ISBN : 978-623-331-387-2

Screening of Indigenous Rhizobacteria for Drought Stress Tolerance on Maize (*Zea mays* L) Seed Germination

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ABSTRACT

Drought stress is still often a problem in maize cultivation, from germination to vegetative and generative growth. The germination process is hampered when there is drought, because imbibition is not perfect. Rhizobacteria have the potential to produce exopolysaccharides that can increase maize germination under drought stress conditions. This study aims to select indigenous rhizobacteria isolates that are able to provide better germination of maize seeds in drought conditions, using PEG 6000 10%. The number of selected rhizobacteria isolates was 20 isolates, and the study was repeated 2 times. Germination parameters observed were germination percentage (%), seedling height (cm), number of plumule leaves (strands), radicle length (cm), number of root hairs, wet weight of seedling (g), and dry weight of seedling (g). Observations were made when the sprouts were 7 days after planting (DAT). The results obtained 3 (three) rhizobacteria isolates, namely isolates $B_{4(6)}$, $B_{4(8)}$, and $B_{5(19)}$ which can increase seed viability and vigor under drought stress conditions. Isolate $B_{4(8)}$ increased germination percentage, seedling height, radicle length, number of root hairs and seedling wet weight. Isolate $B_{5(19)}$ can improve the percentage of germination, radicle length, number of root hairs, wet weight and dry weight of seedlings. Isolate $B_{4(6)}$ increased the number of root hairs and dry weight of seedlings.

Keywords:

Drought stress, rhizobacteria, germination, maize

INTRODUCTION

Corn development in dry land often experiences drought stress due to the lack of water availability, especially if the water source in dry land is only rainwater. Drought stress affects the imbibition process which can inhibit the germination process. The germination phase as the initial phase of plant growth determines the growth and development of plants. Efforts to optimize the use of dry land in agriculture by using varieties that are tolerant of drought stress. Drought tolerant maize varieties can be seen starting from the germination phase. Seedlings with longer roots, more root branches, and higher root dry weight can grow and produce better [1].

Tolerance to drought stress can also be done through microbial inoculation. Rhizobacteria can overcome drought stress by producing exopolysaccharides (EPS). These compounds are produced as a response of rhizobacteria to biotic and abiotic stresses [2]. The exopolysaccharides produced by bacteria are not only capable of protecting bacteria but also can cause better environmental conditions in the rhizosphere. Rhizobacteria produce exopolysaccharides to form biofilms that can increase the resistance of rhizobacteria cells under drought stress conditions [3]. Exopolysaccharides are able to increase water retention around the root area, so that water is easily absorbed by plant roots, besides that exopolysaccharides also act as adhesives that can increase the stability of soil aggregates, aeration and soil porosity [4]. Application of rhizobacteria can increase the growth of corn plants under drought stress conditions [5].

Methods to obtain drought stress conditions can be done using PEG, which is a material to control water potential and cannot be absorbed by plants. PEG 6000 with a concentration of 10% in germination media was effectively used to see tolerance to drought stress [6]. In this study, PEG 6000 with a concentration of 10% was used in the germination media of corn seeds that had been soaked in rhizobacterial culture. This study aimed to select the ability of rhizobacteria in increasing the growth of corn sprouts in drought stress the rhizobacteria used in this study were isolated and characterized by the rhizosphere bacteria of mustard plants, in previous studies.

METHOD

This research was conducted at the Plant Protection Laboratory, Faculty of Agriculture, University of Borneo Tarakan. The rhizobacteria isolates used were 20 isolates that were tolerant to drought stress conditions in the previous study. The rhizobacteria isolates were $B_{3(2)}$, $B_{3(7)}$, $B_{3(12)}$, $B_{3(16)}$, $B_{3(19)}$, $B_{4(3)}$, $B_{4(5)}$, $B_{4(6)}$, $B_{4(8)}$, $B_{4\ (11)}$, $B_{4(13)}$, $B_{4(15)}$, $B_{5(2)}$, $B_{5(4)}$, $B_{5(6)}$, $B_{5(8)}$, $B_{5(10)}$, $B_{5(12)}$, $B_{5(14)}$, $B_{5\ (19)}$. The corn seed used is Super Sweet sweet corn.

