The Effectiveness of Saline Soil Rhizobacteria from the Coast of North Konawe Regency in Increasing Seed Viability and Vigor of Tomato (*Lycopersicum esculentum* L.)

KALIS AMARTANI[1], GUSTI AYU KADE SUTARIATI[2*], MUHIDIN[2], ANDI BAHRUN[2]
[1]Doctoral Student of Agricultural Sciences, Postgraduate Program,
Universitas Halu Oleo, Kendari, 93232,
INDONESIA
[2]Department of Agrotechnology, Agriculture Faculty,
Universitas Halu Oleo,Kendari Southeast Sulawesi 93212,
INDONESIA
[*]*Corresponding author*

Abstract: - The aim of the study was to obtain potential indigenous rhizobacteria as growth promoters and adaptive to saline soils. The isolate was isolated from the saline soil rhizosphere on the coast in the North Konawe district. The study was conducted at the Agronomy Unit Laboratory, Faculty of Agriculture, Halu Oleo University from April to September 2022. The study was arranged based on a completely randomized design (CRD), consisting of 19 test isolates. The isolates were tested for their effectiveness in increasing the viability and vigor of tomato seeds. The test was carried out using a seed biopriming technique, then the seeds were germinated using a standard germination procedure. The observed variables included maximum growth potential, seed germination, vigor index, seed uniformity, relative growth rate and T50. The results showed that seed treatment using rhizobacteria isolated from saline soil significantly increased tomato seeds' viability and vigor, as seen in the variables of maximum growth potential, germination, vigor index, relative growth rate and reduced T50. From this study, 6 isolates were selected which have the potential to stimulate the growth of tomato seeds, namely KNU2, KNU3, KNU8, KNU13, KNU15 and KNU17. Further research is needed to test the effectiveness of this isolate in increasing the growth and yield of tomato plants under salinity stress conditions in the field.

Key-Words: - Indigenous Rhizobacteria, Seed viability, saline soil, Vigor Indext, biofertilzer

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1 Introduction

Sustainable agricultural development focuses on the use of environmentally friendly technologies in crop cultivation systems. The use of indigenous microbes as biological agents that have multiple benefits, such as biological fertilizers and controllers, has been widely used. The development of technology for the use of biological agents in plant cultivation will continue to be carried out because it can reduce the use of chemical fertilizers and pesticides, [1]. Therefore, various efforts to obtain microbial isolates of biological agents that have specific advantages must continue and be supported because every inch of soil rhizosphere has millions of potential rhizobacteria that can be utilized as PGPR (Plant Growth Promoting Rhizobacteria), [2],[3].

The existence of differences in environmental conditions and host plants that become the habitat of rhizobacteria is thought to be one of the causes of differences in the physiological and biochemical characteristics of the rhizobacteria obtained. Some rhizobacteria species have a dual ability to solubilize phosphate, fix nitrogen and synthesize growth hormone IAA, [4], but some other rhizobacteria species have only one of ability to dissolve phosphate, fix nitrogen or synthesize IAA, [5],[6],[7],[8]. Similar studies will continue to be developed to obtain superior bacterial species both as biofertilizers and biopesticides.

Exploration of rhizobacteria from saline soils on the coast is expected to produce groups of rhizobacteria that can increase plant resistance in environmental conditions experiencing salinity stress. The results of previous studies showed that many salt tolerant PGPR (ST-PGPR) were able to increase the growth of cultivated plants grown on saline soils, [9]. Other studies also show that the rhizobacteria *Bacillus pumilus* and *Exiguobacterium aurantiacum* were potential bioinoculants which were able to increase the growth and yield of wheat in saline soil conditions, [10]. In line with this research, strains of *Bacillus tequilensis* and *Bacillus aryabhattai* showed good potential as PGPR for salinity mitigation in rice cultivation in coastal areas, [11]. Furthermore, stated that salt-tolerant plant growth promoting rhizobacteria (ST-PGPR) isolated from saline soils can be used to overcome the detrimental effects of salt stress on plants, with beneficial effects on plant physiological functions such as growth and yield, [12]. Therefore, the application of microbial inoculants to reduce stresses and increase yields in crops can be an environmentally friendly and low cost option in saline soil management for better crop productivity.

