of non-smoke-treated *S. cretica* seeds from 1.3% to 72.0%. Germination of the seeds of all three species was enhanced by both KAR₁ treatments. Germination percentages of 0.1 μM KAR₁-exposed seeds of *O. caricum* and *S. spinosum* were 3.27- and 3.59-fold higher than those of the respective controls. In addition, *S. spinosum* seeds were responsive not only to KAR₁ but also to MAN; however, MAN treatments did not substantially affect the germination of the other two species. None of the smoke-related treatments negatively influenced the seed germination of any of the three species.

Exogenous GA₃ caused a marked increase in the germination percentage of KAR₁-responsive species relative to control conditions (Table 3). Its effect on germination was also concentration dependent. For instance, while the highest con-

Table 2. Germination response (%) of *Onopordum caricum*, *Stachys cretica* and *Sarcopoterium spinosum* to smoke and smoke-derived compounds. Germination percentages are presented as means ± SE of three determinations. Values in the same column marked with different superscript letters differ at $p < 0.05$ (Tukey's post-hoc test).

Treatment	<i>O. caricum</i>	<i>S. cretica</i>	<i>S. spinosum</i>
Control	28.6 ± 3.2 ^c	1.3 ± 1.3 ^b	24.5 ± 2.5 ^b
SW (2.5%)	77.1 ± 3.8 ^b	72.0 ± 6.1 ^a	83.7 ± 0.9 ^a
SW (5%)	82.5 ± 2.7 ^{ab}	59.7 ± 9.8 ^a	84.0 ± 3.1 ^a
KAR ₁ (0.01 μM)	83.2 ± 1.8 ^{ab}	85.2 ± 2.6 ^a	75.0 ± 8.7 ^a
KAR ₁ (0.1 μM)	93.5 ± 4.2 ^a	85.3 ± 5.8 ^a	88.0 ± 0.5 ^a
MAN (10 μM)	34.7 ± 1.6 ^c	6.7 ± 3.5 ^b	87.4 ± 8.4 ^a
MAN (50 μM)	39.3 ± 3.0 ^c	2.7 ± 1.3 ^b	91.0 ± 5.9 ^a

centration of GA₃ (10⁻³ M) stimulated the germination of *O. caricum*, it inhibited the germination of *S. spinosum*. Additionally, 10⁻⁵ M GA₃ treatment did not markedly alter the germination percentage of *O. caricum* and *S. cretica* but elevated that of *S. spinosum*. Overall, positive or negative effects of GA₃ on the germination were observed in a concentration range between 10⁻⁵ and 10⁻³ M.

As we had limited numbers of seeds of *S. cretica* and *S. spinosum*, AT and ROS-generating compounds were applied solely to *O. caricum*. However, seeds of all three species were exposed to H₂O₂. None of the AT, H₂O₂, MD or MV treatments caused a significant change in the germination percentage of *O. caricum* (Fig. 1A and B). In addition, H₂O₂ treatments did not affect the germination percentage of the *S. cretica* seeds (Fig. 1C). In contrast, the highest concentration of H₂O₂ markedly increased the germination of *S. spinosum* (Fig. 1D). These results unequivocally indicate that the germination of only KAR₁-sensitive species, such as *O. caricum* and *S. cretica*, was not triggered by ROS. On the other hand, ROS enhanced the germination in both KAR₁- and cyanide-responsive seeds of *S. spinosum*, indicating that ROS seem to be involved in cyanide-stimulated seed germination.

In *O. caricum*, the 10⁻⁴ M PAC treatment reduced the germination percentage compared with that in the control conditions (Fig. 2). The application of PAC also resulted in a considerable reduction in the germination capability of *O. caricum* seeds subjected to smoke (Fig. 2). However, PAC-induced inhibition of germination in the seeds treated and not treated with SW was reversed by exogenous GA₃. The germination assay results of *S. cretica* demonstrate that both

PAC treatments (10⁻⁴ and 3 × 10⁻⁴ M) suppress KAR₁-induced germination stimulation. Similar to the findings in *O. caricum*, the application of exogenous GA₃ (10⁻⁴ M) reversed the germination inhibition caused by PAC in KAR₁-exposed *S. cretica* seeds (Fig. 3). Overall, these results emphasize the requirement for GA biosynthesis in KAR₁-mediated promotion, showing that PAC hinders the positive effects of smoke or KAR₁ on the germination of *O. caricum* and *S. cretica*.

