JOURNAL OF
EMERGING INVESTIGATORS

Article

# Effects of plant-derived smoke water on germination and growth in stressed plants

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## SUMMARY

**Approximately 30 million additional people joined the vast number of people experiencing food insecurity in 2021 due to climate change. It is paramount to find ways to increase seed germination and plant growth under climate change-related stresses. Previous research has shown that smoke water, the chemical extract of all substances in smoke that is created when burning plants, can stimulate seed germination and plant growth. However, little research has been done on the effect of smoke water treatment on seed germination and plant growth when exposed to abiotic stresses. Thus, our goal was to examine the effect of plant-derived smoke waters on radish, buckwheat, barley, and pea plant germination and growth under heat stress, salinity stress, drought stress, and excessive water stress. We hypothesized that plant seeds germinated with plant-derived smoke water and exposed to abiotic stress would have higher germination and growth rates than those plant seeds not germinated with smoke water and exposed to stress. We derived smoke water from radish, buckwheat, barley, and pea plants, then we germinated and grew the plants with different smoke waters. We found that smoke water derived from certain plants—particularly buckwheat, pea, and radish—can positively influence plant germination and growth under certain environmental stress conditions. The plant-derived smoke water has the greatest influence on increased drought and excessive water stress followed by salinity stress but has little influence on increased temperature stress. Thus, our research provides evidence that smoke water can aid plants germinating and growing in stressed conditions.**

## INTRODUCTION

Climate change is an urgent issue in the world today, and we are at a defining moment as its impacts are widespread and unprecedented (1). Climate change alters otherwise consistent weather patterns, thus increasing abiotic stressors that weaken plant resilience and disrupt ecosystems (2, 3). Climate change's effects on the environment and agriculture are extensive; the unpredictable weather patterns and high temperatures reduce crop yields and biodiversity due to limited adaptability (1). Global warming causes flooding due to extreme weather and rising sea levels as well as drought from the drying out of soil and vegetation from enhanced evaporation (4, 5). Meanwhile, the salt remains in the soil, leading to increased salinity (6). When temperatures reach uncomfortably high limits—which vary between different plant species—photosynthesis and respiration become unbalanced (7). Without adequate water due to drought, biological processes are greatly reduced, meaning decreased energy and consequently diminished plant growth (8). Salinity affects production in crops since high concentrations of sodium and chloride ions in the soil may be toxic to plants, inhibiting growth and reproduction (9). Excessive water in the soil from flooding decreases oxygen levels, which hinders respiration (10). Taken together, these environmental stressors due to climate change have negative effects on agriculture, increasing the threat to food security and the food crisis (11).

Smoke water is a chemical extract used to stimulate the germination and growth of plant species. Plant-derived smoke can induce the activity of amylase, an enzyme which converts starch and glycogen into simple sugars, thus allowing for increased respiration and energy production (12). Furthermore, amylase contributes to increased DNA replication and β-tubulin accumulation, providing structural support, transporting carbohydrates, and aiding cell division (12). These processes promote seed germination and break seed dormancy. Additionally, protein, carbohydrate, and chlorophyll contents are also increased in response to smoke-water exposure, contributing to the necessary cellular processes of the plant (13).

Several studies have demonstrated that a major cue for germination with plant derived smoke is due to several of its chemical compounds, including karrikinolides, cyanohydrins, butenolides, and hydroquinones (13, 14). Increased seed germination by these chemical compounds is due to increased activity of hydrolytic enzymes. These enzymes can degrade the fungal cell wall, cause the cell lysis of fungal pathogens, and suppress the function of abscisic acids: plant hormones that inhibit germination (13). However, no studies have been done on the effect of smoke water treatment on seed germination undergoing abiotic stresses associated with climate change and the variations in the effects on different plants under different types of stressors.

In an age where feeding growing populations is becoming more of a challenge and climate change has reduced production of plant biomass due to increasing drought, increasing flooding, increasing salinity, and increasing temperatures, it has become paramount to find ways to increase seed germination and plant growth under stress conditions (15, 16). A previous study has shown that smoke derived from plant material induces seed germination and plant growth in various plant species when the plants are not exposed to stress conditions (13). However, the plants that respond best to their own smoke water by encouraging the most growth, survival, and reproduction in their own species remain insufficiently investigated. Additionally, the differences in responses to smoke exposure between different plant species under stress conditions are not well studied (13).

