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Geobacillus Stearothermophilus as Growth Booster and an Agent of Heat Resistance for the Germination of Cold-tolerant Rice Varieties

Archie Urbiztondo
Surigao State College of Technology, Philippines

Abstract— *The study aimed to test the potential of Geobacillus stearothermophilus in aiding the rice seeds to grow faster and germinate in high temperatures. Geobacillus stearothermophilus is a gram-positive thermophilic (heat loving) bacteria characterized by an inner cell membrane and a thick cell wall, organisms that grow in extremely hot places [1]. The study utilizes an Experimental design (CRD) and focused only on the collected samples of rice seeds, G. stearothermophilus, and testing the said bacteria on three different set-ups with a certain variety of rice seeds. A total of 2400 rice seeds from three (3) cold-tolerant and one (1) local rice varieties were collected from the Philippine Rice Research Institute treated with bacterium G. stearothermophilus purchased at the Philippine National Collection of Microorganisms, at University of the Philippines - Los Banos, Laguna. The recorded results in the rate of germination at 40^{O}C, 35^{O}C, and 28^{O}C exposed with Geobacillus stearothermophilus shows 100% rate of germination true to all variety of rice. However, the control (BP 125) rice variety (cold-tolerant rice variety), without Geobacillus stearothermophilus, surprisingly showed a 3% rate of germination on the first (40^{O}C) and third (28^{O}C) treatment, while it showed a 5% rate of germination on the second (35^{O}C) treatment. Since the bacteria was mixed with the rice seeds, the first three rice varieties became heat-resistant and have a higher rate of germination than the control rice variety. The results indicate optimal growth for roots and shoot of the said cold-tolerant rice seed varieties.*

Keywords— *Growth Booster, Geobacillus stearothermophilus, cold-tolerant rice varieties, germinating agent.*

I. INTRODUCTION

The Philippines is one of the many countries most vulnerable to adverse effects of climate change. It has only become warmer in the last few decades but it has also become drier. Data show a 6% decline in annual rainfall for the past century. The Philippines experiences an average of 2,325 mm of rainfall and 23.7^{o}C of annual mean temperature [4][17].

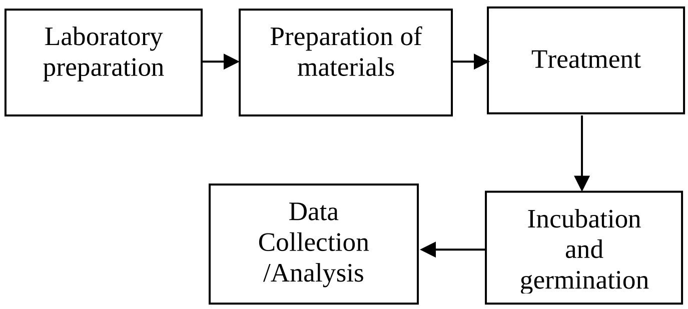
Change in rainfall patterns and more frequent floods or droughts can cause changes in the cropping season and changes in the crop growing period. Acute water shortage and thermal stresses due to drought can lead to lower agricultural crop productivity, particularly in rice. [16],[17]

The increasing temperature and low crop yield caused by climate change prompted the researchers to develop a growth booster and an agent of heat-resistance for the germination of cold-tolerant rice.

Geobacillus stearothermophilus (formally *Bacillus stearothermophilus*) is a rod-shaped, Gram-positive bacterium and a member of the division Firmicutes. The bacteria is a thermophile and is widely distributed in soil, hot springs, ocean sediment, and is a cause of spoilage in food products. It will grow within a temperature range of 30-75 degrees Celsius. Some strains are capable of oxidizing carbon monoxide aerobically.

The aid of *Geobacillus stearothermophilus*, a thermophile, can be of great help in the possibility of producing a growth booster and an agent of heat-resistance for the germination of cold-tolerant rice. This can survive within the temperature range of 20-75 degrees Celsius. It has "amazingly been observed to have formed endospores and survived in high temperature (as high as 140 degrees Celsius)."

