Potential of Karrikins as Novel Plant Growth Regulators in Agriculture

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Received: 18 November 2019; Accepted: 24 December 2019; Published: 26 December 2019

Abstract: Karrikins (KARs) have been identified as molecules derived from plant material smoke, which have the capacity to enhance seed germination for a wide range of plant species. However, KARs were observed to not only impact seed germination but also observed to influence several biological processes. The plants defected in the KARs signaling pathway were observed to grow differently with several morphological changes. The observation of KARs as a growth regulator in plants leads to the search for an endogenous KAR-like molecule. Due to its simple genomic structure, Arabidopsis (Arabidopsis thaliana L.) helps to understand the signaling mechanism of KARs and phenotypic responses caused by them. However, different species have a different phenotypic response to KARs treatment. Therefore, in the current work, updated information about the KARs effect is presented. Results of research on agricultural and horticultural crops are summarized and compared with the findings of Arabidopsis studies. In this article, we suggested that KARs may be more important in coping with modern problems than one could imagine.

Keywords: karrikins; seed germination; crops; Arabidopsis

1. Introduction

Agriculture of the twenty-first century must face new challenges, which require novel solutions [1]. The use of industrial fertilizers, pesticides, and new varieties boosted the green revolution in the last century [2]. This century is even more challenging due to its faster-changing environment than civilization has ever faced, which makes us seek modern means of food and material production [3,4]. Not only does agriculture meet new challenges, but climate change brings along more and more forest and grassland fires [5]. Understanding how these will change biomes all around the world and what mechanisms are hidden behind changes is therefore crucial.

From the time when the evidence of a germination cue created by burning plant material was reported the first time [6], many studies exploring its nature have been performed. We now understand that a group of butenolide compounds isolated from smoke, the first member of which was identified independently by two researchers’ teams [7,8], plays a major role in germination promotion. Further on, another five analogs were added to this group [9], and the group was named KARs according to the word of Australian aborigines for smoke “karrik” [10]. In addition to KARs,
cyanohydrins were identified as a germination cue, which can stimulate the germination of some KAR-insensitive species [11].

Smoke was observed to stimulate the germination of many species from different families [10,12]. KARs are the major germination promoting compound found in smoke, and the KAR receptor is present in all phylogenetic taxa of plants, including mosses, liverworts, or green algae [13]. Therefore, the germination of smoke-responsive species is very likely enhanced by KARs. Indeed, many studies reported that KARs elevated germination of dicotyledonous, as monocotyledonous plants belonging to different plant life forms—annuals, perennials, woody plants, and different importance for people—weeds or agricultural and horticultural crops [14–22]. KARs are a promising new plant growth regulators [23,24], and yet unidentified endogenous molecule perceived through the same signaling pathway is a potential new phytohormone [25].

However, not only germination stimulators, but also germination inhibitors belonging to butenolides are present in smoke [26]. The study of individually derived smoke from 27 different plant species revealed differences in seed germination of Themeda triandra [27]. Quantification of KARs in smoke water using ultrahigh-performance liquid chromatography-tandem mass spectrometry revealed differences in both total and relative levels of KAR [28]. The smoke water prepared by different protocols needs to be dissolved in different ratios due to the variable content of germination stimulators and inhibitors [29]. Therefore, utilization of KARs rather than aerosol smoke or smoke-water is convenient for research and agriculture, despite the lower price of aerosol smoke and smoke-water compared to KARs [30].

Another possible means of KARs application to agriculturally utilized soils is indirectly through biochar, where KARs have been identified just recently [31]. Biochar has several other advantages in addition to KARs content. It improves nutrient sorption and water holding capacity of the soil. Carbon sequestration is another nonnegligible property of biochar [32]. Nevertheless, KARs content of biochar, like in case of smoke, is dependent on charred material and the used technology of pyrolysis. The individual plant species response relates to its sensitivity to KARs and inhibitory compounds within the biochar [31].

In the current review, up-to-date known effects of KARs on Arabidopsis thaliana in different stages of ontogenesis are summarized and compared with plants of agricultural and horticultural importance.

2. Chemical Properties of Karrikins

The structure of KARs is very similar to phytohormones strigolactones (SLs). Butenolide ring of KARs and lactone D ring of SLs are closely related [33]. The KARs structure combines a six-membered pyran ring with a five-membered butenolide ring. The differences between the six known KARs are based on methyl substitutions (Figure 1). These KARs are described as KAR1 to KAR6 [34]. Only C, H, and O are present in the two-ring structures of KARs. The pure KARs have a melting point of 118–119 °C, and they are the substances of crystalline character. KARs can be quickly dissolved in organic solvents and mildly in water [35].

KARs relate to SLs because they share a specific type of lactone known as a butenolide fused to a pyran ring with the systematic name 3-methyl-2H-furo[2,3-c]pyran-2-one [35]. The plant-made signaling compounds SLs are synthesized from carotenoids. To date, the structure of at least 20 different naturally occurring SLs has been characterized [36]. In contrast, KAR molecules are not produced by the plant itself but are formed by heating or combustion of carbohydrates, such as cellulose [37]. Various SL analogs abbreviated as GR have been synthesized, of which GR24 is the most active and widely used in SL research [38,39].

Even though just six KARs so far showed physiological activity in plants, almost 50 analogs of KARs with different substitutions have been synthesized [40–42].
3. Perception of Karrikins by Plants

Although KARs were discovered 15 years ago, the exact mechanism of perception remains a mystery. However, it does not mean that we do not have any clue about signaling cascade, which begins by the sensing of KARs and ends by morphological and physiological responses. Genetic studies indicated that KARs are perceived by the KARRIKIN INSENSITIVE 2 (KAI2) receptor. KAI2 interacts with MORE AXILLARY GROWTH2 (MAX2), which leads to complex degrading SUPPRESSOR OF MAX2 1 (SMAX1) and SMAX1-LIKE2 (SMXL2). These reveal transcription factors from suppression and response to KARs occurs (Figure 2) [43–46].