We simulated environmental conditions projected under climate change. We chose a variety of crop species to assess the effects of smoke water on various plant families and to help identify methods to reduce climate change impacts on global food production. Radish, buckwheat, barley, and pea were chosen because each plant belongs to different plant families, allowing us to evaluate the varied effects of smoke water on a wide range of plants. Each plant is also a commonly cultivated crop that many people around the world consume as part of their diet so more information about improved methods for growing it would be beneficial to minimizing the impact of climate change on crop production. We hypothesized that radish, buckwheat, barley, and pea plant seeds germinated with smoke water derived from burning their own plant species and exposed to heat stress, salinity stress, drought stress, or excessive water stress while germinating and growing would have an increased germination and growth rate than those plant seeds not germinated with smoke water and exposed to the above climate change-related stresses. We discovered that germination and growth rate are higher under certain stresses but not all and for certain plant species when treated with smoke water compared to no-smoke water, which suggest that various plants have differential responses to stressor conditions.

## RESULTS

In our study, we applied smoke water derived from each plant species exclusively to that same species. Specifically, smoke water produced from peas was used for treatment only on peas, from radish only on radish, from buckwheat only on buckwheat, and from barley only on barley. To examine germination percentage, we germinated the four seed types with either its respective smoke water or non-smoke water for two days under no stress or with increased temperature, salinity, drought, or excessive water to represent different stressors. Then, the seeds were planted and grown for 14 days under the same stress and water conditions used during germination. For each seed type within a given stress and water treatment, we planted 20-30 seeds. To examine plant height, we measured and recorded the distance from the plant base to its highest point. .

All four plant species under no stress had a significant increase in germination percentage on Day 1 when grown with smoke water compared to no smoke water (buckwheat: 32% with smoke water, 0% with no smoke water; pea: 11% with smoke water, 0% with no smoke water; barley: 53% with smoke water, 0% with no smoke water; radish: 94% with smoke water, 62% with no smoke water) ($p < 0.05$, two-tailed *t*-test, **Figure 1A**). For Day 2, only buckwheat and peas under no stress had a significant increase in germination percentage when grown with smoke water compared to no smoke water (buckwheat: 75% with smoke water, 52% with no smoke water; pea: 19% with smoke water, 0% with no smoke water) ($p < 0.01$, two-tailed *t*-test, **Figure 1A**). Barley, buckwheat, and pea under the four stress conditions showed no significant germination percentage difference ($p > 0.05$) between smoke water and no-smoke water (**Figures 1B, 1C, 1D, and 1E**). However, under increased salinity stress, radish showed a significant increase in germination percentage between smoke water and no-smoke water (48% with smoke water, 11% with no smoke water) ($p < 0.01$, two-tailed *t*-test, **Figure 1D**).

Significant differences in height between plants given smoke water and not given smoke water were found for increased drought on Day 5 for buckwheat ($p = 0.002$), pea ($p = 0.001$), barley ($p = 0.000103$), and radish $p < 0.00001$). Additionally, significant differences were found for excessive water for Day 5 buckwheat ($p = 0.007667$), Day 5 radish ($p = 0.023$), and Day 10 barley ($p = 0.011$) **(Figure 2 A, 2C**). Also, Day 5 radish under increased salinity showed a significant difference in height ($p = 0.009$) **(Figure 2B**). Under no stress, barley, radish, and buckwheat showed no significant difference ($p > 0.05$) in plant height (**Figure 2**). Only pea with smoke water had a significant increase ($p < 0.01$) in height compared to pea with no smoke water (**Figure 2D**). However, all four plants under increased drought conditions showed a significant increase ($p < 0.01$) in height in plants given smoke water compared to those not given smoke water (**Figure 2**). Additionally, buckwheat, barley, and radish under excessive water conditions showed a significant difference ($p < 0.05$) in height between plants given smoke water and not given smoke water (**Figures 2A, 2C, and 2D**). Also, radish under increased salinity showed a significant difference ($p < 0.01$) in height (**Figure 2B**). In general, smoke water produced taller plants under drought and excessive water stress compared to under increased temperature or increased salinity stress.

Regarding percent survival, peas under no stress had the only significant difference ($p < 0.05$) in percent survival comparing smoke water vs no-smoke water treated plants (**Figure 3A**). Peas treated with smoke water under no stress had 48% survival compared to a 21% survival watered without smoke water ($p = 0.012$). Under no stress conditions, all other plants treated with smoke water had no significant difference ($p > 0.05$) in percent survival in comparison to plants treated with no-smoke water (**Figure 3A**). Under drought stress, barley had 80% survival with smoke water vs 38% without, radish had 64% survival with smoke water vs 10% without, buckwheat had 80% with smoke water vs 0% without, and peas had 53% with smoke water vs 0% without ($p = 0.002$; $p < 0.00001$; $p < 0.00001$; $p < 0.00001$). Under drought stress, all four types of plants had significantly higher ($p < 0.01$) percent survival with smoke water than without it (**Figure 3B**). There was no significant difference ($p > 0.05$) in percent survival for any plants under excessive water and increased temperature stress between smoke water plants and no-smoke water plants (**Figure 3C, 3E**). For salinity stress, radish had a 41% survival with smoke water compared to a 0% survival without smoke water ($p < 0.00001$ For salinity stress, radish was the only plant to perform significantly better ($p < 0.00001$) when grown with smoke water (**Figure 3D)**.

