

Improvement of Sugarcane Seeds Drought Stress Tolerance by Invigoration using of *Trichoderma* as Bio-Primer

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Received:- 13 April 2023/ Revised:- 17 April 2023/ Accepted:- 24 April 2023/ Published: 30-04-2023

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Abstract— *Trichoderma* used in this study resulted in a density of 1.4×10^{-9} with their morphology had septate mycelium, rounded or oval conidia shape and attached to each other, has a bright green color and branched conidiophores. The biopriming treatment had a significant effect on germination, germination rate, vigor index and total chlorophyll content of sugarcane that was stressed by drought. Soaking for a long time (24 hours) caused a decrease in the percentage of germination in sugarcane seeds by 12.69%, the vigor index of 1.06 and short immersion also caused a decrease in the percentage of germination by 10.61% and a decrease in the average vigor index by 21.74. The 12-hour soaking treatment was a better treatment than control to increasing germination rates under drought stress, it was 12.5% specifically and fast germination rate of 3.76 days. Sugarcane germination rates was reduced if the soaking process is carried out in brief time and vice versa. The highest vigor index value was produced by biopriming treatment for 12 hours, which was 81.74. Biopriming treatment using secondary metabolites of *Trichoderma* was increasing the total chlorophyll content in sugarcane germination by 0.22 g/ml compared to without soaking treatment.

Keywords— *Fungi; Germination; Priming.*

I. INTRODUCTION

Sugarcane growth consists of germination, budding, vegetative growth, and maturity phases. Determination of the next phase is determined by the germination phase which is influenced by seed quality, glucose, nitrogen, water, seed treatment [1]. The problem in the field, especially in West Borneo, availability of superior sugarcane planting material was limited and forcing farmers to buy seeds from overseas. As that result, the seeds fall on deteriorate and the quality of the seeds decreases due to the delivery process which takes several days. In the shipping process, especially in the tropics, temperature fluctuations will occur, with the recalcitrant nature of sugarcane seeds, the seeds cannot withstand storage conditions at 10°C and above 35°C so they will be through chilling injury and reduce their viability [2]. Seeds that have low quality can reduce their vigor and viability, which in turn reduces plant productivity. In West Borneo during the long dry season experienced drought conditions so that water supply was limited. Though sugarcane seeds require a lot of water, one of which is during the germination phase. Drought stress in the germination phase affects the quality, growth in the next phase. Each sugarcane clone produce a different expression in response to drought stress. Research by [3] showed that the PS881 clone was able to adapt to conditions drought stress in the early growth phase in unfavorable seed conditions experiencing storage. The main key in increasing crop production is healthy seeds that have viability and vigor of more than 85%. According to [4] that the highest growth and yield was produced by sugarcane seeds that were not stored.

The existence of the above problems, one of the strategies to solve them can be through biopriming techniques using biological agents *Trichoderma* spp by utilizing the secondary metabolites produced. This technique is often used in food crops, but not many have applied this technique to plantation seeds, especially sugarcane. Biopriming is the immersion of seeds in a suspension of biological agents so as to activate signals to maintain stress and increase vigor [5]. *Trichoderma* was also able to increase resistance to abiotic stresses, one of which is drought, nutrient absorption, and increasing root growth and development. *Trichoderma* produces phytohormones such as Indole Acetic Acid (IAA) which is stimulating root and shoot growth [6].

Trichoderma priming technique gave a significant increase in the content of phenol, proline, stomata conductance, photosynthesis, chlorophyll content of wheat plants under drought stress conditions [7]. This technique had also been increasing the size of the seed germination, vigor index and root-shoot growth [6]. Biopriming of seeds using *Trichoderma harzianum* for 4 hours at 40% concentration resulted in a germination rate of 78%, an index of germination speed of 9.2 in chickpeas [8]. Priming for 24 hours increased vigor, average germination time and increased okra yield up to 55% [9]. In another study, *Azoospirillum* 20% biopriming treatment for 12 hours was the best treatment for maize germination and vigor [10].