The analyses of crystallography and ligand-binding experiments of KARs recognition by KAI2 revealed that KARs is capsulized through geometrically defined aromatic-aromatic interactions. KARs attachment induces the changes in KAI2 conformation at the active site entrance. The KARs ligand is located marginally at an active site distal from the catalytic triad (Ser95-His246-Asp217). Such location is consistent with the lack of detectable hydrolytic activity by purified KAI2 [45]. Just a single nucleotide mutation on KAI2 can considerably reduce the KAR-binding activity of KAI2. Mutation of codon 219 causing a change from alanine to valin alters biochemical features of KAI2 and makes a plant severely or completely insensitive to KARs [47]. The KAI2 receptor protein is lost or degraded by a mechanism requiring a yet unidentified cell compartment, but it is independent of 26S proteasome or MAX2. Such loss is probably through enzymatic degradation, and it is rather the result of signaling than its cause [48].

F-box protein MAX2 has a shared role in KARs and SLs signaling, but plants can recognize SLs from KARs and react accordingly [43]. The SLs are synthesized from carotenoids and perceived via the α/β hydrolase DWARF 14 (D14) and the F-box protein MAX2 [33,49,50]. In contrast, KAR molecules are not produced by the plant itself but are formed by heating or combustion of carbohydrates, such as cellulose [37], and there is strong evidence that the MAX2-KAI2 protein complex might also recognize so far unknown plant-made KAR-like molecules [42]. Receptor KAI2 is important for cotyledon expansion, shortening petioles, and leaves to achieve wild type size, anthocyanins, and chlorophylls’ accumulation and enhanced expression of CHLOROPHYLL A/B.
BINDING PROTEIN 3 and CHALCONE SYNTHASE, which are light-responsive genes [51]. The proposed endogenous KAI2 ligand (KL) is not produced by the known SL biosynthesis pathway via carlactone [52].

Recent investigations of host perception in parasitic plants have demonstrated that SL recognition could evolve following gene duplication of KAI2. There are striking parallels in the signaling mechanisms of KARs, SLs, and other plant hormones, including auxins, jasmonates, and gibberellins (GAs) [24].

**Figure 2.** Model of signaling and effect of karrikins (KARs) and strigolactones (SLs) on Arabidopsis thaliana in different stages of ontogenesis. KARs produced by the burning of plant material and yet unidentified KAI2 ligand (KL) are perceived by the KAI2 receptor, which by interaction with F-box protein MAX2, causes degradation of SMAX1 and SMXL2. SMAX1 represses seed germination, SMAX1 and/or SMXL2 repress cotyledon expansion, root straightness, root width, and root hair development, and promote lateral root development and root skewness and hypocotyl elongation by reduction of seedling light sensitivity; SMAX1 promotes expansion of rosette leaves blade under long-day conditions (*LD), SLs are perceived by receptor protein D14, which interacts with MAX2 and causes degradation of SMXL6,7,8. SMXL6,7,8 promotes cotyledon expansion, branching, and lateral root development; SMXL6,7,8 represses petiole and leaf blade elongation under long-day conditions (*LD) [46,53–56].

**4. Effect of Karrikins on Arabidopsis**

*Arabidopsis thaliana* (L.) has great value as a model plant with the sequenced genome [57] for studying all aspects of flowering plant life with a number of advantages [58]. It was an important finding that *Arabidopsis* is a KAR-sensitive plant, despite it not being a fire-following species [53].

The primary dormancy of *Arabidopsis* seeds can be overcome by KARs as it perceives KARs quickly and sensitively. KARs are an effective stimulator of seed germination, but they do not overcome the requirement for synthesis or perception of GAs. Amounts of GAs and abscisic acid (ABA) in seeds of *Arabidopsis* do not get changed in response to KARs during pre-germination [53]. KARs is the most effective KAR in germination stimulation and inhibition of hypocotyl elongation of *Arabidopsis* [53,54].
Inhibition of hypocotyl elongation and cotyledon expansion are light-dependent responses to KAR treatment. Under continuous red light, the KARs were observed to positively influence the accumulation of chlorophyll a and b in Arabidopsis thaliana [53]. KARs alone regulate germination and hypocotyl elongation of plants, whereas KARs together with SLs help in the regulation of leaf morphology in Arabidopsis. SLs repress branching and lower auxin transport [55].

Some of the root architecture features, which had been previously credited to SLs are actually regulated by KARs or by the interaction of SLs and KARs. KARs are responsible for hair root development, the direction of root growth, root diameter, and root waving. KARs and SLs together influence the density of lateral roots [56]. Previous confusion in the role of SLs was caused by the use of GR24 as a racemic mixture like an SL analog to study changes in plant development. This mixture is at the same time a potent activator of the SL signaling pathway due to the presence of natural stereoisomer GR24\textsuperscript{cis} and the KAR signaling pathway by the non-natural stereoisomer GR24\textsuperscript{ent} [48, 59].

5. Effects of Karikins on the Crops’ Growth and Development

Experiments of the KAR treatment effect has been done not only with model plants, but also with several crops as presented in Table 1. These studies are more valuable from a practical point of view as they provide cues about the advantages of KAR treatment for sustainable food production.

KARs stimulates germination of Arabidopsis seeds under favorable conditions, but it can inhibit germination in the presence of osmolytes or at elevated temperature. KAI2 signaling may inhibit germination under unfavorable conditions as protection against abiotic stress [60]. However, germination and seedling growth of tef, an African cereal crop, under high temperature, and low osmotic potential were observed to be enhanced by KAR treatment [61]. The enhanced germination and improved tomato seedling development in temperature extremes connected with KAR utilization were also reported [62]. These facts show that the reactions of a model plant and crops can be different.