## DISCUSSION

Although previous studies have discovered the benefits of smoke water on plant germination and growth, very little is known about smoke water's impact on plants under stress conditions that are representative of the effects of climate change. Thus, the objective of our research was to determine the effect of plant-derived smoke water on radish, buckwheat, barley, and pea plant germination and growth under heat stress, salinity stress, drought stress, and excessive water

https://doi.org/10.59720/23-165

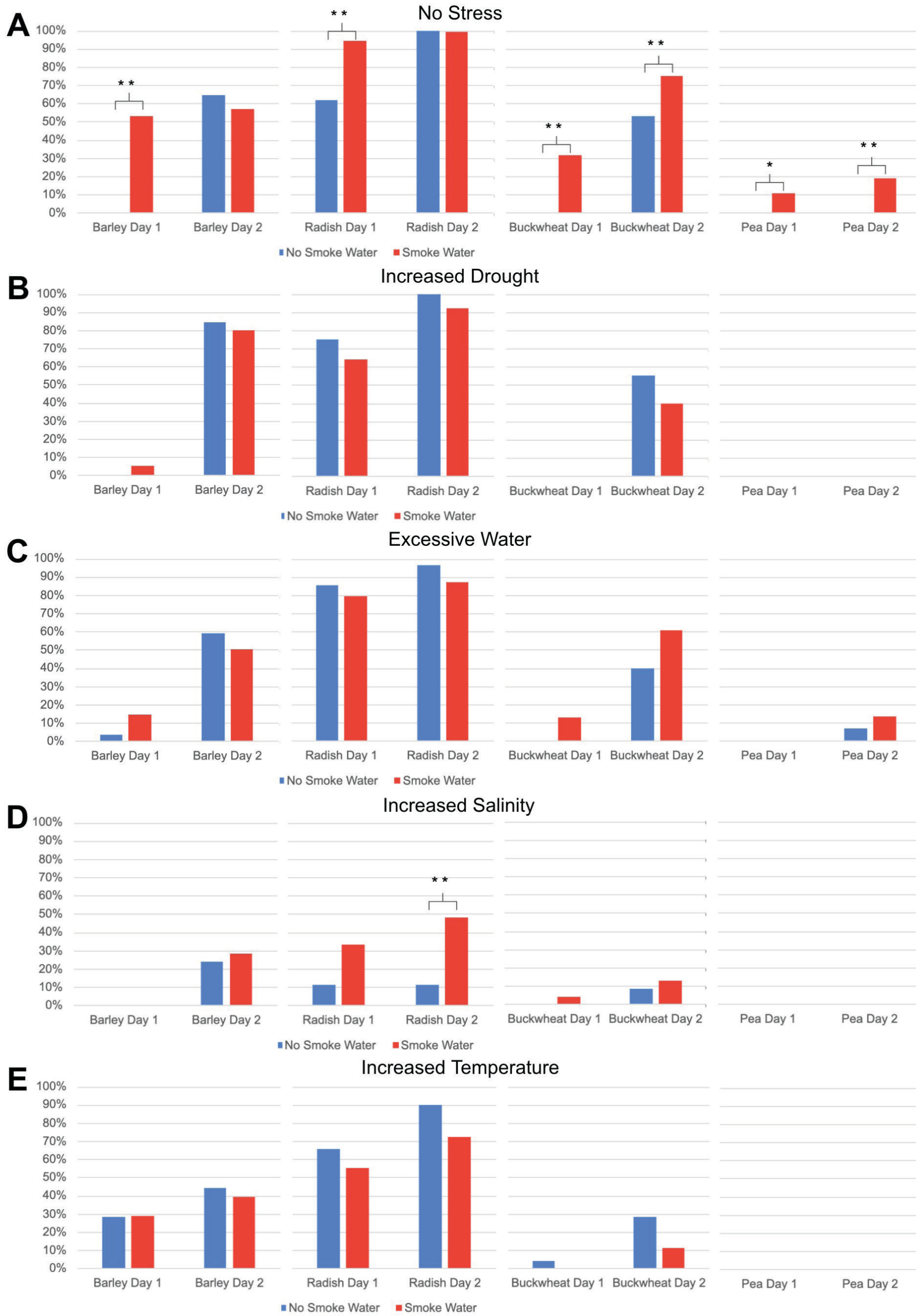


**Figure 1: Germination percentage of plants under various stresses with smoke water and with no smoke water.** Germination percentage data collected over two days for barley, radish, buckwheat, and pea plants under A) no stress, B) increased drought, C) excessive water, D) increased salinity stress, and E) increased temperature stress. Total seed numbers per species and condition are provided in Table 2. *$p < 0.05$, **$p < 0.01$, two-tailed *t*-test.

stress. This information may help minimize the consequences of loss of plant productivity.

