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Article

Seed priming with melatonin improves drought tolerance in maize

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SUMMARY

Maize is the most important agricultural species in sub-Saharan Africa, supplying approximately 50% of the calories and proteins ingested. Due to increasing drought conditions from climate change, maize crop yields are projected to decline by 24% in the next decade. New strategies to aid farmers in coping with drought circumstances are urgently needed. Seed priming or presoaking, soaking seeds in a solution before planting, has been proposed to improve drought tolerance. Melatonin, a plant growth regulator, improves drought tolerance through multiple mechanisms. We investigated if priming maize seeds with melatonin could improve germination and drought tolerance. We hypothesized that priming maize seeds in a melatonin solution would significantly improve plant drought tolerance. We created three groups of 60 maize seeds: control (no priming), priming with water, and priming with a melatonin solution. We primed seeds for 6 hours and allowed them to germinate. We planted seeds and exposed them to drought conditions for 14 days. Cumulative percent germination was higher in the water and melatonin-primed groups and lowest in the control group. Cotyledon (first leaf) emergence was earliest for the melatonin-primed group and latest for the control group. The melatonin-primed group was superior to the control and water-primed groups in plant height, weight, and viability after drought exposure. The results of this study demonstrate that seed priming with melatonin improved plant height, weight, and viability under drought conditions when compared to priming with water or no priming. Priming seeds with melatonin may offer a simple and inexpensive method of improving drought tolerance in maize plants.

INTRODUCTION

According to the Intergovernmental Panel on Climate Change, severe drought conditions continue to threaten the food security of many developing countries (1). By 2050, approximately 50% of arable lands are expected to be under drought stress (2). A critical food source for developing nations at risk is maize (corn) (3). Due to increasing drought conditions, maize crop yields are projected to decline by 24% starting in the next decade (4). In sub-Saharan Africa, maize is the most important agricultural species, supplying 40–50% of the calories and proteins ingested (5). By 2050, the demand for maize in the developing world is expected to double (6). Due to the growing population, novel methods to help farmers combat drought and improve maize production are urgently needed.

One feasible, low-cost method of mitigating plant drought stress is seed priming or presoaking. Seed priming is a technique by which seeds are soaked in a liquid until the root emerges from the seed before planting. Melatonin, a hormone that helps regulate the sleep cycle in humans, has recently been described as an important plant growth regulator produced by plants in response to drought conditions (7). Melatonin enhances drought tolerance in plants through multiple mechanisms, including delaying leaf senescence, regulating water balance, promoting seed germination, and maintaining the integrity of both leaves and chloroplast (8-11).

Exogenous application of melatonin solution to soil has been shown to improve drought tolerance in maize (12). However, adding a melatonin solution to soil may not always be optimal. Growing plants in melatonin-treated soil for extended periods may negatively affect the growth of the plant and the surrounding soil microbiome (13). Priming maize seeds with a melatonin solution may help improve drought tolerance and avoid the limitations of soil application. One study demonstrated that priming wheat seeds with melatonin during drought improved yield by promoting root development and delaying leaf senescence (14). Furthermore, melatonin-priming has been shown to enhance the tolerance of cotton to drought stress by modulating the antioxidant system (15). Recently, it was observed that priming maize seeds with melatonin improved drought tolerance parameters by alleviating the negative effects of reactive oxygen species (16).

In this study, we explored whether priming seeds with melatonin can help improve drought tolerance in maize plants. We hypothesized that priming maize seeds in a melatonin solution would significantly improve plant drought tolerance when compared to priming with water or no priming. The results of this study demonstrate that seed priming with melatonin improved plant height, weight, and viability under drought conditions when compared to priming with water or no priming. These findings underscore its potential as an effective strategy for enhancing crop resilience in water-scarce environments.