The seeds were cleaned from the pesticide layer and sterilized using 2% Sodium Hypochlorite for five minutes, then washed with distilled water three times and dried in LAF for one hour. Each rhizobacteria isolate was cultured on NB media and incubated for 48 hours. Corn seeds were immersed in a rhizobacteria suspension with a cell density of 10^8 cells/ml. The soaked seeds were 10 seeds for each rhizobacterial suspension, soaked for 3 hours and for control the seeds were immersed in sterile distilled water. Furthermore, the seeds were planted on sterile sand media that had been doused with 10% PEG 6000 solution until moist. The experiment was repeated 2 times. Parameters observed were percentage of germination (%), seedlings height (cm), radicle length (cm), number of root hairs, wet weight of seedlings (g), and dry weight of seedlings (g). Observations were made at the age of 7 days after planting. Data analysis was performed by calculating the average value of each parameter and the control was used as a comparison for each treatment of rhizobacteria isolates.

RESULT AND DISCUSSION

The results showed that several rhizobacteria isolates gave a higher average value than the control (K0) on several observed parameters (Table 1). The rhizobacteria isolates that gave a higher percentage of germination than the control treatment was isolates $B_{4(8)}$ and $B_{5(19)}$ (80%). Germination percentage parameter indicates the ability of seeds to germinate normally under optimum environmental conditions. The germination process begins with the imbibition process. Under conditions of drought stress, the imbibition process will be disrupted, thereby reducing seed germination. The addition of PEG 6000 causes a decrease in the potential value of water so that the metabolism of the seeds is disrupted due to insufficient water required. The rhizobacteria isolates gave a better percentage of germination, due to the ability of rhizobacteria to produce exopolysaccharides (EPS). The production of EPS increases the ability to absorb water so that it can increase water retention. This causes the treatment of rhizobacteria isolates to provide more water for the imbibition process. The difference in the amount of water that can be provided by rhizobacteria depends on the amount and composition of EPS produced by rhizobacteria. The amount and composition of EPS varies greatly depending on the genus and species of bacteria, in some cases depending on the environmental conditions of bacterial growth [7].

Table 1. Average Germination Parameters in Drought Stress

T	PG	SH	RL	RH	WW	DW
K0		5,00	4,95	18,4	0,465	0,135
$B_{3(2)}$	50	2,70	3,31	8,7	0,395	0,130
$B_{3(7)}$	60	2,50	3,11	5,2	0,425	0,125
$B_{3(12)}$	20	0,20	0,66	0	0,350	**0,140**
$B_{3(16)}$	50	1,31	2,74	3,6	0,290	0,125
$B_{3(19)}$	10	0,88	0,67	3,2	0,280	0,130
$B_{4(3)}$	40	1,92	2,82	8,3	0,370	**0,145**
$B_{4(5)}$	40	1,64	2,30	7,3	0,375	**0,140**
$B_{4(6)}$	50	3,78	4,70	**21,6**	0,460	**0,170**
$B_{4(8)}$	**80**	**6,43**	**6,13**	**29,8**	**0,530**	0,130
$B_{4(11)}$	60	2,85	3,70	10,9	0,425	**0,145**
$B_{4(13)}$	70	4,52	**7,06**	**26,5**	0,455	0,115
$B_{4(15)}$	60	4,31	3,61	**36,8**	0,355	0,120
$B_{5(2)}$	40	2,84	3,08	10,4	0,395	0,120
$B_{5(4)}$	30	2,18	2,32	10,5	0,315	0,125
$B_{5(6)}$	50	3,08	3,92	11,1	0,365	0,110
$B_{5(8)}$	40	1,87	2,73	8,4	0,305	**0,145**
$B_{5(10)}$	50	1,37	3,64	11,8	0,375	**0,155**
$B_{5(12)}$	50	2,67	1,95	11,4	0,395	**0,140**
$B_{5(14)}$	70	4,39	5,47	**22,1**	0,390	0,130
$B_{5(19)}$	**80**	4,01	**7,12**	**40,8**	**0,565**	**0,160**

Note: T = Treatment; PG = Percentage of Germination; SH = Seedling Height; RL = Radicular Length; RH = Root Hair; WW = Wet Weight; DW = Dry Weight.