Our results suggested that smoke water derived from certain plants can positively influence plant germination and growth under certain environmental stress conditions. We also saw that buckwheat, pea, and radish react better to their own smoke water than barley regarding germination percentage and growth in plants under stress. This variety of responses could be due to the diverse nature of chemical compounds present in the smoke water of plants that affect measures of growth differently (13, 14). Since species capable of producing smoke-water with positive effects have not been fully identified, it is possible that effects of smoke on seed germination may depend on the plant species (17). It is also possible that smoke water derived from peas and buckwheat produce a superior effect on their respective plant species in comparison to smoke water obtained from barley or radish. Future research is needed to determine why pea

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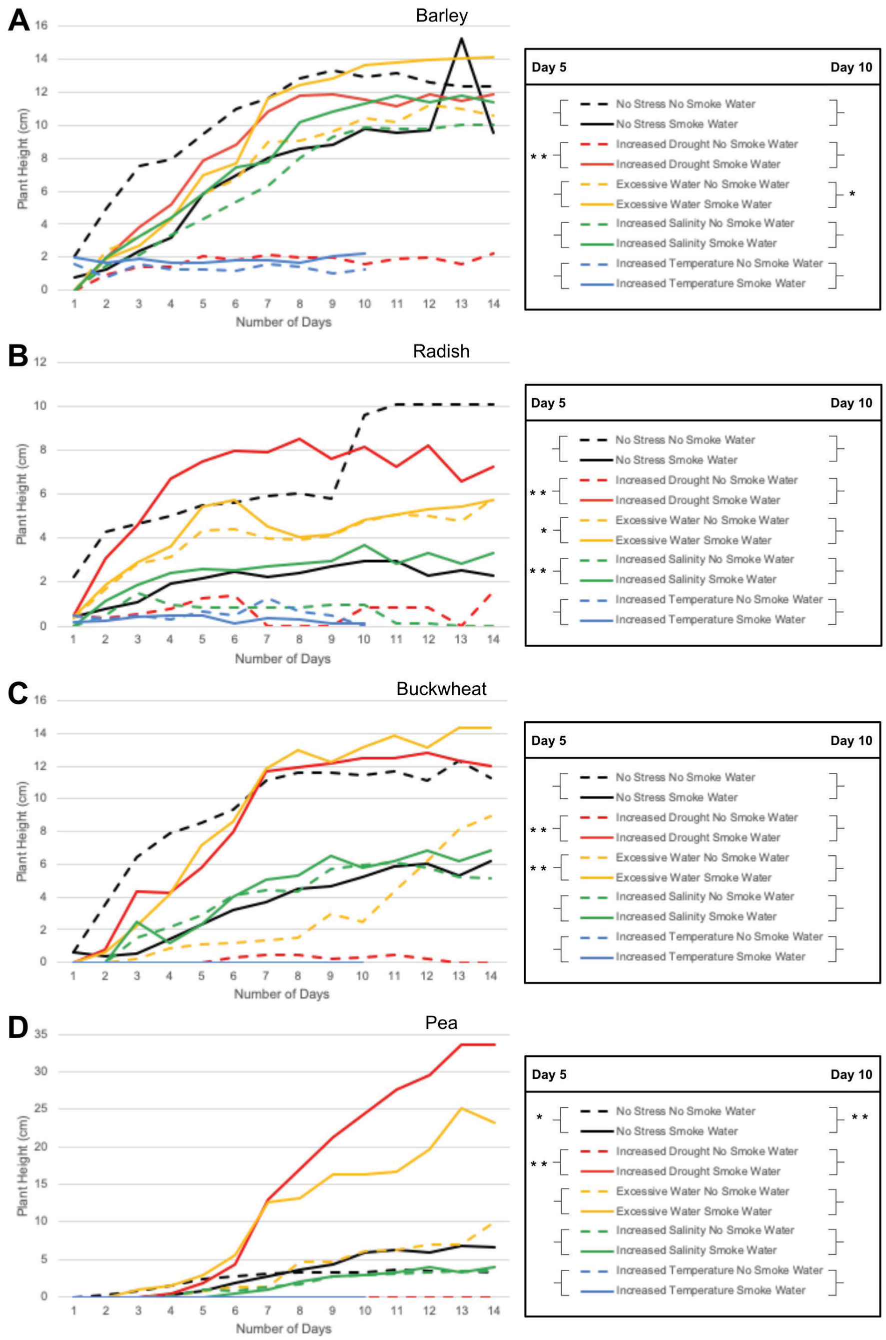