Discussion

Combustion of woody biomass leads to the release of various promoter and inhibitor compounds that regulate different stages of plant growth and development (Burger *et al.* 2018, Waters & Nelson 2023). Plant-derived smoke containing such compounds can exert positive, negative, or no effect on seed germination, depending on dormancy type and exposure concentration (Moreira & Pausas 2018, Gupta *et al.* 2020). The positive effect of smoke on seed germination is mainly attributed to a group of compounds known as KARs and cyanide-containing glyconitrile (Ma *et al.* 2020). We examined the effects of smoke water (SW), KAR₁, and mandelonitrile (MAN) on seed germination of three species (*O. caricum*, *S. cretica*, and *S. spinosum*), which have previously been reported as smoke-sensitive (Çatav *et al.* 2012, 2014, 2015, 2018). Our results indicate that both SW and KAR₁ stimulate germination of all three species. Moreover, *S. spinosum* was shown to respond to cyanohydrin mandelonitrile. By integrating current and previous data, we conclude that germination in these species is strongly dependent on smoke and its components.

Table 3. Germination response (%) of *Onopordum caricum*, *Stachys cretica* and *Sarcopoterium spinosum* to gibberellic acid (GA₃). Germination percentages are presented as means ± SE of three determinations. Values in the same column marked with different superscript letters differ at *p* < 0.05 (Tukey's post-hoc test).

Treatment	<i>O. caricum</i>	<i>S. cretica</i>	<i>S. spinosum</i>
Control	29.9 ± 2.8 ^b	2.7 ± 1.3 ^c	15.8 ± 2.4 ^b
GA ₃ (10 ⁻⁶ M)	30.6 ± 7.8 ^b	0 ^c	15.9 ± 2.6 ^b
GA ₃ (10 ⁻⁵ M)	25.7 ± 3.7 ^b	5.2 ± 2.6 ^c	70.8 ± 6.4 ^a
GA ₃ (10 ⁻⁴ M)	83.4 ± 4.3 ^a	75.6 ± 4.2 ^a	17.8 ± 1.1 ^b
GA ₃ (10 ⁻³ M)	93.1 ± 3.5 ^a	48.0 ± 4.0 ^b	0 ^c

