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Karrikins Reduce the Hypocotyl Length of Rapeseed (*Brassica napus napus* L.) under Continuous Red Light †

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† Presented at the 1st International Electronic Conference on Plant Science, 1–15 December 2020; Available online: https://iecps2020.sciforum.net/.

Published: 1 December 2020

Abstract: Karrikins (KARs) are relatively simple molecules originating from the burning of plant material, which have the ability to enhance the germination of many species from fire-prone environment, but also species, which life cycle is not connected with fire, one example being Arabidopsis (*Arabidopsis thaliana* L.). KARs do not only improve germination but also alter several physiological processes and morphological features of plants. One of the changes of morphology observed on Arabidopsis seedlings is the shortening of hypocotyl when grown under continuous red light. So far, six biologically active compounds belonging to the KARs group were identified. Most of the plants show the strongest response to the first identified KAR, KAR_1, but Arabidopsis exhibits the strongest reaction to KAR_2. In our experiment, we focused on seedlings' hypocotyl length of Rapeseed (*Brassica napus napus* L.), an economically important plant from the same family as Arabidopsis, Brassicaceae. Our results show that the hypocotyl of eight days old seedling of Rapeseed grown under continuous red light was significantly shortened by both KARs examined by us, KAR_1 as well as by KAR_2. Therefore, we can conclude that similarly to Arabidopsis, Rapeseed's seedlings possess the light-dependent response of development to KARs, what proves, that these compounds have to date not fully understood roles in plants' life moreover to germination induction of fire following species and it is very unlikely that combustion of plant material is the only source of KARs occurring in nature.

Keywords: karrikins; Rapeseed; hypocotyl length

1. Introduction

Karrikins (KARs) are molecules present in the smoke, which are produced by the burning of plant material during fires [1]. Their chemical structure is relatively simple as they consist of five-membered butanolide and six-membered pyran ring and contain only carbon, hydrogen, and oxygen atoms [2]. The first identified member of the KAR family, which was later named KAR_1, was identified as an effective germination promotor of lettuce (*Lactuca sativa* L.) and smoke-responsive Australian species [3]. Up to date, six biologically active compounds belonging to the group of KARs have been identified [4]. KARs do not improve only the germination rate of species originating from the fire-prone environment, but also species, which life cycle is not connected to fire, one example being model plant Arabidopsis (*Arabidopsis thaliana* L.) [5]. Arabidopsis and its mutants enabled the recognition of other effects of KARs on the plant's physiology and morphology. One of such changes is a light dependent shortening of seedling hypocotyl when grown under continuous red light conditions [6]. Despite the majority of the KAR responsive plants show the strongest reaction to

KAR_1, which is present in the smoke in the highest amount, Arabidopsis reacts the most readily to KAR_2 [5,6].

Rapeseed (*Brassica napus napus* L.) is the second most produced oilseed crop worldwide, which is used for the production of vegetable oil for human consumption as well as for the production of biofuels. Rapeseed and Arabidopsis both belong to the same plant family, *Brassicaceae* [7,8].

Despite the response of Arabidopsis seedlings to KARs is known for years, the response of seedlings' hypocotyl length to KARs under continuous red light was not studied in another species. Therefore, we investigated the effect of two KARs, KAR_1 and KAR_2 on the length of rapeseed seedlings' hypocotyl under continuous red and white light as well as under constant darkness conditions.

2. Methods

2.1. Plant Material

Seeds of rapeseed (*Brassica napus napus* L.) variety LG Architect treated by insecticide Lumiposa were obtained from the local farm Klasy s. r. o. (Kuralany, Slovakia).

KAR_1 and KAR_2 were purchased from OlChemIm s. r. o. (Olomouc, Czech Republic). Stock solutions with the amount of KAR 1 mM were prepared by dissolving in acetone and stored at −20°C until further use. Experimental, 1 μM, solutions were prepared from the stock solutions by dissolving in distilled water on the day of experiment initiation. The control solution was prepared by dissolving the equivalent amount of acetone in distilled water.