RESULTS

We evaluated the impact of melatonin priming on plant growth by creating three groups of 60 maize seeds in each group: control (no priming), priming with water, and priming with a melatonin solution (100 μM). Based on a dose titration study demonstrating a 100 μM of melatonin was most beneficial for improving plant tolerance to drought stress conditions, we used a melatonin concentration of 100 μM (17). We primed seeds for 6 hours and then allowed the seeds

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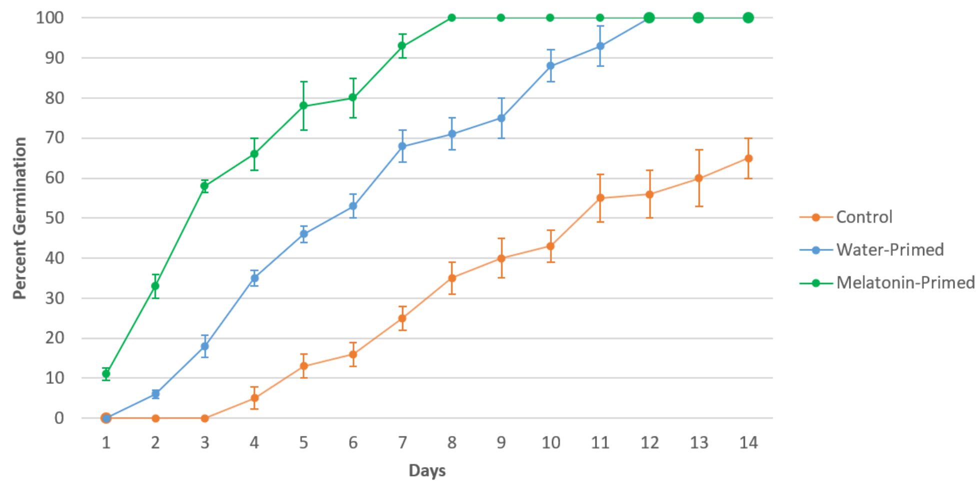

Figure 1: Melatonin-primed seeds germinate more quickly than water-primed and unprimed seeds. Cumulative percent germination of seeds was measured over the course of 14 days for the three groups: control (orange, n = 60), water-primed (blue, n = 60), and melatonin-primed (green, n = 60). The control group had significantly less germination than both the water-primed ($p < 0.05$) and melatonin-primed ($p < 0.05$) groups which were not significantly different from each other. Error bars represent standard error of the mean.

to germinate for 14 days. During this time, we monitored seeds for germination (root emergence). Cumulative percent germination of seeds was measured over the course of 14 days (Days 0–14) for the three groups: control (n = 60), primed with water (n = 60), and primed with melatonin (n = 60). The percent germination of the three groups after 14 days was compared using the Fisher exact probability test. The control group had significantly less germination than both the water-primed ($p < 0.001$) and melatonin-primed ($p < 0.001$) groups which were not significantly different from each other ($p = 0.99$) (**Figure 1**). Next, the seeds were planted and exposed to a 14-day water tapering schedule (Days 15–28). We measured cotyledon emergence of seeds over the course of the water tapering period for the three groups: control (n = 60), primed with water (n = 60), and primed with melatonin (n = 60). We compared the percent with cotyledon emergence on Day 28 of the three groups using the Fisher exact probability test. The control group had significantly less cotyledon emergence than both the water-primed ($p < 0.001$) and melatonin-primed ($p < 0.001$) groups which were not significantly different from each other ($p = 0.99$) (**Figure 2**). This finding may help improve drought tolerance since early germination allows for earlier root system development and potential access to water from deeper soil layers with moisture present. Earlier germination may allow plants to utilize available soil moisture before becoming depleted during drought conditions. Early cotyledon emergence may enable the plant to start photosynthesis earlier, allowing for energy storage to help it survive periods of drought stress.