The level of ABA in imibed seeds of Arabidopsis was not affected by KARs treatment [53]. That was not a case of Avena fatua kernels treated by KAR, which showed a one-third decrease in the level of ABA after 16 hours of imbibition. A similar result was recorded for GA treatment. The promotion of germination in Avena fatua can be related to an increase in reactive oxygen species concentration, which may be a result of lower catalase and superoxide dismutase activity in the aleurone layer [63]. However, the endosperm of maize and cotyledons of bean showed higher antioxidation activity from the third day on, although antioxidant enzymes activity of roots, mesocotyl, and coleoptile of maize or embryo and shoot of the bean was either without change or lower. The improved seedling growth may be due to the movement of starch from storage parts of seeds to growing parts, and the increased activity of amylase in roots and aboveground parts [64]. Another study with different results than above-presented reports delayed germination of soybean after KAR treatment through enhancement of ABA biosynthesis and GA biosynthesis impairment [65]. This all shows the need for a study using one protocol to examine changes in germinating seeds of different species. In the absence of such studies, it is impossible to draw conclusions about the effect of KARs on biochemical changes during seed imbibition and germination of various species.

No significant influence of KARs has been reported on the primary root length of Arabidopsis [56], whereas a positive effect of KAR treatment on rice, tomato, okra, bean, maize, and carrot root was reported (Table 1). Not only the root length enhancement of rice was observed, but also the increased number of lateral roots was found [66]. This is the opposite of effect on Arabidopsis, where KARs repress lateral root development [56]. Thus, the effect of KARs on the root architecture of monocotyledonous and dicotyledonous plants may differ significantly.

DWARF14LIKE, which is an Arabidopsis KAI2 analog in rice, is necessary for the initiation of colonization events by arbuscular mycorrhizal fungi, but KARs was not effective in colonization enhancement of wild-type roots by arbuscular mycorrhizal fungi [67]. Whether other KARs play some role in plant-fungi symbiosis or what another signal is perceived by KAI2 is for now unclear.
Arabidopsis seedlings react more sensitively to light after the treatment by KARs, which results in shorter hypocotyl [54]. Interestingly, the majority of studied crops reacted by increased seedling height (Table 1). This seemingly opposite reaction can be explained as a response of seedlings under KAR treatment by the most convenient growth [54]. It is known that KARs are involved in the regulation of auxins biosynthesis [49,68,69]. Therefore, variability in growth may be caused by the different effects of KARs on auxins level in plants of different species.

Both kai2 and max2 Arabidopsis mutants exhibit drought sensitivity. Max2 and kai2 mutants have larger stomatal aperture due to ABA-hyposensitivity, and both mutants also have a thinner cuticle. These result in higher water loss during dry periods. The rate of chlorophyll leakage in max2 and kai2 was observed to be higher than in wild type plants, suggesting that the evaporation through the cuticle of mutants is faster [70,71]. KAR improved the seedling performance of tomato and tef grown in lowered osmotic potential conditions [61,72]. These indicate the potential of KARs treatment for mitigation of drought stress effect on crops.

KARs stimulate chlorophyll concentration in Arabidopsis, tef, and carrot [21,54,73]. KARs not only influence the chlorophyll content, but also enhance net photosynthesis rate, probably as a result of increased stomatal conductance and higher intercellular CO₂ concentration, which was found in KAR-treated carrot plants [73]. However, foliar application of KARs on amaranth caused a reduction in chlorophylls content [74]. The mechanism behind the KARs influence on chlorophyll concentration and photosynthesis is, for now, unknown, but the method of application may be decisive.

KAR signaling can also influence secondary metabolism. Kai2 mutant of Arabidopsis has lower anthocyanin content as a result of transcription misregulation of the anthocyanin biosynthesis pathway [71]. Ascorbic acid and β-carotene content were increased in carrot roots grown from KAR-primed seeds [73]. The content of tashinone I, pharmacologically active terpenoid, was significantly increased in hairy roots of Salvia miltiorrhiza by a signaling pathway involving nitric oxide and jasmonic acid [75].

Even though KARs improved plant height, weight, stem thickness, and the number of leaves of tomato, it did not increase the yield of fruits. However, fruits were observed to appear earlier on KAR-treated plants than on the control plants, which can be advantageous for seasonal growers [76]. Similarly, grain yield of tef was not significantly improved, but stem thickness and plant height increased, which indicates the potential of higher hay yield interesting for animal farms [21]. Experiments with carrots indicate the considerable potential of KARs utilization for root yield quantity and quality enhancement. The carrot roots grown from KARs presoaked seeds were bigger, heavier, and contained more pigments than control plants [73].

KARs were tested for genotoxicity and mutagenicity on Salmonella typhimurium [77], in Vicia faba and Persea Americana metabolic activated Ames assay [78] and in juice from KARs treated onion by Ames assay [79]. The results of all tests do not show any genotoxicity nor mutagenicity. Therefore, KARs can be considered as safe for use in agriculture and horticulture.

