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Article

Presoaking Seeds with Vinegar Improves Seed Development and Drought Tolerance in Maize Plants

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SUMMARY

Maize is a very important source of food for developing nations. Climate change has contributed to increasing temperatures and drought conditions around the world posing a grave threat to maize crops. Novel methods to help farmers combat drought conditions are urgently needed. Two methods proven to help combat drought stress effects in plants are presoaking seeds in a liquid before planting and applying acetic acid (vinegar) to soil. The purpose of this experiment was to determine whether combining these two methods by presoaking seeds with a vinegar solution can improve seed development and plant drought tolerance of maize plants during drought conditions. We used three experimental groups: Group 1 (control, no presoaking), Group 2 (presoaking with water), and Group 3 (presoaking with vinegar solution). Seed development was determined by measuring the percentage of seeds that germinated and the rate of cotyledon emergence. Plant viability was measured by counting the number of viable plants and measuring final plant height. Our results revealed that presoaking maize seeds with a vinegar solution (Group 3) was superior in improving seed development and plant drought tolerance when exposed to drought conditions. We conclude that presoaking with a vinegar solution may offer a simple and inexpensive method for improving drought tolerance in maize seeds and plants.

INTRODUCTION

Maize is one of the most important food crops in the world (1). In Sub-Saharan Africa, maize is consumed by 50 percent of the population. Worldwide, maize is the preferred food for one-third of all malnourished children and 900 million impoverished people (2). Maize crops are threatened worldwide by increasing annual temperatures due to climate change. Average global temperatures are now one degree Celsius higher than pre-industrial levels, largely due to human activities (3), and some continents are warmer now than they were 100 years ago (4). Droughts are not new, but they appear to be more severe and have lasted longer than ever before (5). Experts expect that future drought conditions will worsen as the temperature continues to rise and precipitation remains unreliable. Drought conditions are already posing a grave threat to the crops and food security in continents such as Africa and will likely become worse as the population is expected to more than double by the year 2050 (6). The effects of climate change will likely increase drought conditions and threaten to decrease the yield of maize crops in many developing countries (2).

Novel methods to help farmers combat drought conditions are urgently needed. One method of combating plant drought stress effects is seed presoaking, a technique by which seeds are soaked in a liquid before planting (6). Germination is the process of a seed sprouting a radicle, the embryonic root of a plant. Drought stress has been shown to delay seed germination, reduce the rate of seed germination, and completely inhibit seedling emergence (7). Seed presoaking has exhibited an increase in germination rate, greater germination uniformity, and greater total germination percentage (7).

Plant production of acetate has also been shown to help with drought tolerance (8). Vinegar is composed of roughly 5% acetic acid. A recent study by Kim et al. helped to elucidate a newly discovered biological pathway that is used by plants to help survive during drought conditions (6). This pathway produces acetate, the main component of vinegar, during drought conditions. The researchers observed that the amount of acetate produced by the plants during drought conditions correlated directly with their survival. The higher the acetate level the better the survival of plants during drought. Next, the researchers tested the addition of acetic acid added to the soil of plants growing in drought conditions. They observed that plants that had acetic acid added to the soil during drought conditions had a higher chance of survival at 14 days. Another study showed that adding vinegar (acetic acid) to the soil of plants under drought conditions helped the plants survive longer when compared to water or other organic acids (8). However, adding acetic acid to soil may not always be realistic, and growing plants in acetic acid-treated soil for long periods of time can negatively affect plant growth as well as the soil and microbiome (9). Using vinegar for seed presoaking to improve germination and plant drought tolerance in maize has not been studied to date. Based on previous research, we hypothesized that presoaking maize seeds in a vinegar solution (5% acetic acid) will improve seed development and plants' drought tolerance. Our results revealed that presoaking maize seeds with a vinegar solution was superior in terms of improving seed development and plants' drought tolerance when exposed to drought conditions.

