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Project Title:

Increasing the Desiccation Tolerance of *Eragrostis tef* through Exogenous Application of Abscisic Acid to Ensure Food Security

Rationale:

With the world population expected to reach almost ten billion by 2050 and the large loss of agricultural land due to climate change, food security has become one of the largest issues for governments and farmers today. This loss of viable farming land is due to drought or lack of rainfall, which causes crop death. Drought has now become the most expensive and destructive natural disaster for farmers in developing countries (FAO, 2017). Between 2005 and 2015, drought cost the developing world agriculture industry $29 billion (FAO, 2017). In the United States, $10 to $14 billion are lost each year to drought (Kuwayama, 2019). Currently, about 11 percent (1.5 billion hectares) of land on Earth is used for farming (FAO, 2015). The United Nations Environment Programme (UNEP) published a study that found that arable land is being lost at 30 to 35 times the historical rates. Meanwhile, the world population continues to increase, consequently increasing food demand. Up to 52 percent of the land used for agriculture is moderately or severely affected by soil degradation (UN Sustainable Development Goals, 2018). Additionally, the world loses 23 hectares of arable land worldwide every minute to drought and desertification; yearly, this adds up to 12 million hectares lost (UNEP, 2018). To ensure that the growing population will have sufficient food, the world will need to see a 69 percent increase in food calories production, but it is becoming harder and harder for farmers to increase their yield as more and more land is lost to drought (Ranganathan, 2013).

Eragrostis tef, the focus species of this study, is a tall grass and a staple crop for Ethiopians. It is primarily found in Ethiopia and in certain parts of South Africa. It is gluten free

as well as highly nutritious, making it valuable for agriculture. However, it is an orphan crop, meaning that it isn't internationally traded; therefore, it gets less research funding and has not been as extensively studied as other crops (Mechael, 2015). It is drought tolerant, meaning it can survive with low environmental water availability while maintaining high internal water content (Ntuli, 2012). However, it is only partially desiccation tolerant, defined as the ability of a living structure to survive drying to equilibrium with low (< 50%) relative humidity and maintain low intracellular water concentrations (Ntuli, 2012). A drought-tolerant organism that is not desiccation-tolerant will die if it loses much of its water, whereas a desiccation-tolerant organism will survive under the same conditions (Ntuli, 2012). Increasing its desiccation tolerance facilitates greater survival in areas with little water.

Given the social and economic impacts of climate change, more research is being done to explore how to increase desiccation of crops and promote food security; especially in developing nations. As *Eragrostis tef* is an important staple crop for several developing nations, increasing its desiccation tolerance could provide food security for millions of people.

Research Question:

Does exogenous application of Abscisic Acid increase the desiccation tolerance of *Eragrostis tef* and could this provide food security for the future?

Hypothesis:

Exogenous application of Abscisic Acid will increase the desiccation tolerance of *Eragrostis tef*.

Procedures:

Part 1: Optimization of Experiment

1. Preparation of Dilutions (6 concentrations)
    a. 0 µM, 5µM, 10 µM , 15 µM, 20 µM (stock solution)
2. Preparing the seeds
    a. Put seeds and ABA in refrigerator for four days prior to experiment
    b. Set up 24 petri dishes and put 4 layers of filter paper in each one

c. Label each dish with the seed order number and concentration of ABA
d. For each genotype, separate the seeds into six different groups with equal amounts of seeds in each one, and place each group in a petri dish
e. Add 10 mL of each respective concentration
f. The next day, add 10 mL more to ensure that the seeds stay soaked

3. Planting
    a. Mix fertilizer into soil as directed
    b. Fill pots up with spoil until 2 inches are left
    c. Label each pot with seed type and divide each pot into two equal parts, for two different concentrations (0μM and 5μM, 10μM and 15μM, 20μM and 1mM)
    d. Place 5 seeds in each division of the pot with a pipette and leave equal amounts of space between each seed
    e. Place pots on a light cart and leave on a 16-8 hour light timer, at 23℃
4. Data Collection
    a. Relative Water Content
    b. Electrolyte Leakage

Part 2: Experimental Procedure

Role of mentor

The mentor will teach the student how to create the callus-inducing and regeneration media, as well as how to culture the calli. Additionally, the mentor will help performing the stressor assays.

Role of student

Culturing the Seeds:

- 800 mL of 2-4 D callus-inducing medium will be made and poured into petri dishes
- The *Eragrsotis tef* seeds will be decontaminated in the Laminar Air Flow
- 100 *Eragrsotis tef* seeds will be cultured in the 2-4 D medium-containing petri dishes
- There will be 20 dishes, each with five seeds

- The petri dishes will be Parafilmed shut to ensure sterility and wrapped in foil to ensure no light stress affects the plants
- The dishes will be checked after three days for contamination

Creation of Solutions:

- A stock solution of 0.2M will be created
- It will then be diluted to a 20μM solution by using 1 mL of stock solution in 10 mL of water

Application of Treatments:

- After four weeks of induction, the treatments will be applied to the calli
- 1 mL of either water or ABA solution will be applied to each petri dish
- The control group will be left unopened
- After application, the dishes will be shut with Parafilm and wrapped in foil again, then placed in the Conviron
- The treatments will be left for 72 hours

Seedling Regeneration:

- After 72 hours, the calli will be transferred into petri dishes containing regeneration medium to allow germination
- The calli will grow for a week, then will dry under the Laminar Air Flow for 48 hours as a desiccation stress

Stressor Assays:

- Three different stressor assays will be run: relative water content, electrolyte leakage, and chlorophyll and carotenoid content
- An ANOVA and post-hoc Tukey test will be performed to calculate significance in treatments.

Risk and Safety

a. Supervision: The mentor will supervise the student at all times when working in the laboratory.
b. Safety Precautions: The student and mentor will wear laboratory coats, gloves, and goggles at all times when handling chemicals.
c. Methods of Disposal: The hazardous waste will be placed in labeled red biohazard waste bins

Data Analysis

After the three stressor assays are performed, all the data will be recorded and then analyzed using Prism Graphing. An ANOVA test and Tukey test will be performed to calculate the significance of each treatment.

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