Research on isolates obtained from saline soil rhizosphere has been widely carried out to develop biological fertilizers. The advantages of rhizobacteria from each region have differences, especially in their role as plant growth promoters. Its effectiveness as a growth promoter is also influenced by the ecological conditions of each region, which may differ in the ability of rhizosphere bacteria to stimulate plant growth and to act as a biofertilizer, biostimulant, and bio-protection. The utilization of indigenous plant rhizosphere microorganism isolates will be better because these microorganisms have

In connection with this study, previously 58 rhizobacteria isolates were selected from saline soil rhizosphere along the coast in North Konawe district. Based on the results of the initial selection, 19 potential isolates (from 2 sub-district namely Lasolo sub-district and Sawa sub-district) were selected for the next stage of testing. This research activity is an advanced selection to get the best isolates that will be used for ST-PGPR, especially for agricultural commodities which are cultivated on marginal lands experiencing salinity stress. This study aims to examine the effectiveness of rhizobacteria originating from saline soils in boosting the growth potential (MGP), germination percentage (GP) and vigor index (VI) of tomato seeds under saline conditions.

2 Materials and Methods

2.1 Place and Time

The research was carried out at the Agrotechnology Laboratory, Faculty of Agriculture, Halu Oleo University from April to September 2022.

2.2 Research Design

The research design used was a completely randomized design, carried out in series according to the sampling location. A total of 9 isolates were tested from Lasolo sub-district and 10 isolates from Sawa sub-district. Thus there were a total of 20 treatment units (plus one control, without inoculation).

2.3 Seed Treatment with Rhizobacteria

Rhizobacteria isolates were grown in solid TSA (Bacillus spp.) media and incubated for 48 hours. The growing bacterial colonies were suspended in a suitable liquid medium until they reached a population density of 10^9 cfu ml^{-1}, [13]. Before use, tomato seeds were disinfected with 1% sodium hypochlorite for five minutes, washed three times with sterile water, then air-dried in a laminar air flow cabinet for one hour. Furthermore, 1 g of seed was soaked for 24 hours in a suspension of each rhizobacterial isolate (50 ml) at 28 ºC. After treatment, the seeds were air-dried again in a laminar air flow cabinet until they reached their initial weight and the seeds were ready to be used for testing.

2.4 Seed Biomatriconditioning Treatment

Before being treated, the isolates were grown on the media and suspended in sterile distilled water until they reached a density of 109 cfu/ml, then mixed with husk charcoal powder. Tomato seeds were disinfected with 2% Hypochlorous Sodium for 5 minutes, then washed three times using sterile water and dried in laminar air flow for 1 hour. The seeds that had been dried were then treated by incorporating the seeds into a mixture of moist solid media and husk charcoal powder containing bacterial suspension for ± 6 hours. After treatment, the seeds were again air-dried in laminar air flow and then the germination test was carried out in the germination tub.

2.5 Test the Effect of Seed Inoculation with Saline Soil Rhizobacteria Isolates on Tomato Seed Viability and Vigor

Tomato seeds that have been treated with rhizobacteria were germinated in a plastic tub measuring 20 cm x 15 cm x 10 cm (length x width x height) containing sterile husk charcoal as a germination medium. In each treatment 25 seeds were planted, with three replications.

1. Maximum growth potential (MGP), observed by calculating the number of seed germinated in the late observation (14 dap), [14].
2. Germination percentage (GP), depicting seed potential viability, was measured based on the

percentage of normal seedlings (NS) in the late observation (14 dap), [14].

3. Vigor index (VI), depicting the growth rate vigor, was measured based on percentage of normal seedlings at the first observation (i.e. 7 dap), [15].
4. Relative growth rate (RG-r), depicting seed vigor, is the ratio of growth rate (RG) to maximum growth rate (RG-m). The maximum growth rate itself was obtained from the assumption that at the first observation, normal seedlings had reached 100%.
5. T50, depicting seed vigor, is the time required to reach 50% of the total emergence of sprouts, observed by counting the number of seeds that germinate every day. T50 describes seed vigor (Sadjad, 1999), calculated by the formula, [15]:

$$T50 = ti + \frac{(n50\% - ni)}{nj - ni}(tj - ti)$$

Notes:
ti = time between, at or before the seeds germinate 50%
tj = time between, after the seeds germinate 50%
n50% = number of germinated seeds (50% of total germinated seeds)
ni = number of seeds germinated at time tj
nj = number of germinated seeds at time ti

2.5 Data Analysis

Data were analysed using ANOVA and if it showed a significant effect, then continued with Duncan's Multiple Range Test 0.05.