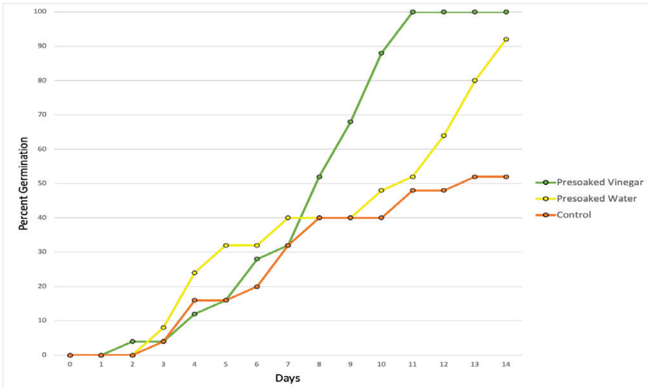

Figure 1: Cumulative percent germination of seeds. Cumulative percent germination of seeds was measured over the course of 14 days for the three groups: control, (orange, n=25), presoaked in water (yellow, n=25), and presoaked in vinegar (green, n=25). The control group had significantly lower cumulative percent germination than the other two groups (p<0.05). One-way ANOVA test was conducted to determine statistically significant differences..

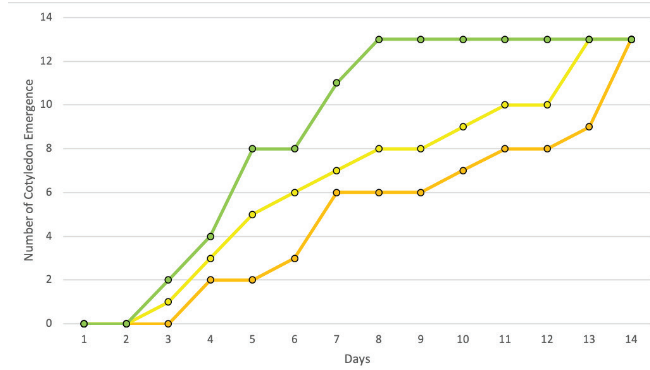

Figure 2: Cotyledon emergence. Cotyledon emergence of the seeds was measured over the course of 14 days for the three groups: control (orange, n=13), presoaked in water (yellow, n=13), and presoaked in vinegar (green, n=13). Cotyledon emergence was earliest for the vinegar treated group and latest for the control group but not significant (p=0.09) One-way ANOVA test was conducted to determine statistically significant differences.

This experiment helped demonstrate that presoaking seeds with a vinegar solution can be used by farmers facing drought conditions to help protect their crops and improve overall food security.

RESULTS

We created three groups of maize seeds as follows: Group 1 (control) seeds were not presoaked before planting, Group 2 seeds were presoaked for 6 hours with distilled water, and Group 3 seeds were presoaked for 6 hours with white vinegar (5% acetic acid). We monitored the three groups for cumulative percent germination over 14 days. Cumulative percent germination was significantly higher in Group 3 and lowest in Group 1 (p value < 0.05, one-way ANOVA) (Figure 1). Group 3 also displayed a sharp increase in cumulative germination on day 8 of the germination period, whereas Groups 1 and 2 had a more gradual increase. Group 3 reached 100% germination, whereas the other groups had less than 100 percent germination.

To investigate if cotyledon (first leaf) emergence was affected by presoaking seeds during drought conditions, we observed the three groups for 14 days after being planted. Cotyledon emergence was earliest for Group 3 and latest for the Group 1, but this was not statistically significant (p=0.09), one-way ANOVA) (Figure 2). To evaluate the effects of drought on plant viability and height, we removed the plants from the pots after 14 days of drought conditions and recorded the following: pliability of stem and roots, firmness, and the presence of a visually intact stem color (Figure 3). If the plant