Research on the use of secondary metabolites of *Trichoderma* in the invigoration of drought stress tolerant sugarcane clones using biopriming technique can be used to improve the quality of sugarcane seeds after experiencing setbacks due to storage and increasing plant resistance in drought stress conditions and can be further developed in sugarcane cultivation in suboptimal land. This study aimed to analyze the effect of *Trichoderma* biopriming on drought stress sugarcane clone germination, determine the appropriate duration of *Trichoderma* biopriming for drought stress sugarcane clone germination, determine drought stress sugarcane seed vigor index with *Trichoderma* biopriming application.

II. RESEARCH METHODS

The research was carried out at the Plantation Plant Science Laboratory and Greenhouse, Pontianak State Polytechnic, West Borneo, for 3 months

2.1 Materials and Equipment

The materials used were *Trichoderma* spp, potato, dextrose, aquadest, water, aluminium foil, mineral soil, dolomite, cow manure compost, fungicide, West Kalimantan, Indonesia local sugarcane budchips, NaClO. The equipment used were soil sieve, hoe, ruler, thermometer, test tube, Erlenmeyer, beaker glass, stirrer, microscope, preparations, clear nail polish, 21D spectrometer, analytical balance.

The study was structured using a Completely Randomized Block Design with 4 times replications. The treatments used were variations of biopriming, T0: Without immersion, T1: 6 hours, T2: 12 hours, T3: 18 hours, T4: 24 hours.

2.2 Research Implementation

Biopriming treatment by immersing the budset into a suspension of *Trichoderma* with the appropriate time of treatment. Before the biopriming treatment, sugarcane buds had been stored for 7 days were disinfected using 2% NaClO for 5 minutes and rinsed thoroughly with distilled water. *Trichoderma* was propagated using Potato Dextrose Broth (PDB) media and incubated for 5 days until conidia formed [11]. Determination of spore density was calculated using a haemocytometer by dripping a suspension of conidia that had been diluted to 10^8 with 400 times magnification [12]. The spore density had been calculated by the formula:

$$C = \frac{t \times d}{n \times 0,25} \times 10^6 \quad (1)$$

Annotation:

C	= conidia density per ml of solution
t	= number of conidia in the observed box
n	= number of boxes sample
0,25	= small-scale sample box correction factor on haemocytometer
d	= dilution factor
10^6	= conidia constant value

After application, the budset was germinated into a seedling basket with Podsolic soil mixed with compost and then maintained for 30 days. Observations made in the study are as follows:

2.2.1 Seed Germination (SG)

To measure the viability of the observed budset daily.

$$SG = \frac{\sum \text{normal sprouts}}{\sum \text{Total seed planted}} \times 100\% \quad (2)$$

2.2.2 Germination Rate (GR)

To see the ability to germinate speed [13]

$$GR = \frac{N_1T_1+N_2T_2+\dots+N_xT_x}{\Sigma \text{total sprouts}} \quad (3)$$

Annotation:

N = number of budset germinated at day x

T = germination at day x

2.2.3 Vigor Index (VI)

To measure the ability of the budset to grow [14]

$$VI = \frac{\Sigma \text{normal sprouts}}{\Sigma \text{total sprouts}} \quad (4)$$

2.2.4 Total Chlorophyll Content (TCC)

Indicator of plant tolerance in response to drought stress measured using a UV-VIS spectrophotometer with wavelengths of 645 nm and 663 nm [15]

$$TCC = 20,2 A_{645} + 8,02 A_{663} \quad (5)$$

2.2.5 Relative Water Content (RWC)

This observation was carried out with fresh weight (FW), dry weight (DW) and leaf turgor weight (TW) obtained by soaking the leaves in water for 24 hours [7]

$$RWC = \frac{(FW-DW)}{(TW-DW)} \times 100\% \quad (6)$$

2.3 Statistical Analysis

Observational data were analyzed by ANOVA and if there was a significant effect, then further tested with the Least Significant Differences (LSD) test at 5% level.