Following the water tapering period, we exposed the seeds to drought conditions for an additional 14 days (Days 29–42). To evaluate the effects of drought and seed priming on plant viability, height, and weight, the plants were removed from the pots after Day 42 of drought conditions. The following

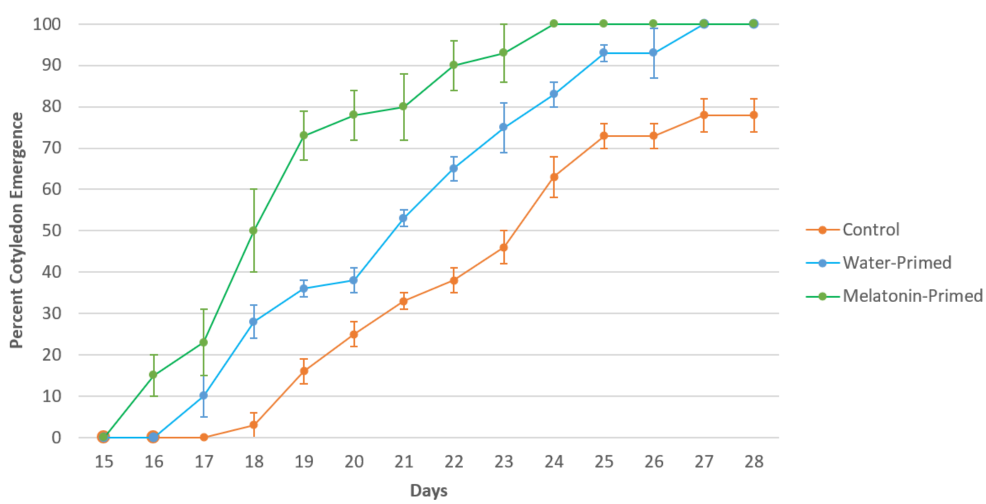

Figure 2: Melatonin-primed seeds displayed earlier cotyledon emergence than water-primed and unprimed seeds. Percent cotyledon emergence of the seeds was measured during the watering tapering schedule for the three groups: control (orange, n = 60), water-primed (blue, n = 60), and melatonin-primed (green, n = 60). The control group had a significantly lower percentage of cotyledon emergence compared to the water-primed ($p < 0.05$) and melatonin-primed ($p < 0.05$). Error bars represent standard error of the mean.

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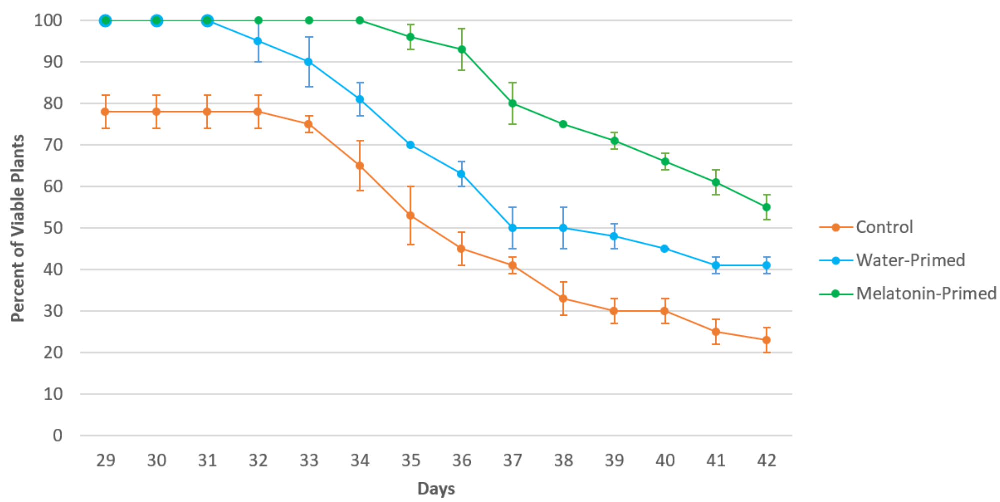

Figure 3: Melatonin-primed seeds resulted in a higher percentage of viable plants than the water-primed and unprimed seeds. The percent of viable plants was measured during drought conditions for the three groups: control (orange), water-primed (blue), and melatonin-primed (green). The control group was not significantly different from the water-primed group and the control and water primed groups had significantly less viable plants than the melatonin-primed group ($p < 0.05$). Error bars represent standard error of the mean.

were recorded: pliability of stem and roots, firmness, and the presence of a visually intact stem color. If the plant fulfilled all three criteria, it was deemed viable. The percentage of viable plants was measured on Day 42 for the three groups.