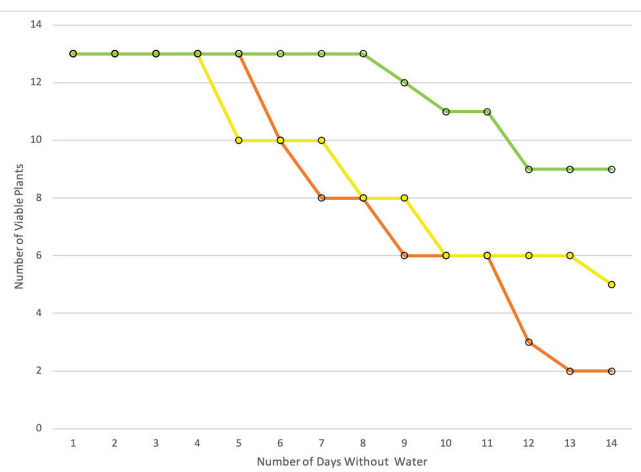

Figure 3: Number of viable plants during drought conditions. The number of viable plants was measured at 14 days of drought conditions for the three groups: control (orange), presoaked in water (yellow), and presoaked in vinegar (green). The number of viable plants at day 14 was significantly different between the groups. (p < 0.05, One-way ANOVA test was conducted to determine statistically significant differences).

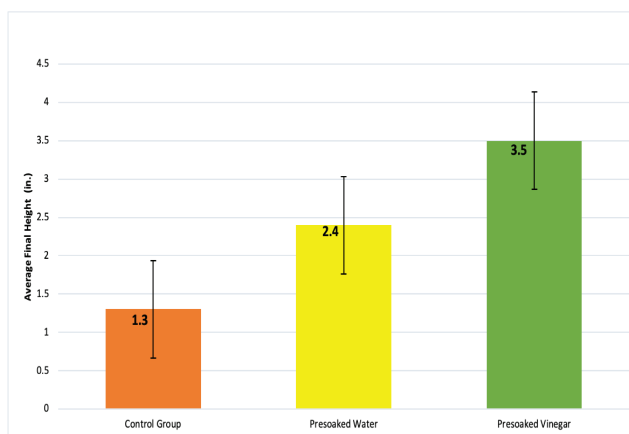

Figure 4: Final plant height. The final plant height was measured at 14 days of drought conditions for the three groups: control (orange, n=2), presoaked in water (yellow, n=5), and presoaked in vinegar (green, n=9). Plant height was greatest in the vinegar-treated group and significantly different than the control group (p <0.5, One-way ANOVA test was conducted to determine statistically significant differences). Error bars represent the standard deviation of the plant heights.

Figure 5: Photograph of all three groups demarcated with a distinct color, Orange dot (Group 1, control), Yellow dot (Group 2, presoaked with water), and Green dot (Group 3, presoaked with vinegar).

fulfilled all three criteria it was deemed viable. The number of viable plants during drought conditions at day 14 was greatest for Group 3. The lowest number of viable plants was seen in Group 1. Group 3 had twice as many viable plants as Group 1 at the end of the 14 days of drought (p value <0.05, one-way ANOVA). The average final height (measured from soil to the tip of the longest leaf) was recorded at the end of the 14-day drought period (Figure 4). The final average plant height was greatest for Group 3 which was significantly taller than Group 1 (p value < 0.5, one-way ANOVA test).