**Figure 2: Plant height of plants under various stresses with smoke water and with no smoke water.** Plant height data collected over a course of 14 days. A) Barley, B) radish, C) buckwheat, and D) pea under no stress, increased drought stress, excessive water stress, increased salinity stress, and increased temperature stress. Total numbers of plants per condition are provided in Table 2. No plant height measurements were recorded beyond Day 10 for the increased temperature stress condition, as all plants exposed to this treatment exhibited complete wilting and death by Day 10 across all species. Statistical analysis was done on Day 5 and Day 10 for each plant and stress condition to compare the heights of plants with smoke water and plants with no smoke water. **p* < 0.05, ***p* < 0.01, two-tailed *t*-test.

and buckwheat smoke water increased germination better than barley or radish. Additionally, in this study, smoke water had the greatest influence on plant height, with a lesser effect percent survival and a minimal impact on germination percentage. Smoke water also had the most influence on increased drought and excessive water stress, followed by salinity stress, but had little effect on increased temperature stress. However, we were unable to test each type of plant-derived smoke with each type of plant for these experiments. This would be an interesting future study because treating plants with smoke water derived from a different species could have a greater influence on the measures of development that we investigated.

Based on our experiment, an area for further study is why

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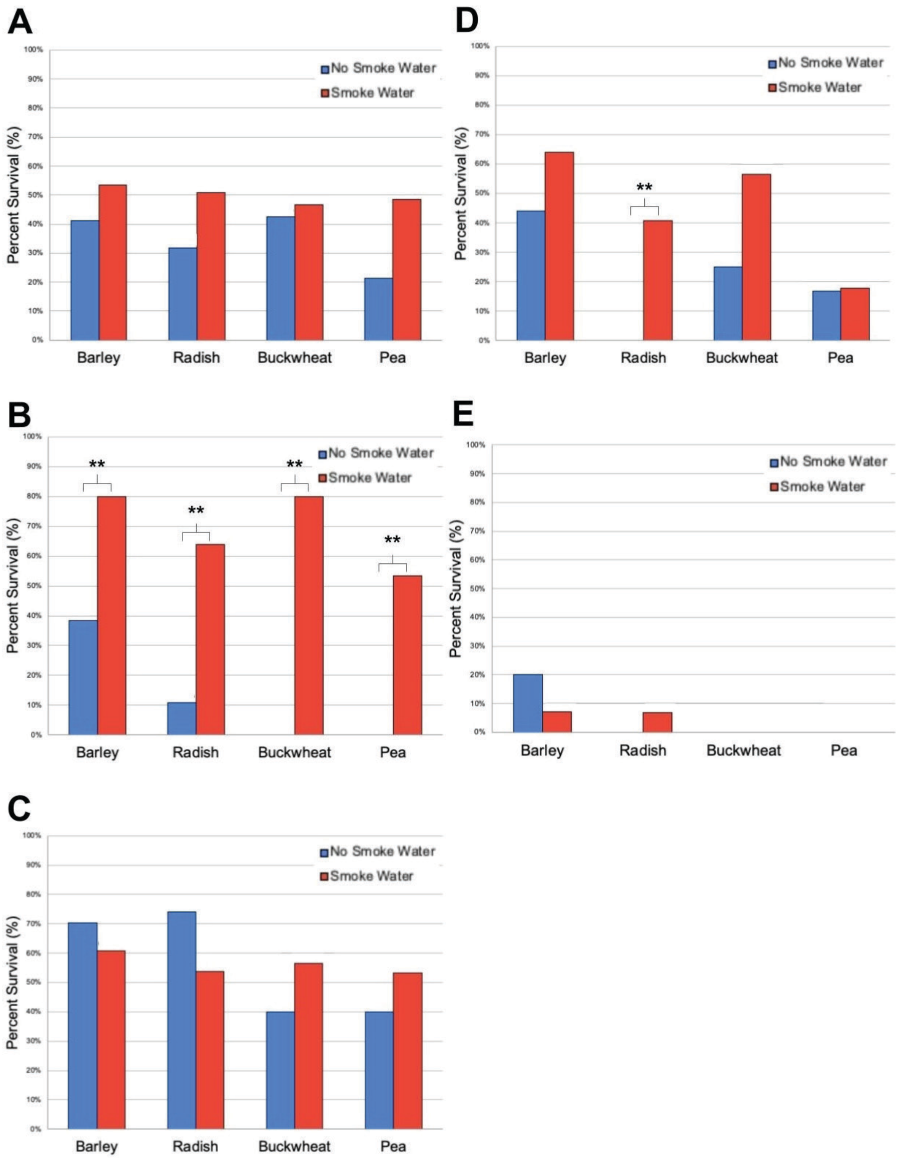