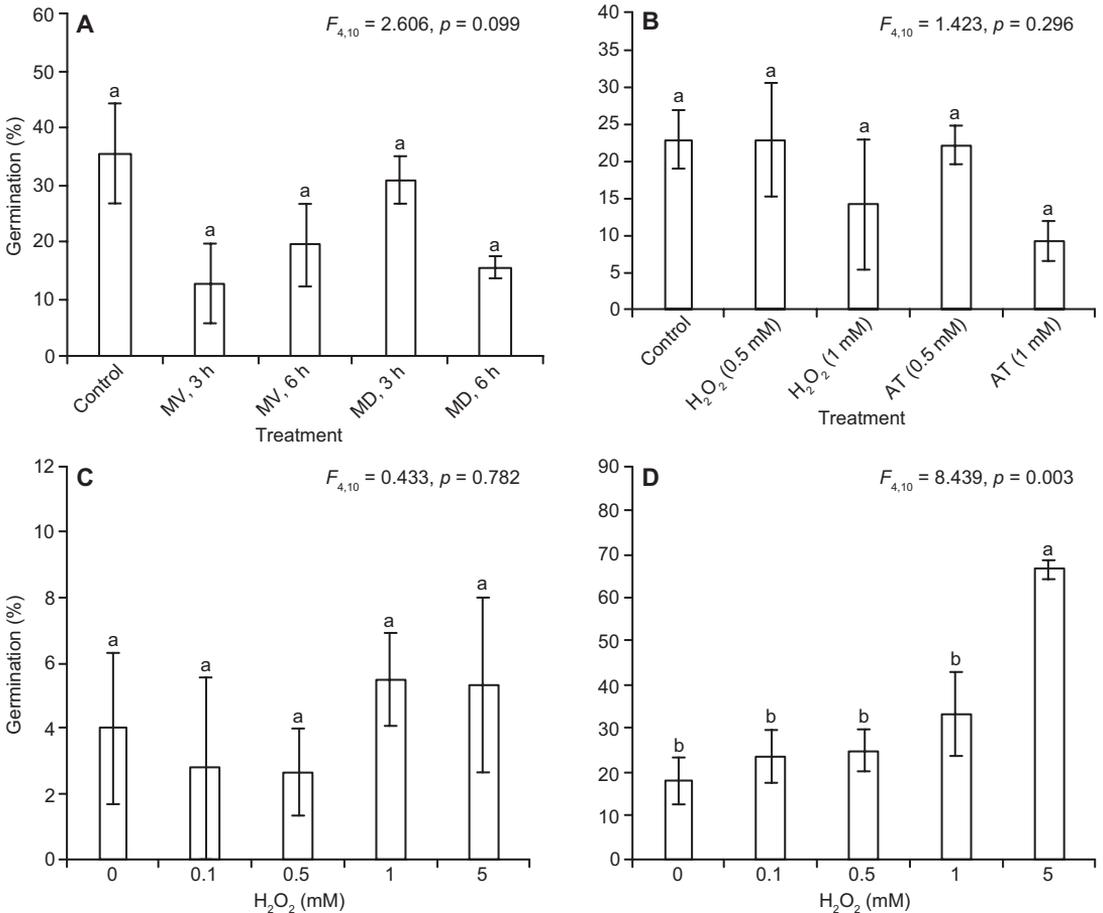


Fig. 1. Germination response of *Onopordum caricum* (**A** and **B**), *Stachys cretica* (**C**), and *Sarcopoterium spinosum* (**D**) to reactive oxygen species (ROS) generating compounds and H₂O₂; data are presented as mean \pm SE ($n = 3$). Different letters above the error bars indicate differences at $p < 0.05$ (Tukey's post-hoc test) among treatment groups. The concentrations of methyl viologen (MV) and menadione (MD) were 0.1 mM and 1 mM, respectively.

Phytohormones, such as abscisic acid (ABA), ethylene, and GAs play critical roles in seed dormancy and germination. For instance, it is widely recognized that increased sensitivity of seeds to ABA induces dormancy (Tuan *et al.* 2018, Yan & Chen 2020). Previous studies demonstrated that smoke and KARs can modify the endogenous levels of ABA and GAs in seeds. The ABA content in *Avena fatua*, *Brassica oleracea* and *Nicotiana attenuata* seeds decreased following exposure to KAR₁ or smoke (Schwachtje & Baldwin 2004, Sami *et al.* 2019, Kępczyński *et al.* 2021). However, there were mixed results for GAs levels in seeds treated with KAR₁ and smoke. Endogenous GA₁, GA₃, and GA₄ concentrations in seeds of various plants were found to

increase, decrease or remain constant with KAR₁ or smoke application throughout the different phases of germination (Schwachtje & Baldwin 2004, Nelson *et al.* 2009, Sami *et al.* 2019). Moreover, the studies on the effect of exogenous GA₃ on seed germination of smoke- and KAR₁-sensitive species also produced mixed findings (for more details see Çatav *et al.* 2018). In our study, the application of GA₃ promoted the germination of all KAR₁-responsive species and our results suggest that GAs may play a role in KAR₁-induced germination. However, the effect of GA₃ on germination seems to be dependent on the concentration used. It should be also noted that seeds of some GA₃-sensitive species germinate in response to smoke or KAR₁ after

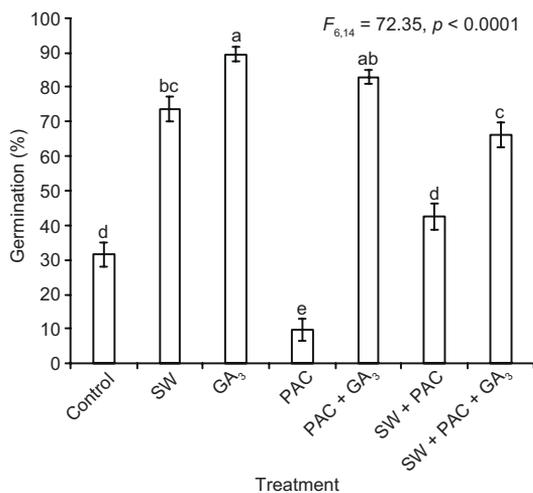


Fig. 2. Germination response of *Onopordum caricum* to paclobutrazol (PAC, 10^{-4} M), smoke water (SW, 5%), and gibberellic acid (GA₃, 10^{-4} M) treatments; data are presented as mean \pm SE ($n = 3$). Different letters above the error bars indicate differences at $p < 0.05$ (Tukey's post-hoc test) among treatment groups.