II. MATERIALS AND METHODS



1. Preparation of Bacterial solution

A loopful of *G. Stearothermophilus* was inoculated at the 100 mL distilled water that underwent heating process at about 50^{o}C

2. Preparation of Rice Seeds

The rice seeds were separated into their respective varieties and were separated into twenty-four (24) petri dishes for three (3) treatments and two (2) replicates). Every petri dish has one hundred (100) rice seeds respectively.

3. Application of Bacteria

The rice seeds were transferred into the bacterial solution and only those viable seeds were included. They were stirred to let the *Geobacillus stearothermophilus* be equally distributed in the solution. The rice seeds were soaked at the bacterial solution and were left in an isolation room at room temperature for 24 hours.

4. Preparation of the Treatments

After a long day of soaking, 100 rice seeds in every beaker were placed in a paper towel and the bacterial solution in every beaker was put into a sprayer. The rice seeds in the paper towel

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were rolled and put into different treatments and were treated with 10 mL bacterial solution in their respective varieties. The treatments are as follows:

Treatment 1 - Rice seeds with bacterial solution put into 40ºC set incubator

Treatment 2 - Rice seeds with bacterial solution put into 35ºC set incubator

Treatment 3 - Rice seeds with bacterial solution put into 28ºC set incubator (room temperature during summer)

5. Incubation and Germination

The treatments were laid into an incubator and in an isolation room. The first treatment was sprayed with bacterial solution (10 mL) every two (2) hours to prevent the paper towel to dry up; the second treatment was sprayed with bacterial solution (10 mL) every three (3) hours; the third treatment was sprayed with the same solution (10 mL) every three (3) hours, and the control rice variety was sprayed with only distilled water (10 mL) instead of the prepared bacterial solution. The spraying was necessary to ensure that the paper towel would avoid being dried up.

6. Data Collection

The experiment was performed using a completely randomized design (CRD) in Philippine Rice Research Institute at Basilisa, Remedios T. Romualdez, Agusan del Norte in three repetitions. The treatment included 4 varieties that include RC 46, GSR 12A, RC 44, and BP 125 rice variety.

Three levels of temperatures (28°C, 35°C, and 40°C) as suggested to be heat-tolerant and high-yielding after a series of field and laboratory heat stress screening. [1],[5]

The length of the roots and shoots were measured in every sample treatment in millimeters (mm), then average values were recorded. All statics were performed with SPSS program (Version 16). Two-way ANOVA with replications was used for different temperatures and different treatments and performed a Duncan multiple range test for the comparisons of means in a significance level of 1 percent.