Utilization of KARs in dose 2–20 g ha⁻¹ as weed control measure was proposed for agriculture [16]. Such use of KARs seems to be highly improbable as the cost of KARs would have to decrease thousands fold to reach an affordable level, and, even then, economic benefit for farmers would be questionable. More likely, KARs can be used as a priming agent for seeds of agricultural and horticultural crops in order to enhance germination and early seedling growth to establish a steady field under conditions of climate change. Priming of seeds is an efficient mean of application, and the positive effect of KARs on the vigor of plants grown from primed seeds endures for at least three months [72]. However, more studies are needed, which should be performed not only in the laboratory but mainly in field conditions, before agricultural practice accept such utilization as beneficial.
Table 1. Effects of karrikins (KARs) on the agricultural and horticultural crops growth, development and photosynthetic properties.

| Plant                     | Conc. [M] | Means of Application                        | Examined Features                                      | Effect of KAR | Ref.  |
|--------------------------|-----------|---------------------------------------------|-------------------------------------------------------|---------------|-------|
| Rice (Oryza sativa L.)   | $10^{-10}$−10$^{-8}$ | grown in Petri dishes with KARs solution | seedling weight, vigor index                            | +             | [66]  |
|                          |           |                                             | root and shoot length, no. of lateral roots            | +             |       |
| Tomato (Lycopersicon esculentum Mill.) | $10^{-7}$ | grown in Petri dishes with KARs solution | germination                                             | 0             |       |
|                          |           |                                             | % of abnormal seedlings                                | −             |       |
|                        |           |                                             | vigour index, seedling weight                           | +             |       |
|                        |           |                                             | hypocotyl and radicle length                           | +             | [80]  |
|                        |           |                                             | weight of 10 embryonic axis                            | +             |       |
| Tomato (Lycopersicon esculentum Mill.), Okra (Abelmoschus esculentus L.), Bean (Phaseolus vulgaris L.) and Maize (Zea mays L.) | $10^{-7}$ | with KARs solution, maize kernels for growth experiment were presoaked in KARs solution for 1 h | germination experiment: root and shoot length | + |       |
|                        |           |                                             | seedling weight of tomato, okra and maize              | +             |       |
|                        |           |                                             | seedling weight of bean                                 | 0             |       |
|                          |           |                                             | vigour index                                             | +             |       |
| Tomato (Lycopersicon esculentum Mill.) | $10^{-7}$ | grown in Petri dishes with KARs solution in different temperatures | shoot and root length                                  | 0             |       |
|                          |           |                                             | shoot fresh and dry weight of okra                      | 0             |       |
|                        |           |                                             | root dry weight of okra                                 | −             |       |
| Tomato (Lycopersicon esculentum Mill.), Okra (Abelmoschus esculentus L.) | $10^{-7}$ | spraying by KARs solution to the point of runoff in four days intervals from eight day after seed sowing | shoot and root fresh weight of tomato                  | +             |       |
|                          |           |                                             | shoot and root dry weight of tomato                     | 0             | [81]  |
|                          |           |                                             | no. of leaves and total leaf area of okra               | +             |       |
|                          |           |                                             | stem thickness                                           | 0             |       |
|                          |           |                                             | seedling vigour and absolute growth rate                | 0             |       |
| Tomato (Lycopersicon esculentum Mill.) | $10^{-7}$ | seeds were primed in KARs solution for 24 h, blotted dry and grown in different temperatures or salt concentrations or osmotic potentials | vigor index (salt concentration = 0, 100, 125, 150 mM) | + | [72]  |
|                          |           |                                             | vigor index (%Ψ = 0, −0.05, −0.15, −0.30, −0.49 MPa) | +             |       |
|                          |           |                                             | vigor index (t = 10, 15, 20, 25, 30, 35 °C)            | +             |       |
| Tef (Eragrostis tef Zucc.) | $10^{-8}$ | imbibed or grown in Petri dishes with KARs solution in different temperatures or osmotic potentials | imbibition (%Ψ = 0, −0.5 MPa)                          | 0             |       |
|                          |           |                                             | imbibition (%Ψ = −0.15, −0.30, −0.49 MPa)              | +             |       |
|                          |           |                                             | germination                                              | 0             | [61]  |
| Tomato (Lycopersicon esculentum Mill.) | $10^{-9}$ | irrigation by KARs solution twice a week | seedling length (t = 20 °C)                            | 0             |       |
|                          |           |                                             | seedling length (t = 25, 30, 35, 40, 30/15 °C)         | +             |       |
|                          |           |                                             | seedling length (%Ψ = 0, −0.5, −0.15, −0.30 MPa)       | 0             |       |
|                          |           |                                             | seedling length (%Ψ = −0.49 MPa)                        | +             |       |
| Onion (Allium cepa L.)    | $10^{-30}$ | grown in pots drenched by KARs solution twice a week | plant height, plant weight                             | +             |       |
|                          |           |                                             | no. of leaves, stem thickness                           | +             |       |
|                          |           |                                             | fruit appearance (days)                                 | −             | [76]  |
|                          |           |                                             | harvest index                                            | +             |       |
|                          |           |                                             | ascorbic acid, β-carotene and lycopene content          | 0             |       |

Effect of KAR: + indicates enhancement, − indicates suppression.
6. Conclusions

KARs are relatively simple molecules affecting several physiological and morphological features of different species. Their structure and signaling pathway are like plant hormones SLS. Finding that Arabidopsis is one of the KAR-responsive species enabled to study signaling cascade of KAR perception. Analysis of mutants shows that receptor KAI2 in complex with F-box protein MAX2 can degrade repressors SMAX1 and SMXL2, which release the number of genes from repression. That stimulates germination and cause morphological responses of aboveground and belowground organs. KARs can also stimulate the germination of several crops under optimal and suboptimal
conditions. Responses of the model plant, *Arabidopsis*, and agricultural and horticultural crops are not always the same. Therefore, more studies on crops, mainly in field conditions, are needed to discover possible benefits of KARs use in the challenged nowadays agriculture.

**Author Contributions:** M.A., O.S., and M.B. wrote the manuscript. M.A. and O.S. prepared illustrations, figures, tables, and references. M.A., A.R., and O.S. conceptualized the overall structure; M.B. and A.R. contributed critical comments to the draft and approved the manuscript. All of the authors have read the proof version and agreed the version to be published.