Rapeseed seeds were placed in a transparent plastic box with proportions 100 × 90 × 80 mm (length × width × high) designated for in vitro cultivation on four filter papers wetted by 15 mL of either control or experimental (KAR_1 or KAR_2) solution. Six boxes for each treatment (control, KAR_1, and KAR_2) with 20 seeds each were prepared. Boxes were placed for eight days in a growth chamber with a temperature 23 °C and relative air humidity 60%. Two boxes of each treatment were placed in light-proof boxes (darkness), two to white light, and two to red light. Spectra of the light conditions were measured by SpectraPen mini (Photon Systems Instruments s. r. o., Drasov, Czech Republic) spectroradiometer. White light of growth chamber LED tubes was of photon flux density ~100 μmol m^{-2} s^{-1}. The red light was obtained by installing a light filter effectively filtering out all wavelengths under 570 nm with the main peak of the transmitted spectrum at ~610 nm and a secondary peak at ~710 nm. The photon flux density of red light was ~20 μmol m^{-2} s^{-1}.

2.2. Hypocotyl Length Measurement

Fifteen seedlings from each box were after eight days stuck to the transparent tape, covered by another piece of transparent tape, and scanned by an office scanner on black background. Hypocotyl length of 30 seedlings from each treatment and each light condition was measured from pictures by use of software Fiji (LOCI, University of Wisconsin).

2.3. Statistical Analysis

Analysis of variance and Tukey HSD test were performed to establish statistically significant differences between treatments for each light condition. The level of statistical significance was established to $p < 0.05$. Statistical analysis was performed by the use of RStudio version 1.1.456 (RStudio Inc., Boston, MA, USA).

3. Results and Discussion

The growth of hypocotyl to length is a basic skotomorphogenic response that enables young seedlings to push through the soil to get exposure to light [9]. The response of seedling growth to KARs is independent of KARs' effect on seed germination [6] Results of our experiment show that neither KAR_1 nor KAR_2 significantly altered the hypocotyl length of Rapeseed seedling (Figure 1A). These results are the opposite of results obtained earlier in an experiment with Tomato (*Lycopersicon esculentum* Mill.), Okra (*Abelmoschus esculentus* L.), and Bean (*Phaseolus vulgaris* L.), in which longer hypocotyls of crops grown in darkness were found after use of KAR_1 [10]. Our results are in concordance with the results obtained with Arabidopsis seedlings grown in darkness, which revealed no change of hypocotyl length after the use of KAR_1, KAR_2, KAR_3, or KAR_4. However, the same experiment reports hypocotyl elongation inhibition in the case of wild turnip (*Brassica tournefortii* Gouan.) [6]. The exact reason for such different responses of seedling growth in darkness to KARs remains elusive and needs more examination.

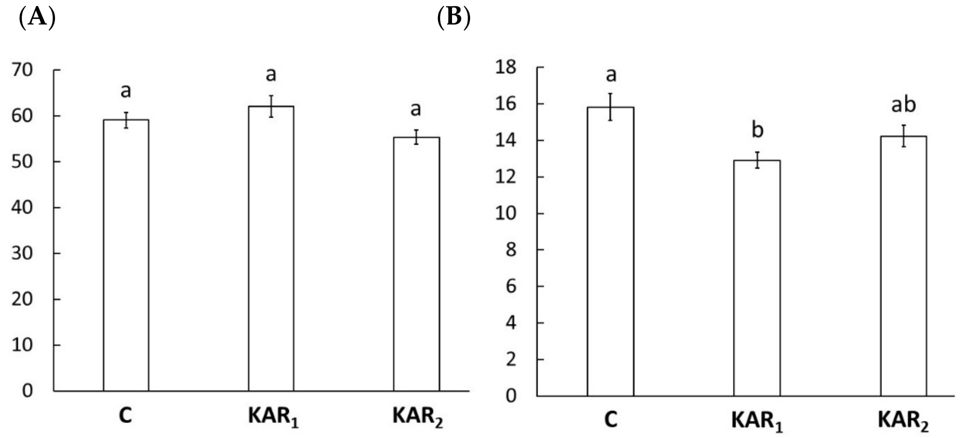

Figure 1. Hypocotyl length [mm] of 8 days old Rapeseed (*Brassica napus napus* L.) seedlings treated by 1 μM $karrikin_1$ (KAR_1), 1 μM $karrikin_2$ (KAR2), or control solution (C) grown under: (**A**). constant darkness; (**B**). continuous white light. Bars represent the mean of 30 seedlings ± standard error. Different letters indicate statistically significant differences at the level $p < 0.05$.