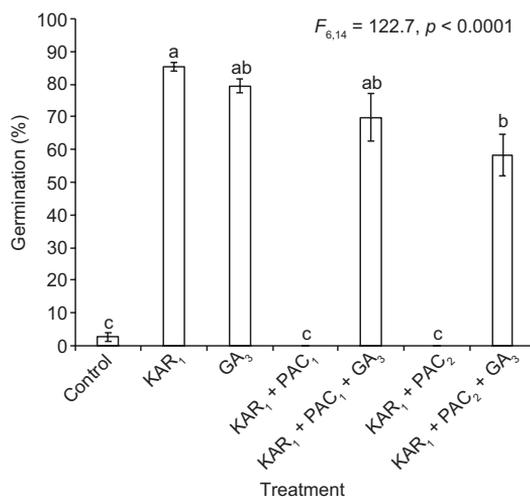


Fig. 3. Germination response of *Stachys cretica* to paclobutrazol (PAC₁, 10^{-4} M; PAC₂, 3×10^{-4} M), karrikinolide (KAR₁; 0.1 μ M), and gibberellic acid (GA₃, 10^{-4} M) treatments. Data are presented as mean \pm SE ($n = 3$). Different letters above the error bars indicate differences at $p < 0.05$ (Tukey's post-hoc test) among treatment groups.

other dormancy-alleviating treatments, such as soil burial, dry after-ripening, and warm stratification (Commander *et al.* 2008). Therefore, the contradictory results from previous works may be linked to the applied concentration(s) of GA₃ and/or seed dormancy levels. In view of our results and available literature, we suggest that the germination response of fire-adapted species should be tested for GA₃ concentrations between 10^{-5} and 10^{-3} M.

Several researchers studied whether the biosynthesis of ethylene and GAs are essential for smoke- and KAR₁-stimulated seed germination, using inhibitor compounds that affect the biosynthesis and action of these phytohormones. While inhibitors of ethylene perception, such as 2,5-norbornadiene and methylcyclopropene have been reported to hinder the positive effect of KAR₁ on germination (Kępczyński & van Staden 2012, Sami *et al.* 2021), ethylene biosynthesis inhibitors, such as α -aminoisobutyric acid and aminoethoxyvinylglycine had minimal or no effect on the germination of KAR₁-treated seeds (Ruduś *et al.* 2019). On the other hand, GA biosynthesis inhibitors, such as paclobutrazol, ancymidol, and AMO 1618 counteracted the induction effects of smoke or KAR₁ (Gardner *et*

al. 2001, Kępczyński *et al.* 2013, Cembrowska-Lech & Kępczyński 2017, Rudus *et al.* 2019). We explored the effect of paclobutrazol treatments on the germination of *O. caricum* and *S. cretica* seeds exposed to smoke and KAR₁, respectively. Our findings align with those of the above-cited works, emphasizing the necessity of GA biosynthesis in KAR₁-triggered seed germination. Overall, we conclude that the perception or biosynthesis of several phytohormones plays a part in the regulation of seed responses to KARs.

