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Karrikinolide Promotes Seed Germination but has no Effect on Leaf Segment Senescence in *Triticum aestivum* L.

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ABSTRACT

Objective: Germination and senescence are the two most important developmental processes in the plant life cycle. While seed germination is an important physiological event for the continuity of species, leaf senescence is also an important developmental process that impacts crop yields. Karrikins are a group of plant growth regulators found in the smoke generated by burning plant material. It has been suggested that karrikinolide (KAR$_1$) is generally the most active karrikin in terms of stimulating germination.

Materials and Methods: In this study, the effect of karrikinolide on germination and leaf segment senescence in wheat was investigated. For this purpose, control, 1 nM, 0.01, 0.1, 1, and 10 μM KAR$_1$ solutions were used. Firstly, the wheat seeds were germinated in the dark in these solutions and germination percentages and root lengths were measured. Secondly, 4 of first leaf segments (3cm. each) from 10-day-old wheat seedlings were placed in petri dishes containing 1, 10, 100 μM KAR$_1$ and distilled water as a control. Following incubation, fresh weight, chlorophyll content, cell death amounts and total protein amounts were determined.

Results: The obtained data shows that 1 μM KAR$_1$ promotes germination and root length to the greatest extent. This suggests that karrikins have a promoting effect on the germination of wheat seeds. Our results demonstrate that KAR$_1$ has no effect on leaf segment senescence.

Conclusion: Our study suggests that KAR$_1$ has the potential to be used in agriculture to improve germination and seedling growth of crop species.

Keywords: Seed germination, leaf senescence, KAR$_1$, *Triticum aestivum*

INTRODUCTION

Germination and senescence are the two most important developmental events in plant life. While the life cycle of plants begins with seed germination in higher plants, senescence is the last phase of the plant life cycle. Seed germination is an important physiological event for the continuity of species. Internal and external conditions must be suitable for seed germination. Germination is controlled by external environmental factors and internal factors such as plant growth regulators (1,2). Gibberellic acid (GA) and abscisic acid (ABA) are well-known plant hormones that have an important role in the regulation of dormancy and germination (3-5). In addition, plant-derived smoke has been shown to promote the germination of numerous plant species with different life histories (6-8). These findings led to the discovery of compounds found in smoke (e.g. karrikins and glyceronitrile) that are responsible for the stimulation of germination (6,9). Researchers have found that karrikinolide (KAR$_1$) is the most abundant and active karrikin in smoke and that enhances germination and seedling growth in many plants even at very low concentrations (7,8,10-12).

Leaf senescence is an important developmental stage affected by various internal and external factors, such as leaf age, hormone levels, exposure to darkness, and environmental stresses (13,14). Hormones are internal components that mediate the regulatory effects of environmental factors on leaf senescence. While some hormones such as ethylene, ABA, jasmonic acid (JA) and salicylic acid (SA) stimulate senescence, cytokinins and gibberellins play an important role in delaying senescence (15-19). In addition to these hormones, strigolactones (SLs) appear to be a class of plant hormones that regulate leaf senescence, because SL-deficient and SL-insensitive mutants show a phenotype with delayed leaf senescence (14). Karrikins and strigolactones have a butenolide ring in their structure. Even though both molecules have highly similar signaling mechanisms, it has been suggested that they have different effects on plant growth (20).

In literature, there has been no study showing the effect of karrikins on senescence so far. Our study is thus the first attempt to show the effect of KAR_1 on leaf segment senescence in wheat. For this reason, the aims of the current study were to investigate the effects of KAR_1 on seed germination and leaf segment senescence in wheat.

MATERIALS AND METHODS

Plant Material, Growth Conditions & Hormone Treatments
For the germination experiments, wheat (*Triticum aestivum* L.) seeds were sterilized with 10% commercial bleach and washed 5 times with sterile distilled water. Five replicates of 100 seeds each were placed in Petri dishes containing filter paper imbibed in a solution of KAR_1 (1 nm, 0.01, 0.1, 1, 10 µM) and distilled water as a control and kept in darkness at 25 °C. The wheat seeds were germinated in the dark in these solutions and germination percentages and root lengths were measured.