We compared the percent of viable plants at the end of the drought period for each of the three groups using the Fisher exact probability test. Pliability refers to the flexibility and bendability of plant tissue. We defined pliability as the ability of the plant stem and main root to undergo deformation (manual bending greater than or equal to 45°) without breaking or tearing (18). Firmness refers to the resistance of plant tissue to deformation or compression and is a measure of how rigid or hard the tissue feels. We determined a plant to have adequate firmness if there was no indentation left after gentle manual compression (19). The visually intact plant stem color refers to the uniform appearance and hue of the stem surface without any visible discoloration or blemishes. For young maize plants, the natural coloration of the stem is typically light green with minor variations in shade. We used the above parameters to determine viability based on a literature review (20). While plants were in the soil, only pliability of the stem was utilized.

The control group was not significantly different from the water-primed group (p = 0.677) and the control and water-primed groups had significantly less viable plants than the melatonin-primed group ($p < 0.001$ and $p = 0.005$, respectively) (**Figure 3**). The melatonin-primed group displayed greater viability at the end of the drought period, suggesting that melatonin may have drought-tolerant enhancing effects.

The mean height and weight were measured from the viable plants in each group at the end of the drought period. The plants were removed from the soil and mean plant height, measured from the deepest root to the tip of the longest leaf, was recorded (**Figure 4**). The mean plant heights on

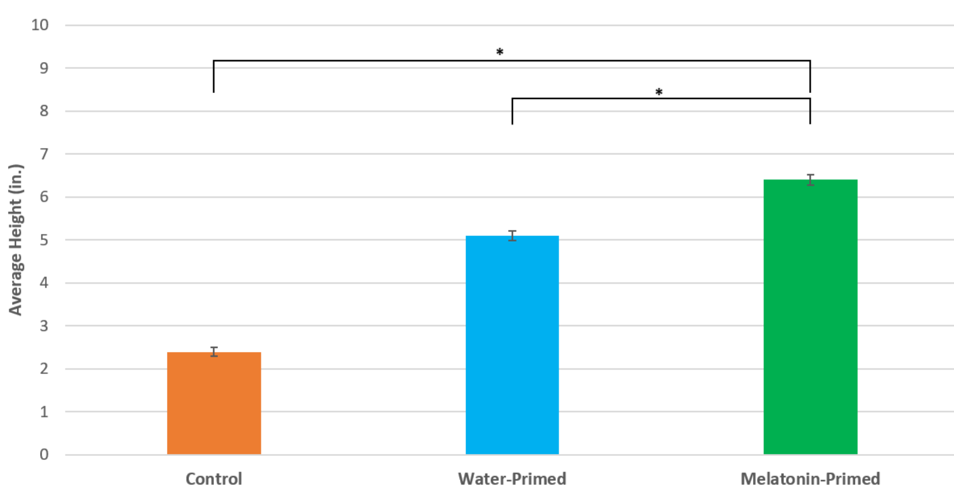

Figure 4: The final plant height was greatest for the melatonin-primed group compared to the water-primed and control groups. Plant height was measured on the final day of drought conditions for the three groups: control (orange, n = 14), water-primed (blue, n = 25), melatonin-primed (green, n = 33). Each mean was significantly different from the other two means. The melatonin-primed height was significantly higher than both the water primed and control groups. Error bars represent the standard deviation of the plant heights. *$p < 0.05$.