III. RESULTS AND DISCUSSION

Trichoderma propagation carried out in this study resulted in a density of 1.4×10^{-9} (Figure 1) with a morphology of septate mycelium, conidia shape that is round or oval and one cell attached to each other, has a bright green color and branched conidiophores. *Trichoderma* is a fungus that is generally found in root areas or free-living soil. *Trichoderma* plays a role not only in reducing the negative effects on plants in the face of drought stress but also increasing growth significantly. This fungi has an easily recognizable character and grows fast and matures at 5 days of growth [16]. *Trichoderma* is able to adapt to different living environments, whether in a humid, dark or dry environment by regulating the growth of conidia and enzyme production [17].



FIGURE 1: *Trichoderma* spp.

TABLE 1
AVERAGE OF SEED GERMINATIONS, GERMINATION RATES AND VIGOR INDEX ON SUGARCANE SEEDS DROUGHT STRESS TOLERANCE BY OF TRICHODERMA SECONDARY METABOLITES AS BIOPRIMER.

Bioprimer Treatment	Seed Germination (%)	Germination Rate (day)	Vigor Index
T0 (without soaking)	81.06 c	5.73 a	49.37 b
T1 (6 hours)	87.31 bc	4.98 a	60.00 b
T2 (12 hours)	93.56 ab	3.76 b	81.74 a
T3 (18 hours)	97.92 a	3.58 b	80.11 a
T4 (24 hours)	85.23 bc	3.68 b	80.68 a
Average	89.016	4.34	70.38

Annotation: Numbers followed by the same letter within a column are not significantly different according to the least significant difference (LSD) test.

2.4 Seed Germination

The results of analysis of variance showed that the bioprimer treatment had a significant effect on the germination of sugarcane under stress. The bioprimer treatment for 18 hours (T3) resulted in the highest germination rate of 97.92%, while the bioprimer treatment for 12 hours (T2) slightly decreased the percentage of germination but statistically, it was not significantly different with 18 hours soaking. Sugarcane seeds without soaking resulted in the lowest germination rate of 81.06%, which was not significantly different from soaking for 6 and 24 hours (Table 1). Soaking too long (24 hours) and too short caused a decrease in the percentage of germination in sugarcane seeds with a decrease of 12.69% and 10.61%, respectively. Soaking sugarcane seeds using *Trichoderma* for too long (24 hours) or too short causes a decrease in germination power of 10.61% - 12.69%. This means that the 12-hour soaking treatment is a good treatment to increase the percentage of sugarcane germination under stress. These results are in accordance with research conducted by [6] who said that the *Trichoderma* isolate test could increase the germination percentage of wheat seeds by 87% compared to the control which only got 72.7% of seed germination. The increase in germination was due to *Trichoderma* produce secondary metabolites in the form of Indole Acetic Acid (IAA), which can stimulate meristem cells to trigger the emergence of roots and shoots. IAA is an unstable auxin that functions in root formation and division and enlargement of plant shoot cells.

2.5 Germination Rate

The treatment of immersion bioprimer *Trichoderma* affects the rate of germination of sugarcane seeds under stress. Sugarcane seed germination takes longer if the soaking process is carried out for a short time and will be slightly faster if soaked for a longer time. Sugarcane clones soaked in *Trichoderma* for 18 hours (T3) resulted in the fastest germination rate of 3.58 days, but it was not significantly different from soaking for 12 hours (T2) and 24 hours (T4), while without soaking it produced the longest germination rate of 5.73 days that were not significantly different with 6 hours of treatment (T1) (Table 1). Sugarcane seeds soaked in *Trichoderma* for 12 hours resulted fast germination rate of 3.76 days. The duration was related to the imbibition process. Therefore, 12 hours of soaking the seeds can complete the metabolic process, including starting from the imbibition process of *Trichoderma* suspension. *Trichoderma* can induce various types of signals that can be transmitted to plants. Its secondary metabolites produced in the form of salicylic acid, jasmonic acid or Reactive Oxygen Species (ROS) can trigger plant defense proteins through the induction of systemic resistance [18]. In the priming process, the plant defenses are not activated directly by *Trichoderma*, but the response of plant defenses to environmental stresses is accelerated, besides that *Trichoderma* can encourage plant growth and development. The faster plant growth is influenced by the production of auxin and the activity of the deaminase enzyme which reduces the ethylene content that accumulates during stress. In addition, *Trichoderma* also produces organic acids that can increase the solubility of phosphate, magnesium, manganese so that it can induce roots in the germination process [19]. The presence of *Trichoderma* which is applied to seeds is useful as a barrier to the physical and chemical environmental stresses of the soil in the form of salinity stress caused by lack of water in the soil or drought. In saline soil conditions due to drought *Trichoderma* is able to produce secondary metabolites in the form of acid phosphatase, harzianolides and peptaibols which stimulate root branching and absorption of plant nutrients [20].