For the leaf segment senescence experiments, wheat seeds were planted in moisturized perlite after surface sterilization with 10% commercial bleach and washed 5 times with sterile distilled water. They were grown in a growth chamber (16 h light, 8 h dark photoperiod and at 25 ± 2 °C). Four of the first leaf segments (3 cm. each) from 10-day-old wheat seedlings were placed in 5 cm diameter petri dishes containing 4 mL of KAR_1 solutions (1, 10, 100 µM). Distilled water was used as a control.

Fresh Weight Analysis for Senescence
After the harvest, the segments of wheat were weighed and placed in 1, 10 and 100 µM KAR_1 solutions. After 10 days, the segments were weighed and the fresh weight change was calculated. It was analysed with 10 replicate tissue samples of 4 bulked leaf segments.

Analysis of Pigment Content for Senescence
The pigments were extracted by grinding the wheat segments in 90% ice-cold acetone with a pestle and mortar and added to a 15 mL tube. The samples were stored at 4 °C in the dark overnight. They were spun at 3000 g for 10 min at 4 °C in a centrifuge and the supernatant was collected in a new tube. The total chlorophyll content was determined spectrophotometrically (Shimadzu 1601) (21). It was analysed with 10 replicate tissue samples of 4 bulked leaf segments.

Measurement of Cell Death for Senescence
Cell death was measured spectrophotometrically using Evans blue to stain the detached leaves (22). The detached leaves were submerged in a 0.1% (w/v) aqueous solution of Evans blue dye (Sigma-Aldrich). They were subjected to two 5-min cycles of vacuum followed by 30 min under vacuum. The leaves were then washed three times with distilled water (15 min each). The dye bound to dead cells was solubilized in 50% (v/v) methanol and 1% (w/v) SDS at 60 °C for 30 min and then quantified by absorbance at 600 nm. For 100% cell death, the detached leaves were heated at 100 °C for 5 min before staining. Four leaves were pooled for each sample. Ten samples were analysed and this experiment was repeated three times with equivalent results.

Analysis of Protein Content for Senescence
The segment samples were homogenized with an ice-cold 0.1 mmol/L sodium phosphate buffer (pH 7.0). The homogenates were centrifuged at 13000 rpm for 30 min at 4 °C and the supernatants were used to determine the total soluble protein content. The protein content of the extracts was determined according to Bradford (1976), using bovine serum albumin as a standard (23). It was analyzed with 10 replicate tissue samples of 4 bulked leaf segments.

Statistical Analysis
Each treatment was analysed with 10 replicate root and segment samples. The data presented here is the mean values ± SE (n=10). All data was evaluated using one-way ANOVA followed by Dunnett's multiple comparison tests using Graph Pad PRISM software. *$p < 0.05$ was considered significant, $p > 0.05$ was considered not significant.

RESULTS

KAR_1 has a Stimulating Effect on the Germination and Root Growth of Wheat Seeds
To investigate the effect of different concentrations of KAR_1 on wheat seed germination we calculated the germination percentage. Our results showed that KAR_1 increased the germination percentage even at 1nM concentration ($p < 0.05$). However, 1 µM KAR_1 was found to be the most effective concentration with 100% germination percentage. It increased the germination by 1.3 times compared to the control (Figure 1). When the root length data was examined, it was seen that the root length compared to the control increased in all concentrations and the most effective concentration was found to be 1 µM KAR_1 ($p < 0.05$) (Figure 2). It was found 2.2 times higher compared to the control at 72th hours. Our data indicated that KAR_1 promotes seed germination and increases root length even at lower concentrations (1 nm KAR_1).
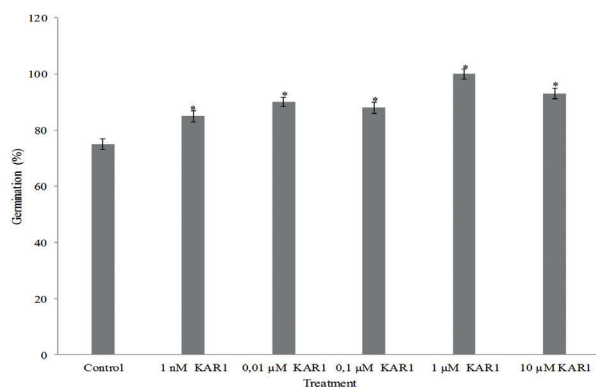
Figure 1. Comparisons of germination (%) after incubation in distilled water (control), 1 nM, 0.01, 0.1, 1 and 10 µM KAR₁ solutions. Values are means± S.E (n=10). (*p < 0.05; compared to control)