**Funding:** This research was funded by the grants VEGA 1/0589/19.

**Conflicts of Interest:** The authors declare no conflict of interest.

**References**

1. IPCC. Summary for Policymakers. In *Climate Change and Land*; An IPCC Special Report on Climate Change, Desertification, Land Degradation, Sustainable Land Management, Food Security, and Greenhouse Gas Fluxes in Terrestrial Ecosystems; IPCC: Geneva, Switzerland, 2019. Available online: https://www.ipcc.ch/scl/ (accessed on 18 November 2019).
2. Jain, H.K. *The Green Revolution: History, Impact and Future*; Studium Press LLC: Houston, TX, USA, 2010. ISBN 1-933699-63-9.
3. Tripathi, A.K.; Roberts, C.D.; Eagle, R.A. Coupling of CO2 and Ice Sheet Stability Over Major Climate Transitions of the Last 20 Million Years. *Science* 2009, 326, 1394–1397.
4. Blunden, J.; Arndt, D.S. State of the Climate in 2018. *Bull. Am. Meteorol. Soc.* 2019, 100, 9.
5. Jolly, W.M.; Cochrane, M.A.; Freeborn, P.H.; Holden, Z.A.; Brown, T.J.; Williamson, G.J.; Bowman, D.M.J.S. Climate-induced variations in global wildfire danger from 1979 to 2013. *Nat. Commun.* 2015, 6, 7537.
6. Wicklow, D.T. Germination Response in *Emmenanthe Penduliflora* (Hydrophyllaceae). *Ecology* 1977, 58, 201–205.
7. Flematti, G.R.; Ghisalberti, E.L.; Dixon, K.W.; Trengove, R.D. A Compound from Smoke That Promotes Seed Germination. *Science* 2004, 305, 977–977.
8. van Staden, J.; Jäger, A.K.; Light, M.E.; Burger, B.V. Isolation of the major germination cue from plant-derived smoke. *S. Afr. J. Bot.* 2004, 70, 654–659.
9. Flematti, G.R.; Ghisalberti, E.L.; Dixon, K.W.; Trengove, R.D. Identification of Alkyl Substituted 2H-Furo[2,3-c]pyran-2-ones as Germination Stimulants Present in Smoke. *J. Agric. Food Chem.* 2009, 57, 9475–9480.
10. Dixon, K.W.; Merritt, D.J.; Flematti, G.R. Karrikinolide—A Phytoreactive Compound Derived from Smoke with Applications in Horticulture, Ecological Restoration and Agriculture. *Acta Hortic.* 2009, 813, 155–170.
11. Flematti, G.R.; Merritt, D.J.; Piggott, M.J.; Trengove, R.D.; Smith, S.M.; Dixon, K.W.; Ghisalberti, E.L. Burning vegetation produces cyanohydrins that liberate cyanide and stimulate seed germination. *Nat. Commun.* 2011, 2, 360.
12. Sweedman, L.; Merritt, D. *Australian Seeds: A Guide to Their Collection, Identification and Biology*; CSIRO PUBLISHING: Clayton, Australia, 2006; pp. 199–219.
13. Douglas, R.B.; Rothfels, C.L.; Stevenson, D.W.D.; Graham, W.S.; Wong, C.K.-S.; Nelson, D.C.; Bennett, T. Evolution of strigolactone receptors by gradual neo-functionalization of KAI2 paralogues. *BMC Biol.* 2017, 15, 52.
14. Daws, M.I.; Davies, J.; Pritchard, H.W.; Brown, N.A.C.; van Staden, J. Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. *Plant Growth Regul.* 2007, 51, 73–82.
15. Kulkarni, M.G.; Sparg, S.G.; van Staden, J. Germination and post-germination response of Acacia seeds to smoke-water and butenolide a smoke-derived compound. *J. Arid Environ.* 2007, 69, 177–178.
16. Stevens, J.C.; Merritt, D.J.; Flematti, G.R.; Ghisalberti, E.L.; Dixon, K.W. Seed germination of agricultural weeds is promoted by the butenolide 3-methyl-2H-furo[2,3-c]pyran-2-one under laboratory and field conditions. *Plant Soil* 2007, 298, 113–124.
17. Long, R.L.; Stevens, J.C.; Griffiths, E.M.; Adamek, M.; Gorecki, M.J.; Powles, S.B.; Merritt, D.J. Seeds of *Brassicaceae* weeds have an inherent or inducible response to the germination stimulant karrikinolide. *Ann. Bot.* 2011, 108, 933–944.
18. Kulkarni, M.G.; Ghebrehiwot, H.M.; Kirkman, K.P.; van Staden, J. Response of Grass Seedlings to Smoke-Water and Smoke-Derived Butenolide in the Absence of Macronutrients (Nitrogen, Phosphorus, and Potassium). *Rangel. Ecol. Manag.* 2012, 65, 31–38.

19. Catav, S.S.; Kucukakayuz, K.; Tavsanoglu, C.; Pausas, J.G. Effect of fire-derived chemicals on germination and seedling growth in Mediterranean plant species. *Basic Appl. Ecol.* 2018, 30, 65–75.

20. van Staden, J.; Sparg, G.G.; Kulkarni, M.G.; Light, M.E. Post-germination effects of the smoke-derived compound3-methyl-2H-furo[2,3-c]pyran-2-one, and its potential as a preconditioning agent. *Field Crops Res.* 2006, 98, 98–105.

21. Ghebrehiwot, H.; Kulkarni, M.G.; Bairu, M.; van Staden, J. Plant-derived aerosol-smoke and smoke solutions influence agronomic performance of traditional cereal crop, *Exp. Agric.* 2013, 49, 244–255.

22. Demir, I.; Ozden, E.; Yildirim, K.C.; Sahin, O.; van Staden, J. Priming with smoke-derived karrikinolide enhances germination and transplant quality of immature and mature pepper seed lots. *S. Afr. J. Bot.* 2017, 115, 264–268.