**Figure 3: Percent survival of plants under various stresses with smoke water and with no smoke water.** Percent survival data collected at the end of the two-week period of growing in pots. Barley, radish, buckwheat, and pea under A) no stress, B) increased drought stress, C) excessive water stress, D) increased salinity stress, and E) increased temperature stress. Total numbers of plants per condition are provided in Table 2. *$p < 0.05$, **$p < 0.01$, two-tailed $t$-test.

most of the plant species under no stress did not grow taller in response to smoke water, but stressed plants did. This difference in response may be because plants react to abiotic stresses on both a molecular and cellular level by modifying the expression of many genes, making it is possible that chemicals in smoke water affect gene expression during certain stress conditions that are not expressed when the plant is not stressed (18). A potential reason why most of the plant species under no stress did not grow taller in response to smoke water in contrast to the results of previous experiments is because prior studies dehydrated the plants used to create smoke water before burning them; however, we did not have the time allowance to do so (12, 13). Dehydration could have affected the chemical composition of the plant material and led to more plant growth even under no stress (19). Another area for future research is why smoke water derived from barley and radish influenced their respective plant heights under stress, when smoke water did not affect germination percentage. One possible explanation for this is that the chemicals derived from smoke water in each plant vary and therefore may have different effects, which is supported by a previous study in which the active compounds in plant-derived smoke from different plants were isolated and showed diversity in the chemical composition of smoke obtained from different plants (13). A potential experiment that could address this area for future studies could be to perform a chemical analysis of smoke water from different plant species to identify the concentrations of key compounds and then correlate the concentrations with observed effects on plant height and germination.

https://doi.org/10.59720/23-165

| Condition Number | Plant | Smoke Water | Stressor |
|---|---|---|---|
| 1 | Barley | No | None |
| 2 | Barley | Yes | None |
| 3 | Barley | No | Increased Drought |
| 4 | Barley | Yes | Increased Drought |
| 5 | Barley | No | Excessive Water |
| 6 | Barley | Yes | Excessive Water |
| 7 | Barley | No | Increased Salinity |
| 8 | Barley | Yes | Increased Salinity |
| 9 | Barley | No | Increased Temperature |
| 10 | Barley | Yes | Increased Temperature |
| 11 | Radish | No | None |
| 12 | Radish | Yes | None |
| 13 | Radish | No | Increased Drought |
| 14 | Radish | Yes | Increased Drought |
| 15 | Radish | No | Excessive Water |
| 16 | Radish | Yes | Excessive Water |
| 17 | Radish | No | Increased Salinity |
| 18 | Radish | Yes | Increased Salinity |
| 19 | Radish | No | Increased Temperature |
| 20 | Radish | Yes | Increased Temperature |
| 21 | Buckwheat | No | None |
| 22 | Buckwheat | Yes | None |
| 23 | Buckwheat | No | Increased Drought |
| 24 | Buckwheat | Yes | Increased Drought |
| 25 | Buckwheat | No | Excessive Water |
| 26 | Buckwheat | Yes | Excessive Water |
| 27 | Buckwheat | No | Increased Salinity |
| 28 | Buckwheat | Yes | Increased Salinity |
| 29 | Buckwheat | No | Increased Temperature |
| 30 | Buckwheat | Yes | Increased Temperature |
| 31 | Pea | No | None |
| 32 | Pea | Yes | None |
| 33 | Pea | No | Increased Drought |
| 34 | Pea | Yes | Increased Drought |
| 35 | Pea | No | Excessive Water |
| 36 | Pea | Yes | Excessive Water |
| 37 | Pea | No | Increased Salinity |
| 38 | Pea | Yes | Increased Salinity |
| 39 | Pea | No | Increased Temperature |
| 40 | Pea | Yes | Increased Temperature |

**Table 1: Experimental groups.** Each unique combination of plant species (barley, radish, buckwheat, and pea), stressor (no stress, increased drought, excessive water, increased salinity, and increased temperature), and smoke water treatment (Yes/No) defines a separate experimental group, resulting in 40 unique conditions.