Reactive oxygen species (ROS) are multifunctional molecules implicated in the regulation of seed dormancy and germination within a concentration range defined as the oxidative window. They participate in various processes during seed germination, including protein carbonylation, mRNA oxidation, and cell wall loosening (Bailey & Merendino 2021, Li *et al.* 2022). ROS have also been associated with dormancy release caused by cyanide (Gniazdowska *et al.* 2010, Yu *et al.* 2022). For instance, Gniazdowska *et al.* (2010) demonstrated that HCN treatment led to a transient increase in the H₂O₂ content of apple embryos, and this, in turn, enhanced the emission of ethylene, a positive regulator

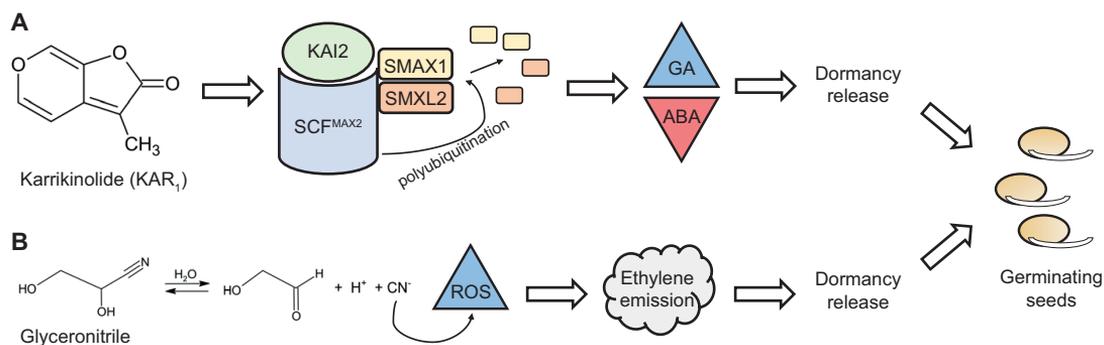


Fig. 4. Mechanisms for KAR₁- and glyconitrile-induced seed germination. — **A:** KAR₁ perception and signaling are mediated by an α/β -hydrolase (KAI2), an F-box protein (SCF^{MAX2}), and the repressor proteins SMAX1 and SMXL2 (Waters & Nelson 2023). Proteasomal degradation of repressor proteins is involved in dormancy release and germination promotion by modulating ABA catabolism and GA biosynthesis (Brun *et al.* 2018, Lamont & Pausas 2023). — **B:** Cyanide is released from cyanohydrin glyconitrile in the presence of water (Flematti *et al.* 2015). Cyanide has been shown to elevate ROS levels, which in turn leads to increased ethylene emission and subsequent dormancy release (Gniazdowska *et al.* 2010).

of dormancy release. Cembrowska-Lech *et al.* (2015) reported that KAR₁-promoted seed germination in *Avena fatua* was mediated by ROS. Here, we examined the germination response of three fire-adapted species to H₂O₂, AT, and ROS-generating compounds MD and MV. Our ROS-related treatments failed to induce germination of KAR₁-only responsive species. On the other hand, 5 mM H₂O₂ markedly increased the germination of *S. spinosum*, a both KAR₁- and cyanohydrin-responsive species. Our findings imply that a positive germination response to ROS is more likely to be associated with cyanide sensitivity rather than KAR₁ sensitivity, and are incompatible with the results of Cembrowska-Lech *et al.* (2015). In their study, similar ROS treatments triggered the germination of KAR₁- and GA₃-responsive *A. fatua* caryopses. In addition, KAR₁ was found to cause a remarkable increase in H₂O₂ and superoxide anion levels of *A. fatua* embryos during the germination period. However, previous studies also revealed that *A. fatua* seeds were sensitive to other environmental signals, including cyanide and strigol (Tilsner & Upadhyaya 1987, Bradov *et al.* 1990). In addition, a rise in the ROS content of KAR₁-treated *A. fatua* caryopses might be related to increased metabolic activity due to higher germination rates (Bailly 2019). Taken together, more studies are required to clarify the role of ROS in KAR₁- and cyanohydrin-stimulated germination.

In this study we showed that smoke and several smoke components play an indispensable role in releasing dormancy in *O. caricum*, *S. cretica*, and *S. spinosum*. In addition, exogenous GA₃ was found to induce the germination of all KAR₁-sensitive species in a concentration-specific way. A dramatic decrease in germination of KAR₁-treated seeds in the presence of PAC suggests that KAR₁ likely stimulates GA biosynthesis. Finally, ROS-related treatments triggered germination in cyanide-responsive seeds, but not in KAR₁-responsive seeds. Our results support the mechanisms proposed in earliest studies (Fig. 4) and highlight the need for mode-of-action research using species with different sensitivities to KAR₁ and cyanide.

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