Figure 2. Comparisons of root lenght (cm) after 24h, 48h and 72h after incubation in distilled water (control), 1 nM, 0.01, 0.1, 1 and 10 µM KAR₁ solutions. Values are means± S.E (n=10). (*p < 0.05; compared to control)
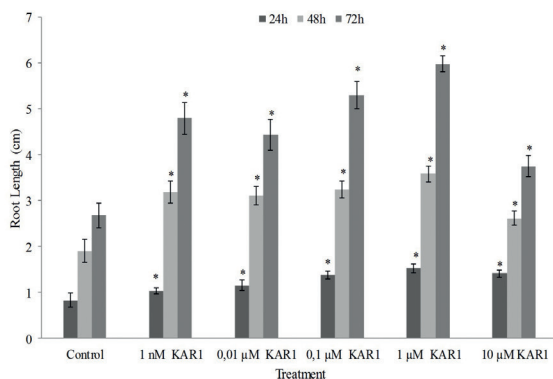

Figure 3. Wheat leaf segments after incubation in distilled water (control), 1, 10 and 100 µM KAR₁ solutions, respectively.

KAR₁ does not Show Any Promoting or Inhibitory Effect on Leaf Senescence

There are no studies showing the relationship between KAR₁ and leaf senescence. We designed this research to investigate the effect of KAR₁ on leaf segment senescence in wheat. The leaf segments were incubated in 1, 10 and 100 µM KAR₁ solutions and distilled water (as a control). We found that there was no significant change between the control and treatment groups (Figure 3). The amounts of fresh weight observed in wheat

segments soaked in 100, 10 and 1 µM KAR₁ solutions are given in Figure 4. Fresh weight was calculated by subtracting the final weights of the segments recorded after being soaked in KAR₁ solutions from their initial weights recorded before being soaked in KAR₁ solutions. It was determined that exposure to the control or KAR₁ treatments did not make a lot of difference ($p > 0.05$) (Figure 4).
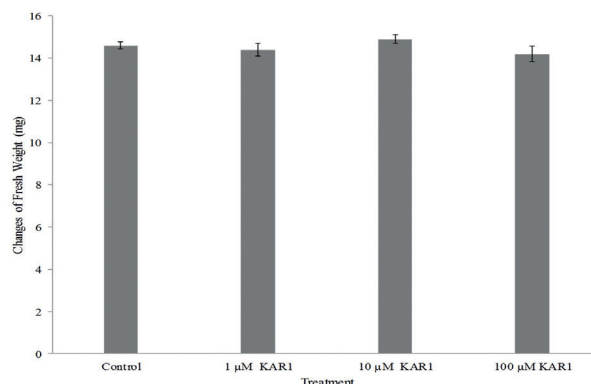

Figure 4. Comparisons of fresh weight after incubation in distilled water (control), 1, 10 and 100 µM KAR₁ solutions. Values are means± S.E (n=10). (p > 0.05; compared to control)