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| Condition Number | Total Seeds | Germination Percentage | Plant Height | Percent Survival |
|---|---|---|---|---|
| 1 | 34 | 22 | 15 | 14 |
| 2 | 56 | 32 | 26 | 30 |
| 3 | 26 | 22 | 11 | 10 |
| 4 | 20 | 16 | 15 | 16 |
| 5 | 27 | 16 | 15 | 19 |
| 6 | 28 | 14 | 18 | 17 |
| 7 | 25 | 6 | 10 | 11 |
| 8 | 25 | 7 | 15 | 16 |
| 9 | 25 | 11 | 6 | 5 |
| 10 | 28 | 11 | 3 | 2 |
| 11 | 47 | 47 | 22 | 15 |
| 12 | 67 | 66 | 27 | 34 |
| 13 | 28 | 28 | 5 | 3 |
| 14 | 25 | 23 | 15 | 16 |
| 15 | 27 | 26 | 13 | 20 |
| 16 | 39 | 34 | 22 | 21 |
| 17 | 27 | 3 | 3 | 0 |
| 18 | 27 | 13 | 13 | 11 |
| 19 | 29 | 26 | 5 | 0 |
| 20 | 29 | 21 | 2 | 2 |
| 21 | 33 | 17 | 13 | 14 |
| 22 | 47 | 35 | 24 | 22 |
| 23 | 20 | 11 | 2 | 0 |
| 24 | 15 | 6 | 12 | 12 |
| 25 | 15 | 6 | 12 | 6 |
| 26 | 23 | 14 | 16 | 13 |
| 27 | 24 | 2 | 6 | 4 |
| 28 | 23 | 3 | 13 | 13 |
| 29 | 25 | 7 | 5 | 0 |
| 30 | 27 | 3 | 0 | 0 |
| 31 | 28 | 0 | 6 | 6 |
| 32 | 37 | 7 | 19 | 18 |
| 33 | 15 | 0 | 0 | 0 |
| 34 | 15 | 0 | 8 | 8 |
| 35 | 15 | 1 | 5 | 6 |
| 36 | 15 | 2 | 5 | 8 |
| 37 | 18 | 0 | 3 | 3 |
| 38 | 17 | 0 | 3 | 3 |
| 39 | 16 | 0 | 0 | 0 |
| 40 | 18 | 0 | 0 | 0 |

**Table 2: Number of seeds per experimental group.** Sample size used to calculate germination percentage, plant height, and percent survival for each of the 40 experimental groups (as defined in Table 1). Total seeds represent total number of seeds (germinated and ungerminated) per condition; germination percentage represents the number of germinated seeds; plant height represents the number of plants that emerged and had a measurable height at Day 10; and percent survival represents the number of plants that were alive (not shriveled up) with a measurable height at Day 14. Differences in sample sizes between plant height and percent survival are due to plant mortality or the growth of new plants between the two measurement dates.

https://doi.org/10.59720/23-165

Regarding the percent survival, the large disparity between plants with smoke water compared to plants with no smoke water could be explained by the presence of karrikins and karrikin receptors in all types of smoke water. Smoke water helps plants retain water because the karrikins and karrikin receptors increase stomatal closures, leading to more conservation of water and less water loss, lessening drought susceptibility (19, 20). Additionally, the karrikins and karrikin receptors help protect against stress-induced cell membrane damage, which leads to cellular electrolyte leakage (20). The variation in increases of percent survival as a result of the treatment of smoke water across the four plant species could be due to the varied amounts of karrikins and karrikin receptors present in different types of plant-derived smoke waters (21). Another potential reason for the substantial difference in percent survival between smoke water-treated plants and non-smoke water-treated plants is the presence of antioxidants in the form of hydroquinone in smoke water (13). Antioxidants could potentially be protecting photosynthetic enzymes from damage caused by reactive oxygen species (ROS), thus preventing harm to plants from stresses (22). Additionally, another possibility is that plants need to turn on genes to get to homeostasis, but since the chemicals in each plant-derived smoke water are different, a different mechanism is used to help turn on the gene, leading to a variance in responses to smoke water.

There are some limitations to our project that may have impacted the results. We grew and maintained 160 pots of plants and were unable to keep them all in the same location, making the control of all variables a challenge, particularly light and temperature. Ideally, we would expose all plants to equal intensity fluorescent lighting, due to the unpredictability of natural light. Due to the constraints of a high school research setting like limited space availability, all the plants were placed by a windowsill, which exposed them to natural lighting. However, this means that the light was not consistent throughout all times of the day and varied day by day with changing weather patterns, such as sunny vs cloudy days. If we had the chance to continue our experimentation, we would also like to expand our project to test other species of plants since our current results show that different types of plants respond to smoke water with varying levels of success in terms of germination percentage and plant height.

In summary, climate change is already affecting every region on Earth in many ways. Many of the changes observed in the climate are occurring at unprecedented levels, and some of them are irreversible. Furthermore, climate change can disrupt food availability, reduce access to food, and affect food quality. Projected increases in temperatures, salinity, drought, and excessive water may all result in reduced agricultural productivity. Therefore, our project is important to find improved ways to produce more crops from existing resources and combat the effects of climate change that are currently taking place.