Isolate $B_{4(8)}$ also gave the best seedling height (6.43 cm) compared to the control and treatment of other rhizobacteria isolates. Seedling height is related to the time it takes for seeds to germinate. Germination time starts from the imbibition process until the appearance of the radicle. $B_{4(8)}$ isolate provided environmental conditions that accelerated the imbibition process and activated enzymes that decompose compounds in the endosperm so that the cell enlargement and elongation process runs faster. The slow imbibition process has implications for the slow activation of enzymes that play a role in germination [8].

The highest average values for the parameters of radicle length, number of root hairs and wet weight of seedlings were shown by the treatment of isolate $B_{5(19)}$. For the dry weight parameter, the isolate $B_{5(19)}$ treatment showed an average value of 0.160 g (second order), the highest was obtained from the isolate $B_{4(6)}$ treatment. It is suspected that isolate $B_{5(19)}$ in addition to producing exopolysaccharides is also capable of producing IAA. IAA phytohormones can affect root development and elongation, increase lateral root and number of root hairs [9]. IAA-producing rhizobacteria isolates were reported to have an effect on the root length of maize seeds [10].

Good root growth and development affects the ability to absorb water and nutrients which will determine the wet weight and dry weight of sprouts. Wet weight is influenced by water content in plant cells, while dry weight is an accumulation of organic compounds synthesized from inorganic compounds. In this study isolates $B_{4(8)}$ and $B_{5(19)}$ produced more exopolysaccharides than other rhizobacteria isolates, so they were able to provide more water. This condition caused the seedlings to absorb a lot of water, so the wet weight of the seedlings in these two treatments was greater than the other treatments. On the dry weight parameter, treatment $B_{4(6)}$ gave the highest average value of 0.170 g. The dry weight of the seedlings is the result of the reshuffle of food reserves (endosperm) and the synthesis of organic compounds from inorganic compounds after forming roots and leaves. In the germination process, the reshuffle of food reserves is used as a building block for new cells so that it contributes to the dry weight of the seedlings [11]. $B_{4(6)}$ and $B_{5(19)}$ isolates were thought to be able to produce gibberellins or activate gibberellins present in corn seeds. Phytohormones that are generally produced by rhizobacteria are auxins, gibberellins, cytokinins, abscisic acid, ethylene and brassino steroids [12]. Each rhizobacteria produces phytohormones with different concentrations. Gibberellins in the germination process play a role in activating enzymes that will remodel the endosperm to produce sugar compounds, amino acids and other simple compounds. If gibberellins are absent or less active, amylase, proteases and other hydrolytic enzymes will not be formed. This causes inhibition of endosperm reshuffle and germination process which affects the dry weight of seedlings.

In this study, isolates of rhizobacteria that could improve viability and vigor of maize seeds under drought stress were isolates that showed higher average values for several germination parameters than control treatments. The parameter of germination percentage (maximum growth potential) is an indicator of seed viability [13]. Seed vigor is the ability of seeds to produce strong roots and shoots under optimum and sub-optimum conditions [14]. The vigor variables observed in this study were seedling height, radicle length, root hairs, wet weight and dry weight of seedlings. Seed viability should not be 100% but also not less than 80% [15]. Germination percentage in the treatment of isolates $B_{4(8)}$ and $B_{5(19)}$ was 80% higher than the control (70%). A high dry weight value of seedlings indicates a high vigor value. Isolate $B_{4(6)}$ gave the highest dry weight value of seedlings from all treatments and the value of the number of root hairs was higher than the control, although for other parameters the value was lower than the control. Seeds that have high vigor are able to produce high seedling dry weights under optimum and sub-optimum conditions [16].

CONCLUSION

Based on this research, it can be concluded that rhizobacteria can improve the germination of sweet corn seeds under drought stress conditions. Isolate $B_{4(8)}$ increased germination percentage, seedling height, radicle length, number of root hairs and seedling wet weight. Isolate $B_{5(19)}$ can improve the percentage of germination, radicle length, number of root hairs, wet weight and dry weight of seedlings. Isolate $B_{4(6)}$ increased the number of root hairs and dry weight of seedlings.

ACKNOWLEDGMENTS

The author would like to thank the Dean of the Faculty of Agriculture, University of Borneo Tarakan, who has provided funds for the publication of this scientific paper, as well as to those who assisted in the implementation of the research.

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