III. RESULTS AND DISCUSSIONS

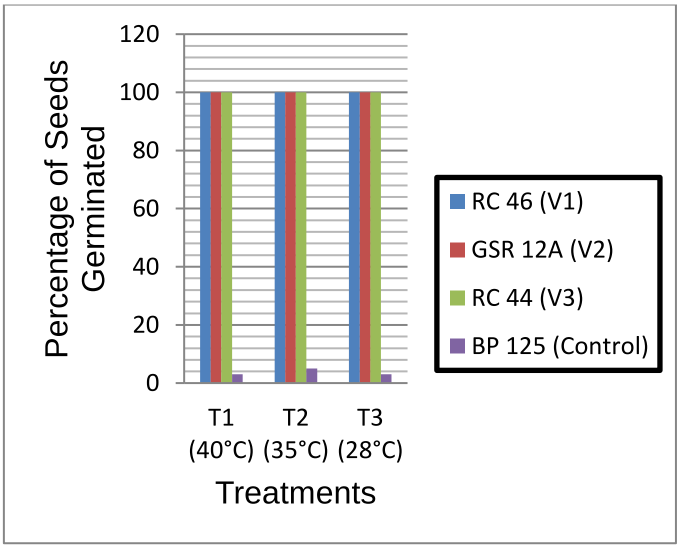

Figure 1. Rate of Germination

The recorded results in the rate of germination at 40^{O}C, 35^{O}C, and 28^{O}C exposed with *Geobacillus stearothermophilus* shows 100% rate of germination true to all variety of rice. However, the control (BP 125) rice variety (cold-tolerant rice variety), without *Geobacillus stearothermophilus,* surprisingly showed a 3% rate of germination on the first (40^{O}C) and third (28^{O}C) treatment, while it showed a 5% rate of germination on the second (35^{O}C) treatment. Since the bacteria was mixed with the rice seeds, the first three rice varieties became heat-resistant and have a higher rate of germination than the control rice variety.

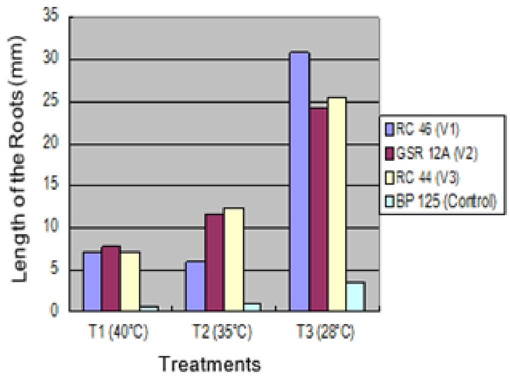

Figure 2. *Length of Roots vs. Different Treatments*

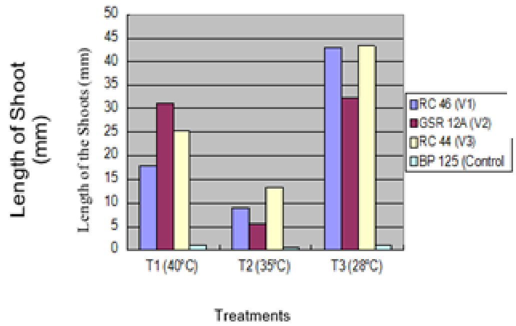

Figure 3. *Length of Shoot vs Different Treatments*

Although the results show a difference in the roots and shoot of the rice seedlings, it also shows the possible potential of *G. stearothermophilus* to be used as a germinating agent and aid the cold-tolerant rice resistance from extreme heat. The first and second treatments explicitly show that the cold-tolerant rice seeds exposed to *G. Stearothermohilus* grew and were able to resist extreme heat. The third treatment explicitly shows that in normal room temperature (28^{O}C) with *G. Stearothermophilus*, rice seeds met optimal growth while in the control set-up without *G. Stearothermophilus* it was clear that growth seem minimal. According to the data given above, the highest average height of roots and shoot is the one in treatment 3 (rice seeds exposed at room temperature). The lowest average height of roots is the one in the first treatment (rice seeds exposed in 40ºC set incubator) and the lowest average height of shoot is the one in the second treatment (rice seeds exposed in 35ºC set incubator). According to the researched literature: "Geobacillus stearothermophilus grows within a temperature range of 30-75 degrees Celsius" The *G. stearothermophilus* helped the rice seeds grow and became heat resistant, therefore, being the basis of the results in all treatments. The first three treatments proved with the height of their roots and shoot and how the cold-tolerant rice varieties adapted to extremely hot temperatures and survived. The fact that the growth performance varied, several other factors also affect the rate of germination such as the exposure to carbon dioxide and oxygen, exposure to sunlight, and temperature, this is now the reason in treatment 3 (28°C) which has optimal growth.

IV. CONCLUSION

The *Geobacillus stearothermophilus* is also a good agent of heat-resistance during the germination of cold-tolerant rice varieties. Results indicate significantly that the three rice varieties with bacterial solution had the greatest percentage of growth than the control rice variety.

The *Geobacillus stearothermophilus* proved to be an effective growth booster during the germination of cold-tolerant rice varieties. The results indicate optimal growth for roots and shoot of the said cold-tolerant rice seed varieties.

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APPENDIX