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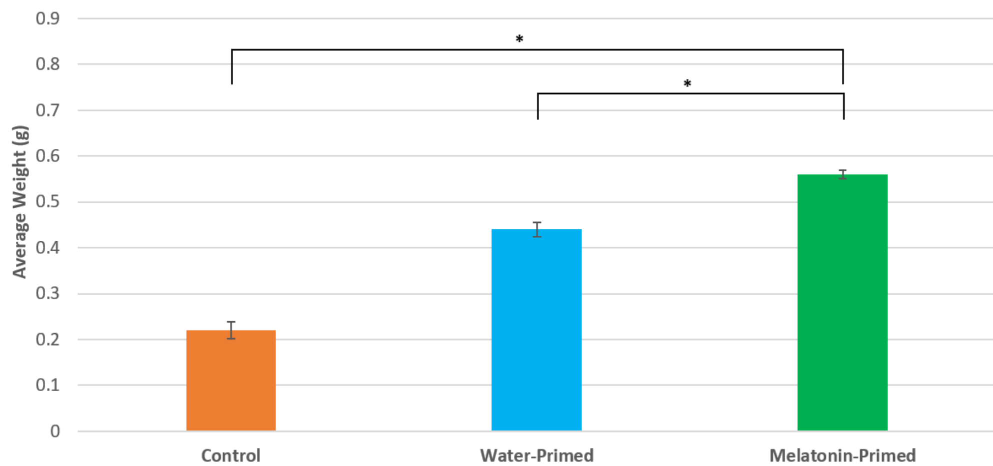

Figure 5: The final plant weight was greatest in the melatonin-treated group compared to the water-primed and control groups. When comparing the three groups, control (orange, n = 14), primed with water (blue, n = 25), and primed with melatonin (green, n = 33). The mean weight of each group was significantly different from the other two groups. The melatonin weight was significantly higher than both the water and control groups. Error bars represent the standard deviation of the plant heights. *$p < 0.05$.

Day 42 for the three groups were compared using one-way ANOVA. The hypothesis of no differences among the groups can be rejected ($p < 0.001$). The Tukey Honestly Significant Difference (HSD) test was then used to compare each pair of means. Each mean was significantly different from the other two means ($p < 0.01$ for each of the three tests). The melatonin-primed height on Day 42 was significantly taller than both the water-primed and control groups.

We compared the mean plant weights on Day 42 for the three groups using one-way ANOVA. The hypothesis of no differences among the groups can be rejected ($p < 0.001$). We compared each pair of means with a Tukey HSD test. The mean weight of each group was significantly different from the other two means ($p < 0.01$ for each of the three sets). The melatonin-primed weight on Day 42 was significantly taller than both the water-primed and control groups (**Figure 5**). These findings of higher plant height and weight of the melatonin-primed group compared to the other groups suggest that melatonin priming enhances the fitness of maize plants under drought conditions, potentially improving crop resilience.

DISCUSSION

This research investigated the impact of melatonin priming on the drought tolerance of maize seeds. The results demonstrated that the seeds primed with melatonin or water had superior germination and cotyledon emergence rates compared to the control group. Priming seeds may improve early plant growth, allowing for improved soil moisture utilization before drought conditions worsen. After a drought period, the group treated with melatonin showed superior plant viability, height, and weight compared to the control and water-treated groups. These findings suggest that melatonin may help farmers in drought-prone regions by enhancing maize crop resilience and food security.

Recently, melatonin has become recognized as an important plant growth regulator in a plant's response to drought stress. The findings of our study suggest that seed priming with melatonin helps improve drought stress in maize. Priming seeds with either water or 100 μM melatonin was associated with 100% seed germination. On the contrary, no priming resulted in 65% seed germination. The results observed in the seed-priming groups are consistent with a prior study demonstrating the benefits of priming seeds during drought conditions (21). Although both seed-priming groups had 100% seed germination, the melatonin-primed group displayed earlier germination. The earlier germination rate of the melatonin group may help explain the higher plant viability of the melatonin group at the study conclusion. This observation is consistent with prior research showing that early germination and seedling development are important and improve plant tolerance and viability during drought conditions (22). Cotyledons can carry out photosynthesis immediately to provide energy for seedling growth, which may help promote drought tolerance (23). Seed priming improved cotyledon emergence in the water and melatonin-primed groups when compared to the control group. The melatonin group had an earlier and more rapid increase in cotyledon emergence when compared to the water-primed group and may help explain the superior drought tolerance parameters of viability, height, and weight of the melatonin-primed group compared to the water-primed group.