A loss of chlorophyll is the first visible symptom of leaf senescence. We measured the chlorophyll content and did not find a significant change ($p > 0.05$) (Figure 5). Cell death was indicated by a loss of plasma membrane integrity. An examination of cell viability showed that cell death was not significantly altered in the treatment group, as measured by Evans blue staining. Evans blue measures cell death for an entire leaf. Our results showed that different concentrations of KAR₁ had no effect on cell death amounts when compared to the control ($p > 0.05$) (Figure 6). Another senescence parameter is total protein amount. The amounts of total protein observed
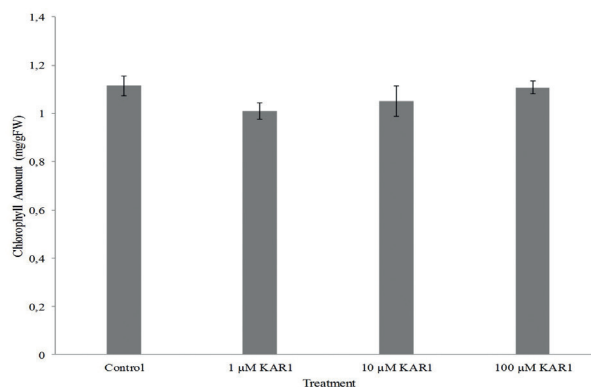

Figure 5. Effects of 1, 10 and 100 µM KAR₁ treatments on chlorophyll content in wheat leaf segments. Values are means± S.E (n=10). (p > 0.05; compared to control)
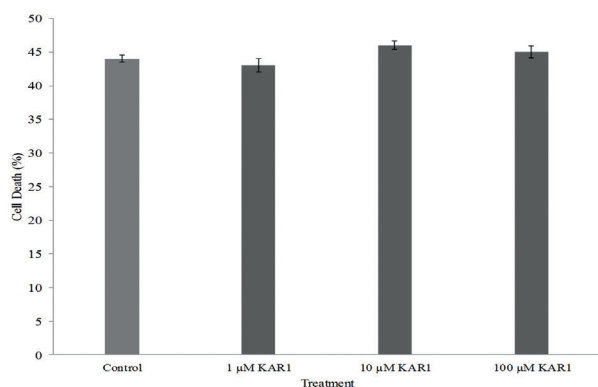
Figure 6. Effects of 1, 10 and 100 μM KAR1 treatments on cell death amount. Values are means± S.E (n=10). (p > 0.05; compared to control)

in wheat segments soaked in 100, 10 and 1 μM KAR1 solutions are given in Figure 7. Our results showed that different concentrations of KAR1 had no effect on total protein amount when compared to the control (*p* > 0.05) (Figure 7).
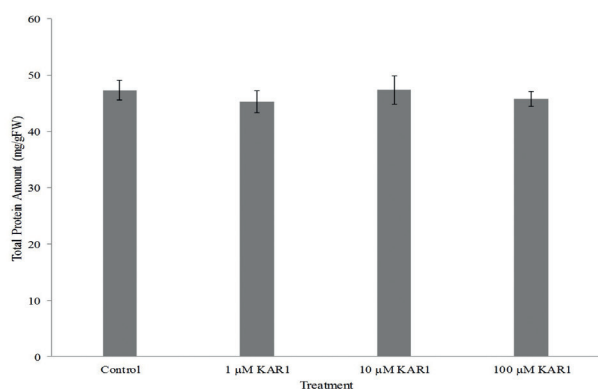

Figure 7. Effects of 1, 10 and 100 μM KAR1 treatments on total protein amount. Values are means± S.E (n=10). (p > 0.05; compared to control)

DISCUSSION

Seed germination and leaf senescence are important developmental processes that are affected by external and internal factors. Many plant hormones regulate seed germination and the initiation of leaf senescence. In recent years researchers have discovered new plant growth regulators such as karrikins. The discovery of karrikins is extremely important because of their potential usage in agriculture and horticulture. KAR1 was discovered in 2004 (6) and following this discovery, studies on the karrikins have always been on the effect of seed germination and seedling growth (11,24-28). When the previous studies are examined, it is seen that there are no studies concerning the effect of KAR1 on the germination of wheat seeds and on leaf senescence.