23. Scaffidi, A.; Waters, M.T.; Bond, C.S.; Dixon, K.W.; Smith, S.M.; Ghisalberti, E.L.; Flematti, G.R. Exploring the molecular mechanism of karrikins and strigolactones. *Bioorg. Med. Chem. Lett.* 2012, 22, 3743–3746.

24. Morfły, N.; Faure, L.; Nelson, D.C. Smoke and Hormone Mirrors: Action and Evolution of Karrikin and Strigolactone Signaling. *Trends Genet.* 2016, 32, 176–188.

25. Conn, C.E.; Nelson, D.C. Evidence that Karrikin–Insensitive2 (KA12) Receptors may Perceive an Unknown Signal that is not Karrikin or Strigolactone. *Front. Plant Sci.* 2016, 6, 1219.

26. Burger, B.V.; Pošta, M.; Light, M.E.; Kulkarni, M.G.; Vivia, M.Z.; van Staden, J. More butenolides from plant-derived smoke with germination inhibitory activity against karrikinolide. *S. Afr. J. Bot.* 2018, 115, 256–263.

27. Baxter, B.J.M.; Granger, J.E.; van Staden, J. Plant-derived smoke and seed germination: Is all smoke good smoke? That is the burning question. *S. Afr. J. Bot.* 1995, 61, 275–277.

28. Hrdlička, J.; Gucký, T.; Novák, O.; Kulkarni, M.G.; Gupta, S.; van Staden, J.; Doležal, K. Quantification of karrikins in smoke water using ultra-high performance liquid chromatography-tandem mass spectrometry. *Plant Methods* 2019, 15, 81.

29. Gupta, S.; Hrdlička, J.; Ngoroyemoto, N.; Nemahunguni, N.K.; Gucký, T.; Novák, O.; Kulkarni, M.G.; Doležal, K.; van Staden, J. Preparation and Standardisation of Smoke-Water for Seed Germination and Plant Growth Stimulation. *J. Plant Growth Regul.* 2019, 1–8. doi:10.1007/s00344-019-09985-y.

30. Nelson, D.C.; Flematti, G.R.; Ghisalberti, E.L.; Dixon, K.W.; Smith, S.M. Regulation of Seed Germination and Seedling Growth by Chemical Signals from Burning Vegetation. *Ann. Rev. Plant Biol.* 2012, 63, 107–130.

31. Kochanek, J.; Long, R.L.; Lisle, A.T.; Flematti, G.R. Karrikins Identified in Biochars Indicate Post-Fire Chemical Cues Can Influence Community Diversity and Plant Development. *PLoS ONE* 2016, 11, e0161234.

32. Mona, S.; Rachna, B.; Deepak, B.; Bala, K.; Nisha, R. Biochar for Reclamation of Saline Soils. In *Microorganisms in Saline Environments: Strategies and Functions*; Giri, B., Varma, A., Eds.; Springer: Cham, Germany, 2019; pp. 451–466.

33. Waters, M.T.; Brewer, P.B.; Bussell, J.D.; Smith, S.M.; Beveridge, C.A. The Arabidopsis Ortholog of Rice DWARF27 Acts Upstream of MAX1 in the Control of Plant Development by Strigolactones. *Plant Physiol.* 2012, 159, 1073–1085.

34. Waters, M.T.; Scaffidi, A.; Sun, Y.K.; Flematti, G.R.; Smith, S.M. The karrikin response system of Arabidopsis. *Plant J.* 2014, 79, 623–31.

35. Flematti, G.; Dixon, K.; Smith, S.M. What are karrikins and how were they ‘discovered’ by plants? *BMC Biol.* 2015, 13, 108.

36. Tokunaga, T.; Hayashi, H.; Akiyama, K. Medicaol, a strigolactone identified as a putative didehydro-orobanchol isomer from *Medicago truncatula*. *Phytochemistry* 2015, 111, 91–97.

37. Flematti, G.R.; Scaffidi, A.; Dixon, K.W.; Smith, S.M.; Ghisalberti, E.L. Production of the Seed Germination Stimulant Karrikinolide from Combustion of Simple Carbohydrates. *J. Agric. Food Chem.* 2011, 59, 1195–1198.

38. Zwanenburg, B.; Pospíšil, T. Structure and Activity of Strigolactones: New Plant Hormones with a Rich Future. *Mol. Plant* 2013, 6, 38–62.