## MATERIALS AND METHODS

### Experimental Design

The plants in this experiment were *Hordeum vulgare* (barley), *Raphanus sativus* (radish), *Pisum sativum* (pea), and *Fagopyrum esculentum* (buckwheat). These four different plant species were obtained from Carolina Biological and used to create four plant-derived smoke water solutions, respectively. Afterwards, for each type of plant, experimental groups were established to test the effects of smoke water under various environmental stress conditions—including temperature, salinity, drought, excessive water, and no stress—with and without the addition of smoke water (**Table 1**). The control group consisted of all containers receiving filtered tap water—water with no added smoke. The experimental conditions were based on projections for the natural environment with consideration of the impact of climate change.

### Smoke Water Creation

For making smoke water, radish, buckwheat, barley, and pea seeds (Carolina Biological) were planted in 12 x 16 cm pots in potting soil obtained from Carolina Biological and allowed to grow to maturity. At maturity, the plants were extracted to create four different smoke waters, one made from each type of plant. Smoke water was made using a modified procedure as previously described (23). In short, one container was sealed with 10 g of plant material from one plant type, and a tube was run from this container to a second container filled with 1 L of distilled water; both containers were sealed at their openings using aluminum foil. The container with plants was placed on a hot plate and heated to 300°C for 10 minutes. Smoke from the heated plants dissolved into the water in the water beaker (**Figure 4**).

### Smoke Water Treatment

Around 25 seeds for a particular plant were placed in a petri dish with a Whatman Grade 1 filter paper soaked with its corresponding smoke water or normal water for germination studies (24). Data collection was done on the first two days of germination—24 hours after the seeds were placed in the petri dish was considered Day 1, and 48 hours afterward was considered Day 2. Percent germination was recorded and compared by dividing the number of seeds with a radicle poking out by the total number of seeds of that plant type in the petri dish and multiplying by

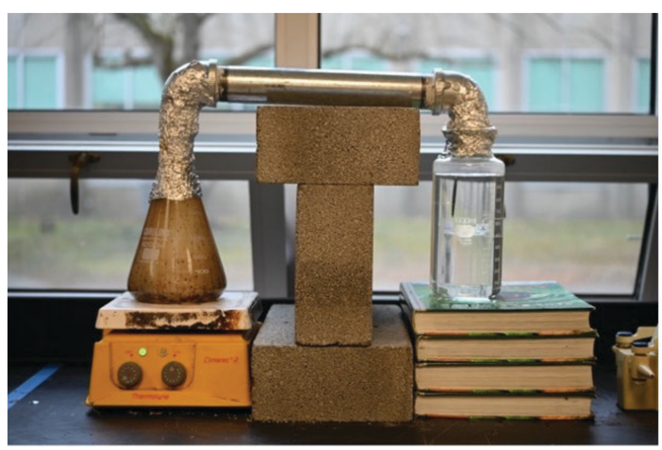

**Figure 4: Smoke solution system.** A flask, which contains the plant material, is located on a hot plate (left). A tube from the plant container connects to a fleaker with 1 L distilled water (right). The system is sealed with aluminum foil to prevent any gas from escaping. Smoke from the heated plants will dissolve into the water in the water fleaker.

100. While different seeds do have differing germination requirements, in order to keep variables consistent in our experiment, the time allotted for germination—two days—was kept constant. Afterwards, all of the plant seeds were placed in planting pots and watered every day with 20 mL of corresponding smoke water or normal water (24).

### Exposure to Stress

Planted pots were exposed to the corresponding abiotic stresses. Plant height was measured and recorded once every day for all of the plants from the base of the plant, where it meets the soil, to its highest point. Percent survival was calculated and compared for all of the plants at the end of the two-week period by dividing the number of plants after two weeks of being planted in pots by the total number of seeds planted and multiplying by 100. Increased temperature was determined by taking the average maximum growing temperatures for each plant species (barley: 25°C, radish: 18°C, buckwheat: 21°C, pea: 16°C) (25-28). To simulate heat stress, four separate environments were maintained at 26°C, 19°C, 22°C, and 17°C using heat lamps set to these specific temperatures and controlled by a timer (25-28). As such, once the temperature falls below the average growing temperature, the lamp turns on, then when it hits the designated temperature, the lamp turns off. Increased salinity was considered 0.21 M of kosher salt (29). Increased drought was considered a 50% decrease from the normal amount of water required by each plant as per growing instructions (30). Excessive water was considered a 150% increase from the normal amount of water required by each plant, and the drainage holes were covered for 48 hours to make sure the excessive water did not drain (31).

### Statistical Analysis

A two-tailed *t*-test was conducted for all plants and conditions to see if there was significant difference between the smoke water test group and the no-smoke water test group within a single species. A significance level of 0.05 was used. The statistical analysis was conducted in Socscistatistics.com.

**Received:** July 13, 2023
**Accepted:** May 13, 2024
**Published:** April 8, 2026

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