White light is composed of lights of all wavelengths including blue light, which is a strong hypocotyl elongation inhibitor [11]. Hypocotyl of Rapeseed seedlings grown under white light was more than two times shorter than hypocotyl of seedlings grown in red light and almost four times shorter than those grown in darkness (Figures 1 and 2). Regardless inhibitory effect of white light, KAR_1 further significantly hindered the growth of hypocotyl (Figure 1B). However, the effect of KAR_2 on hypocotyl length was insignificant.

It has been shown that seedlings of Arabidopsis grown under continuous red light in the presence of KARs in the medium have shorter hypocotyl than control seedlings. This response was observed for different ecotypes and it is independent of germination stimulation reaction [6]. Our results manifest shorter hypocotyls of seedlings grown under continuous red light in the presence of KAR_1 or KAR_2, which is in accordance with results obtained with the model plant Arabidopsis (Figure 2).

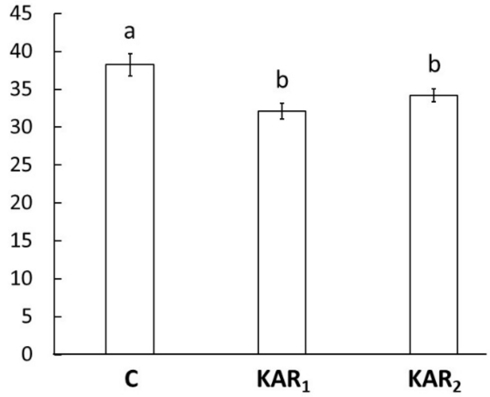

Figure 2. Hypocotyl length [mm] of 8 days old Rapeseed (*Brassica napus napus* L.) seedlings treated by 1 μM karrikin$_1$ (KAR$_1$), 1 μM karrikin$_2$ (KAR$_2$), or control solution (C) grown under continuous red light. Bars represent the mean of 30 seedlings ± standard error. Different letters indicate statistically significant differences at the level $p < 0.05$.

The upregulation of different transcription factors involved in the transduction of signals connected to light conditions was found in Arabidopsis [6]. As we did not perform transcriptional analysis of Rapeseed seedlings, we can just hypothesize that the mechanism of the response of Rapeseed is like Arabidopsis due to their taxonomical relatedness and similar morphology. The exact mechanism needs to be examined by future experiments.

4. Conclusions

Neither of the KARs examined by us had a significant effect on hypocotyl length of Rapeseed seedling grown in constant darkness. Seedling grown under continuous white light in presence of KAR$_1$ had significantly shorter hypocotyls, while KAR$_2$ did not alter the length of the hypocotyl. When grown under continuous red light KAR$_1$ as well as KAR$_2$ caused hypocotyl to be significantly shorter compared to control. Therefore, we can conclude that similarly to Arabidopsis, Rapeseed's seedlings possess the light-dependent response of development to KARs, what proves, that these compounds have to date not fully understood roles in plants' life moreover to germination induction of fire following species and it is very unlikely that combustion of plant material is the only source of KARs occurring in nature. The need for more studies about KARs as plant growth and development regulators as well as searching for KAR-like compounds produced by plants or possibly microorganisms still exists.

Author Contributions: M.A. and M.B. designed the experiment and conceptualized the work. M.A. performed the experiment, analyzed the data, and wrote the paper draft. M.B. supervised the work, reviewed, and improved the draft. Both authors have read and agreed to the final version of the paper.

Funding:

Acknowledgments: This work was financially supported by research project APVV-18-0465 Use of Advanced Phenomic Approaches to Exploit Variation in Photosynthetic Efficiency to Increase Yield under Fluctuating and Stress Environment.

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

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