39. Besserer, A.; Bécard, G.; Jauneau, A.; Roux, C.; Séjalon-Delmas, N. GR24, a Synthetic Analog of Strigolactones, Stimulates the Mitosis and Growth of the Arbuscular Mycorrhizal Fungus *Gigaspora rosea* by Boosting Its Energy Metabolism. *Plant Physiol.* 2008, 148, 402–413.
40. Flematti, G.R.; Goddard-Borger, E.D.; Merritt, D.J.; Ghisalberti, E.L.; Dixon, K.W.; Trengove, R.D. Preparation of 2H-Furo[2,3-
    c]pyran-2-one Derivatives and Evaluation of Their Germination-Promoting Activity. J. Agric. Food Chem. 2007, 55, 2189–2194.
41. Goddard-Borger, E.D.; Ghisalberti, E.L.; Stick, R.V. 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.
42. De Cuypere, C.; Struk, S.; Braem, L.; Gevaert, K.; De Jaeger, G.; Goormachtig, S. Strigolactones, karrikins and beyond. Plant Cell Environ. 2017, 40, 1691–1703.
43. Nelson, D.C.; Scaffidi, A.; Dun, E.A.; Waters, M.T.; Flematti, G.R.; Dixon, K.W.; Beveridge, C.A.; Ghisalberti, E.L.; Smith, S.M. F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in Arabidopsis thaliana. PNAS 2011, 108, 8897–8902.
44. Stanga, J.P.; Smith, S.M.; Briggs, W.R.; Nelson, D.C. SUPPRESSOR OF MORE AXILLARY GROWTH2-1 Controls Seed Germination and Seedling Development in Arabidopsis. Plant Physiol. 2013, 163, 318–330.
45. Guo, Y.; Zheng, Z.; La Clair, J.J.; Chory, J.; Noel, J.P. Smoke-derived karrikin perception by the α/β-
    hydrolase KAI2 from Arabidopsis. Proc. Natl. Acad. Sci. USA 2013, 110, 8284–8289.
46. Stanga, J.P.; Morfý, N.; Nelson, D.C. Functional redundancy in the control of seedling growth by the
    karrikin signaling pathway. Planta 2016, 243, 1397–1406.
47. Lee, I.; Kim, K.; Lee, S.; Lee, S.; Hwang, E.; Shin, K.; Kim, D.; Choi, J.; Choi, H.; Cha, J.S.; et al. A missense
    allele of KARRIKIN-INSUSITIVE2 impairs ligand-binding and downstream signaling in Arabidopsis thaliana. J. Exp. Bot. 2018, 69, 3609–3623.
48. Waters, M.T.; Scaffidi, A.; Flematti, G.R.; Smith, S.M. Substrate-Induced Degradation of the α/β-Fold
    Hydrolase KARRIKIN INSENSITIVE2 Requires a Functional Catalytic Triad but Is Independent of MAX2. Mol. Plant 2015, 8, 814–817.
49. Waters, M.T.; Scaffidi, A.; Flematti, G.R.; Smith, S.M. The origins and mechanisms of karrikin signalling.
    Curr. Opin. Plant Biol. 2013, 16, 667–73.
50. Smith, S.M.; Li, J. Signalling and responses to strigolactones and karrikins. Curr. Opin. Plant Biol. 2014, 21,
    23–29.
51. Sun, X.D.; Ni, M. Hyposensitive to Light, an Alpha/Beta Fold Protein, Acts Downstream of Elongated
    Hypocotyl5 to Regulate Seedling De-Etiolation. Mol. Plant 2011, 4, 116–126.
52. Waters, M.T. Strigolactones and Karrikins. In Encyclopedia of Applied Plant Sciences, 2nd ed.; Thomas, B.,
    Murray, B.G., Murphy, D.J., Eds.; Academic Press: Cambridge, MA, USA, 2017; Volume 3, pp. 466–472.
53. Nelson, D.C.; Risenborough, J.-A.; Flematti, G.R.; Stevens, J.; Ghisalberti, E.L.; Dixon, K.W.; Smith, S.M.
    Karrikins Discovered in Smoke Trigger Arabidopsis Seed Germination by a Mechanism Requiring
    Gibberellic Acid Synthesis and Light. Plant Physiol. 2009, 149, 863–873.
54. Nelson, D.C.; Flematti, G.R.; Risenborough, J.A.; Ghisalberti, E.L.; Dixon, K.W.; Smith, S.M. Karrikins
    enhance light responses during germination and seedling development in Arabidopsis thaliana. Proc. Natl.
    Acad. Sci. USA 2010, 107, 7095–7100.
55. Soundappan, I.; Bennett, T.; Morfý, N.; Liang, Y.; Stanga, J.P.; Abbas, A.; Leyser, O.; Nelson, D.C. SMAX1-
    LIKE/D53 Family Members Enable Distinct MAX2-Dependent Responses to Strigolactones and Karrikins
    in Arabidopsis. Plant Cell 2015, 27, 3143–3159.
56. Villaècija-Aguilar, J.A.; Hamon-Josse, M.; Carbonnel, S.; Kretschmar, A.; Schmidt, C.; Dawid, C.; Bennett,
    T.; Gutjahr, C. SMAX1/SMAX2 regulate root and root hair development downstream of KAI2-mediated
    signalling in Arabidopsis. PLoS Genet. 2019, 15, e1008327.
57. The Arabidopsis Genome Initiative. Analysis of the genome sequence of the flowering plant Arabidopsis
    thaliana. Nature 2000, 408, 796–815.
58. Koornneef, M.; Meinke, D. The development of Arabidopsis as a model plant. Plant J. 2010, 61, 909–921.
59. Scaffidi, A.; Waters, M.; Sun, Y.K.; Skelton, B.W.; Dixon, K.W.; Ghisalberti, E.L.; Flematti, G.R.; Smith, S.M.
    Strigolactone Hormones and Their Stereoisomers Signal through Two Related Receptor Proteins to Induce
    Different Physiological Responses in Arabidopsis. Plant Physiol. 2014, 165, 1221–1232.
60. Wang, L.; Waters, M.T.; Smith, S.M. Karrikin-KAI2 signalling provides Arabidopsis seeds with tolerance
    to abiotic stress and inhibits germination under conditions unfavourable to seedling establishment. New
    Phytol. 2018, 219, 605–618.
61. Ghebrehiwot, H.; Kulkarni, M.G.; Kirkman, K.P.; van Staden, J. Smoke-Water and a Smoke-Isolated
    Butenolide Improve Germination and Seedling Vigour of Eragrostis tef (Zucc.) Trotter under High
    Temperature and Low Osmotic Potential. J. Agron. Crop Sci. 2008, 194, 270–277.
62. Jain, N.; Kulkarni, M.G.; van Staden, J. A butenolide, isolated from smoke, can overcome the detrimental
    effects of extreme temperatures during tomato seed germination. Plant Growth Regul. 2006, 49, 263–267.
63. Cembrowska-Lech, D.; Koprowski, M.; Kepczynski, J. Germination induction of dormant *Avena fatua* caryopses by KAR1 and GA3 involving the control of reactive oxygen species (H2O2 and O2•-) and enzymatic antioxidants (superoxide dismutase and catalase) both in the embryo and the aleurone layers. *J. Plant Physiol.* 2014, 176, 169–179.