2.6 Vigor Index

The vigor index of drought-stressed sugarcane clones was influenced by the immersion of *Trichoderma*. Sugarcane seeds soaked in *Trichoderma* for 12 hours (T2) showed the highest vigor index compared to those without soaking and soaking for 6 hours but not significantly different from 18 hours and 24 hours immersion (Table 1). Short and too long soaking will reduce the average vigor index by 21.74 as well as if sugarcane seeds are soaked in *Trichoderma* for too long it also reduces the vigor index by 1.06. The highest vigor index value was produced by bioprimering treatment for 12 hours, which was 81.74. During the priming process for 12 hours there will be activation of enzymes that play a role in the formation of sucrose so that the germination rate increases through decreased lipid prooxidation activity. The priming duration for 12 hours will increase the germination index and decrease the priming duration will cause a decrease in the germination index [21].

2.7 Total Chlorophyll Content (TCC) and Relative Water Content (RWC)

The immersion of *Trichoderma* in sugarcane budset affected the chlorophyll content, while the relative water content was not affected by the bioprimering treatment. Soaking for 12 hours produced the highest total chlorophyll but not significantly different from 18 and 24 hours immersion. Meanwhile, the budset that was not soaked by *Trichoderma* produced the lowest total chlorophyll which wasn't significantly different from that of soaking for 6 hours. The bioprimering treatment of *Trichoderma* will increase the total chlorophyll content compared to not soaking, but if the immersion time is too long it will decrease the total chlorophyll content by 0.07 g/ml. Chlorophyll is an indicator of drought stress in plants. Plants that experience drought stress will experience chlorophyll damage, both total chlorophyll, chlorophyll a and chlorophyll b due to inhibition of magnesium absorption which is a plant chlorophyll pigment forming. *Trichoderma* spp bioprimering treatment can increase the total chlorophyll content in sugarcane germination compared to without soaking treatment. Based on [22] *Trichoderma* inoculation can increase the total chlorophyll content in tomato plants by 15.04%.

TABLE 2

TOTAL CHLOROPHYLL CONTENT (TCC) AND RELATIVE WATER CONTENT (RWC) ON SUGARCANE SEEDS DROUGHT STRESS TOLERANCE BY OF TRICHODERMA SECONDARY METABOLITES AS BIOPRIMER.

Bioprimering Treatments	TCC (g/ml)	RWC (%)
T0 (without soaking)	0.48 b	85.16
T1 (6 hours)	0.52 b	86.77
T2 (12 hours)	0.70 a	87.37
T3 (18 hours)	0.75 a	85.98
T4 (24 hours)	0.68 a	83.64
Average	0.626	85.78

Annotation: Numbers followed by the same letter within a column are not significantly different according to the least significant difference (LSD) test.

IV. CONCLUSION

The bioprimering treatment of *Trichoderma* had an effect on the germination of sugarcane clones under stress. The duration of bioprimering of *Trichoderma* for 12 hours (T2) is the appropriate time for germination of sugarcane clones under stress. Vigor index of sugarcane seeds under stress with the application of bioprimering *Trichoderma* for 12 hours is 81.74.

ACKNOWLEDGEMENTS

The author would like to express his deepest gratitude to the Research and Community Service Unit (UPPM) of the Pontianak State Polytechnic for funding and supporting this research. We would also like to thank the Department of Agricultural Technology for technical support during the research and publication process.

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