The number of viable plants was greatest in the melatonin primed group. This observation is supported by previous research, which has demonstrated that melatonin has multiple beneficial effects on plants during drought conditions, including improved relative water content, photosynthetic gas exchange parameters, stomatal behavior, and stimulation of the antioxidant system (24). Pre-treatment has also been shown to increase endogenous melatonin production (25). Both the height and weight of the plants after 14 days of drought conditions were significantly greater in the melatonin-primed group than in the other two groups. These findings coincide with previous findings, which reported that pre-treatment with 100 μM exogenous melatonin improved the dry weight and leaf area under drought stress (17). In addition, this research helped demonstrate that creating a melatonin solution is easy to apply.

Future studies may be considered to build on our current research by increasing the sample size, testing different

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concentrations of melatonin, presoaking seeds in melatonin for different durations, and studying the applicability on different crops.

MATERIALS AND METHODS

Design and Seed Soaking

Three groups of maize seeds were treated as follows. The first (control) group consisted of 60 maize seeds not primed before planting, the second consisted of 60 maize seeds primed for 6 hours in 20 mL of distilled water before planting, and the third consisted of 60 maize seeds primed for 6 hours in 20 mL of distilled water and melatonin (100 μM) (Sigma-Aldrich, Inc., St. Louis, MO) before planting. Seeds were presoaked for 6 hours based on a study suggesting longer soaking duration may damage the seeds (22). Maize seeds were obtained from Seeds Needs LLC, New Baltimore, MI. After priming the seeds for 6 hours in their assigned 20 mL solution, the seeds were drained and rinsed once with distilled water. To demonstrate reproducibility, the above procedures were repeated two additional times. Three groups of 60 seeds were created for the control, water-primed, and melatonin primed groups.

After seed priming, the seeds underwent a germination period (Days 0–14) then the seeds were planted for a water tapering period (Days 15–28), followed by a drought period (Days 29–42).

Germination and Planting

After priming, the seeds were covered with germinating paper (Home Microgreen, Syracuse, NY) and rinsed with 5 mL of distilled water twice daily. All seeds received 5 mL of distilled water twice daily. The seeds were maintained at a temperature of 70° F for 14 days, and during this time, they were monitored for germination (root emergence). The percentage of plants that germinated by the end of the 14 days was compared between the three groups. All seeds were planted after the 14-day germination period. Each seed was planted 1.5 inches deep into a planting pot with the root facing down. Three groups were created, each with a total of 60 individual planter pots containing potting soil and one maize seed.

Plant Maintenance

After planting the seeds, the planter pots' positions were rotated daily. All seeds were treated regardless of their stage of development. The seeds underwent a water tapering period for 14 days. The following water schedule was used, 5 mL of distilled water on Days 15–17, followed by 3 mL on Days 18–20, 1 mL on Days 21–28, and no water after to mimic gradual drought conditions. During the water tapering schedule the timing of the appearance of the first cotyledon (leaf) emergence was recorded. From Days 29-42 the plants were maintained without water to simulate drought conditions. Daily light was supplied to each pot using a grow lamp (Lovebay 18 WLED Growth Light) for 12 hours with targeted wavelengths of 460 nm and 660 nm to ensure uniform and consistent light exposure to each planting pot.

Plant Height

After the drought period (Days 29–42), the following were measured and recorded: the number of viable plants, plant height (in), and weight (g). After removing each plant, full extension was secured, and the plant height and weight were measured and recorded. Plant viability was determined if the following three criteria were met: pliability of stem and roots, firmness, and visually intact stem color.

Statistical Analysis

Microsoft Excel was used for statistical analysis. Fisher exact probability test was used to compare the percent germination, percent cotyledon emergence, and viability of the three groups. A two-way analysis of variance (ANOVA) test was conducted to examine the effects of the control, water-primed, and melatonin-primed on plant weight and height exposed to drought conditions. The Tukey HSD test was used to determine if there was a significant difference in the results between the individual groups.

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