Global warming is causing a reduction in the productivity and survival of plants - including crops (29). It also adversely affects seed germination. Due to the role of wheat in nutrition, promoting the germination of wheat seeds is very important for yielding more crops from cultivated areas. For these reasons, it is important to identify new substances that will promote seed germination and to investigate their effectiveness. There is limited information on the effect of KAR_1 on germination in wheat seeds. To investigate the effect of KAR1 on the germination of wheat seeds, we used different concentrations of KAR_1, ranging from 1 nm to 10 μM. Researchers found that 10 nm KAR_1 was an effective concentration for germination of lettuce seeds (26, 30). Our results showed that 1 μM KAR_1 was an effective concentration for wheat seeds. 1 μM KAR_1 accelerated both seed germination and root length. Our results indicate that KAR1 is effective in stimulating root growth as previously suggested (31).

Senescence is a developmental process that results in the death of a cell, organ or organism. Considering the remobilisation and recycling of important nutrients such as nitrogen, sulphur, phosphorus and potassium, we can clearly see the vital importance of senescence in the plant life cycle. These nutrients are remobilised from the senescing leaves to the actively growing tissues, thus providing for the growth and reproduction of the plant (32,33). The photosynthetic capacity of the leaf suddenly drops due to the loss of chlorophyll during senescence. The production of carbohydrates, amino acids and other molecules is displaced by the degradation of macromolecules such as protein, lipids and nucleic acids (DNA and RNA), and the released nutrients are mobilised to plant parts such as new buds, young leaves, developing fruits and seeds or to storage organs for the future growing season (34,35).

Initiation of leaf senescence is affected by various factors including age, abiotic and biotic stress, and plant hormones (36,37). Effects of plant hormones on senescence such as auxin, cytokinin, gibberellin, ethylene ABA, SA and JA are well-known (29,35,36). Moreover, the effect of KAR1 on leaf senescence is still lacking in the literature. To test the effect of KAR_1 on wheat leaf segment senescence, different concentrations of KAR_1 from 1 to 100 μM were used. We measured important leaf senescence parameters such as, chlorophyll amount, fresh weight changes, cell death amount and total protein amount.

Changes in fresh weight are one indication of leaf senescence because nutrients remobilise from senescing leaves to storage organs during senescence (38,39). The fresh weight tends to decrease when leaf senescence starts. Our data showed that there were no significant changes between the control and treatment groups (Figure 4). The dramatic metabolic transition from anabolism to catabolism, including the increased hydrolysis of macromolecules occurs during leaf senescence (38). Leaf cells are subject to structural and biochemical changes during senescence (40-44). Because of this, the changes of fresh weight are an important parameter for leaf senescence.

Another important leaf senescence indicator is a decreased chlorophyll amount. During leaf senescence, the death of the photosynthesizing tissues occurs and this results in chlorophyll catabolism (42-44). A yellowing of the leaves is the most obvious phenotypic change in leaf senescence (45). It is caused by the dismantling of the pigment-protein complexes of chloroplasts and a degradation of the constituent chlorophyll (46). We measured the chlorophyll amounts and did not see any significant changes compared to the control (Figure 5).

Membrane integrity is crucial to cell viability. High levels of membrane integrity loss are clear symptoms of cell death (47, 48). The percentage of death cells is an important parameter for leaf senescence. In parallel with our results, we did not detect any changes in cell death amounts compared to the control groups (Figure 6). Our results related to leaf senescence show that KAR$_1$ does not have any effect on leaf segment senescence in the 1-100 μM concentration range.

Biochemically, senescence is characterized by the degradation of macro-molecules, such as chlorophylls, proteins, membrane lipids and RNA, and metabolically these events replace carbon assimilation (49). In our study we found no changes in protein content following the KAR$_1$ application (Figure 7).

CONCLUSION

In the light of this data, this study suggests that KAR$_1$ promotes seed germination even at 1 nM in wheat. Our results indicate that 1 μM KAR$_1$ is more effective for promoting seed germination in wheat seeds. However, neither a stimulatory nor inhibitory effect of KAR$_1$ on the leaf segment senescence in wheat leaves was observed.

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