DISCUSSION

The addition of acetic acid to soil during drought conditions has been shown to improve drought tolerance (8). However, adding acetic acid to soil may not always be realistic and growing plants in acetic acid-treated soil for long periods of time can negatively affect plant growth as well as the soil and microbiome (10). The limitations of adding acetic acid directly to soil was the impetus for the present study. By presoaking seeds in vinegar (5% acetic acid), the present study demonstrated the benefits of using acetic acid in drought conditions while avoiding the potential negative effects of adding acetic acid to soil directly. In this experiment, it was observed that presoaking maize seeds with a simple vinegar solution (5% acetic acid) for 6 hours helped promote seed development and drought tolerance at 14 days. Seeds were presoaked for 6 hours based on various recommendations from the literature suggesting longer soaking duration may damage the seeds (11). The results of this experiment supported the initial hypothesis. Furthermore, the results agree with prior studies that demonstrated the benefits of acetic acid application during drought conditions (12). In our study all seeds germinated, which was expected, but Group 3 had earlier germination, which may help explain its higher viability at the study conclusion. Studies have demonstrated that early germination and seedling growth are critical for plant establishment during drought conditions (13). Prior research has demonstrated less than 100% maize crop yield during drought conditions; therefore, it is less likely that all our seeds would germinate if our sample sizes were larger. (14) Cotyledon emergence between groups was not statistically significant (p=0.09), One-way ANOVA test). This finding may have been significant if our population size was larger. A simple vinegar solution (5% acetic acid) was used due to its wide availability and low cost, allowing for potential widespread implementation. It is unclear if a different concentration of acetic acid would be beneficial or detrimental. Future studies examining different concentrations of acetic acid on maize seeds would be helpful. To our knowledge, this is the first application of acetic acid as a presoaking strategy to improve drought tolerance in maize plants. This experiment helped demonstrate that soaking seeds in a vinegar solution is both simple and inexpensive, and can be used by farmers in developing countries facing drought conditions to protect their crops and improve overall food security. Several limitations of this study should be noted. First, the small sample size may limit the applicability to larger crops. Second, it is unclear if the concentration of vinegar (5% acetic acid) used is ideal. Perhaps a more or less concentrated solution would yield different results. Third, it is unknown if presoaking seeds in vinegar for a shorter or longer duration would change the measured outcomes. Finally, it is unknown if our results can be applied to different crops.

MATERIALS AND METHODS

Design and Seed Soaking

We treated three groups of maize seeds as follows. The first (control) group consisted of 25 maize seeds not presoaked before planting. The second group consisted of 25 maize seeds presoaked for 6 hours with distilled water. The third group consisted of 25 maize seeds presoaked for 6 hours with distilled water and white vinegar (concentration 40 parts of distilled water to one-part white vinegar). The vinegar source was distilled white vinegar (The Hain Celestial Group INC., Lake Success NY). The concentration of the undiluted white vinegar concentration was 5% acetic acid with a molarity of 0.8 M. We obtained maize seeds from Seeds Needs LLC, New Baltimore MI. After presoaking the seeds for 6 hours in their assigned 20 mL solution, we drained and rinsed the seeds with distilled water.

Germination and Planting

We covered the seeds with a paper towel and rinsed with 5 mL of distilled water twice each day. We maintained the seeds at a temperature of 70 º F for 14 days, and during this time, the seeds were monitored for germination (root emergence). Once the seeds germinated, we recorded the cumulative percent germination. The first 13 seeds to germinate in each group were planted 1.5 inches deep into the planting pot with the root facing down. We created three groups of 13 planter pots, each containing potting soil and one maize seed (Figure 5).

Plant Maintenance

We rotated the planter pots' positions daily. We planted one seed in each pot. After planting the 3 groups of 13 seeds, we watered the planter pots on days 1 through 3 with 5 mL of distilled water. In order to mimic drought conditions, which typically occurs gradually, we administered 3 mL of distilled water on days 4 through 6, followed by 1 mL of water on days 7 through 14. After day 14, we maintained the plants without water to simulate drought conditions for an additional 14 days. Each of the 13 planting pots with potting soil were placed in a well-lit area such that each planting pot received the same amount of light daily. We supplied daily light to each pot using a grow lamp (Lovebay 18 WLED Growth Light) for 12 hours with targeted wavelengths of 460 nm and 660nm to ensure uniform and consistent light exposure to each planting pot. We recorded the appearance of the first cotyledon (leaf), cotyledon emergence.

Plant Height and Viability

After 14 days, we measured the number of plants viable and plant height (in). After removing each plant, we secured full extension and the plant height was measured and recorded. We determined plant viability if the following three criteria were met: pliability of stem and roots, firmness, and visually intact stem color.

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