64. Sunmonu, T.O.; Kulkarni, M.G.; van Staden, J. Smoke-water, karrikinolide and gibberellic acid stimulate growth in bean and maize seedlings by efficient starch mobilization and suppression of oxidative stress. *S. Afr. J. Bot.* 2016, 102, 4–11.

65. Meng, Y.; Chen, F.; Shuai, H.; Luo, X.; Ding, J.; Tang, S.; Xu, S.; Liu, J.; Liu, W.; Du, J.; Liu, J.; Yang, F.; Sun, X.; Yong, T.; Wang, X.; Feng, Y.; Shu, K.; Yang, W. Karrikins delay soybean seed germination by mediating abscisic acid and gibberellin biogenesis under shaded conditions. *Sci. Rep.* 2016, 6, 22073.

66. Kulkarni, M.G.; Sparg, S.G.; Light, M.E.; van Staden, J. Stimulation of Rice (*Oryza sativa* L.) Seedling Vigour by Smoke-water and Butenolide. *J. Agron. Crop Sci.* 2006, 192, 395–398.

67. Gujjar, C.; Gobbato, E.; Choi, J.; Riemann, M.; Summers, W.; Carbonnel, S.; Mansfeld, C.; Yang, S.-Y.; Nadal, M.; et al. Rice perception of symbiotic arbuscular mycorrhizal fungi requires the karrikin receptor complex. *Science* 2015, 350, 1521–1524.

68. Banerjee, A.; Tripathi, D.K.; Roychoudhury, A. The Karrikin ‘Calisthenics’: Can Compounds Derived from Smoke Help in Stress Tolerance? *Physiol. Plant.* 2019, 165, 290–302.

69. Meng, Y.; Shuai, H.; Luo, X.; Chen, F.; Zhou, W.; Yang, W.; Shu, K. Karrikins: Regulators Involved in Phytohormone Signaling Networks during Seed Germination and Seedling Development. *Front. Plant Sci.* 2017, 7, 2011.

70. Bu, Q.; Lv, T.; Shen, H.; Luong, P.; Wang, J.; Wang, Z.; Huang, Z.; Xiao, L.; Engineer, C.; Kim, T.H.; et al. Regulation of Drought Tolerance by the F-Box Protein MAX2 in Arabidopsis. *Plant Physiol.* 2014, 164, 424–439.

71. Li, W.; Nguyen, K.H.; Chu, H.D.; Ha, C.V.; Watanabe, Y.; Osakabe, Y.; Leyva-González, M.A.; Sato, M.; Toyooka, K.; Voges, L.; et al. The karrikin receptor KAI2 promotes drought resistance in Arabidopsis thaliana. *PLoS Genet.* 2017, 13, e1007076.

72. Jain, N.; van Staden, J. The potential of the smoke-derived compound 3-methyl-2H-furo[2,3-c]pyran-2-one as a priming agent for tomato seeds. *Seed Sci. Res.* 2007, 17, 175–181.

73. Akeel, A.; Khan, M.M.A.; Jaleel, H.; Uddin, M. Smoke-saturated Water and Karrikinolide Modulate Germination, Growth, Photosynthesis and Nutritional Values of Carrot (*Daucus carota* L.). *J. Plant Growth Regul.* 2019, 38, 1387–1401.

74. Ngoroyemoto, N.; Gupta, S.; Kulkarni, M.G.; Finnie, J.F.; van Staden, J. Effect of organic biostimulants on the growth and biochemical composition of *Amaranthus hybridus* L.. *S. Afr. J. Bot.* 2019, 124, 87–93.

75. Zhou, J.; Xu, Z.-X.; Sun, H.; Guo, L.-P. Smoke-Isolated Karrikins Stimulated Tanshinones Biosynthesis in Salvia miltiorrhiza through Endogenous Nitric Oxide and Jasmonic Acid. *Molecules* 2019, 24, 1229.

76. Kulkarni, M.G.; Ascough, G.D.; van Staden, J. Smoke-water and a Smoke-isolated Butenolide Improve Growth and Yield of Tomatoes under Greenhouse Conditions. *HortTechnology* 2008, 18, 449–454.

77. Verschaeve, L.; Maes, J.; Light, M.E.; van Staden, J. Genetic toxicity testing of 3-methyl-2H-furo[2,3-c]pyran-2-one, an important biologically active compound from plant-derived smoke. *Mutat. Res.* 2006, 611, 89–95.

78. Partoens, M.; Kulkarni, M.G.; Light, M.E.; Chukwuokwu, J.C.; Verschaeve, L.; van Staden, J. Genotoxicity studies on plant growth promoting smoke-water and smoke-derived compounds using *Vicia faba* and *Persea americana* S10 metabolic activation. *S. Afr. J. Bot.* 2017, 115, 269–275.

79. Kulkarni, M.G.; Ascough, G.D.; Verschaeve, L.; Baeten, K.; Arruda, M.P.; van Staden, J. Effect of smoke-water and smoke-isolated butenolide on the growth and genotoxicity of commercial onion. *Sci. Hortic.* 2010, 124, 434–439.

80. Jain, N.; van Staden, J. A smoke-derived butenolide improves early growth of tomato seedlings. *Plant Growth Regul.* 2006, 50, 139–148.

81. Kulkarni, M.G.; Ascough, G.D.; van Staden, J. Effects of Foliar Applications of Smoke-Water and a Smoke-isolated Butenolide on Seedling Growth of Okra and Tomato. *HortScience* 2007, 42, 179–182.

82. Demir, I.; Ozuaydin, I.; Yasar, F.; van Staden, J. Effect of smoke-derived butenolide priming treatment on pepper and salvia seeds in relation to transplant quality and catalase activity. *S. Afr. J. Bot.* 2012, 78, 83–87.