3 Result and Discussion

3.1 Viability and Vigor Test of Tomato Seeds Inoculated with Saline Soil Rhizobacteria Isolates from Lasolo Sub-District

The test results of isolates (explored from Lasolo sub-district, North Konawe district) inoculated on tomato seeds showed that all test isolates had the same effect in increasing the viability and vigor of tomato seeds. This can be seen from the observed variables, namely maximum growth potential (MGP), germination percentage (GP) and vigor index (VI) of tomato seeds which were significantly different from the control (Figure 1). The increase in MGP in seeds inoculated with saline soil rhizobacteria isolates reached the range of 50%-63%, while the increase in GP reached the range of 67%-83% and the increase in VI reached the range of 63%-89% compared to controls.

As in the previous three variables (Figure 1), all saline soil rhizobacteria isolates inoculated on tomato seeds gave the same and better effect in increasing the relative growth rate (RG-r) of tomato seeds compared to controls. The increase in RG-r reached the range of 63%-86% (Figure 2). Meanwhile on the T50 variable (the time it takes to reach 50% of tomato seed germination), saline soil rhizobacteria isolates gave a different effect. There are 3 isolates that are better at reducing T50, namely KNU2, KNU3 and KNU8 isolates (Figure 2).

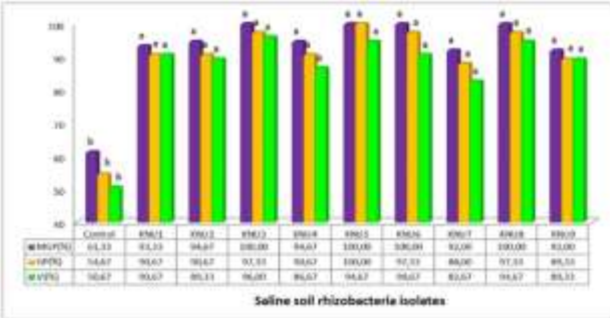

Fig. 1: The effect of rhizobacterial isolates from the rhizosphere of saline soil (explored from Lasolo sub-district) on MGP, GP and VI of tomatoe seed. Note: The same letter notation on the bar chart with the same color shows no significant difference at the 5% significance level according to DMRT.

The data in Figure 1 shows that the highest average maximum growth potential of tomato plants was obtained in the KNU3, KNU5, and KNU8 isolates treatment which differed significantly from the control treatment, indicating that these isolates have the potential to increase the growth of tomato plants in saline soils. The data in Figure 1 also explains that the high maximum growth potential percentage values obtained by isolates KNU3, KNU5, and KNU8 are related to the ability of these isolates to produce IAA. IAA produced by these isolates can stimulate the germination of tomato seeds and functions to encourage cell elongation and increase the ability of cells to absorb water, to increase the water potential of the tissue, which results in cell elongation, [16]. On the germination percentage parameter, the highest average value was obtained in the isolated treatment KNU3, KNU5, KNU6, and KNU8 and was significantly different from the control. The data indicated that these isolates could increase the percentage of seed germination. The increase in the percentage of germination in isolates is inseparable from the role of exogenous IAA produced by rhizobacteria so that they can accelerate seed germination by accelerating the process of root differentiation in forming root hairs and shoots, [17], [18],[19]. The vigor index compares the normal number of sprouts carried out in the first count with the total number of seeds planted. The vigor index measures the strength and ability of seeds to grow normally in the field. The vigor index parameter showed that the isolates KNU3, KNU5, and KNU8 had the highest values compared to the controls. The increase in vigor index values in isolates KNU3, KNU5, and KNU8 is thought to be related to the isolate's ability to produce IAA growth hormone, which can increase the ability of seeds to grow normally in the suboptimal field and environmental conditions. The vigor index value is a value that can represent the speed of germination of a seed so that the seed is categorized as a vigor seed [15].

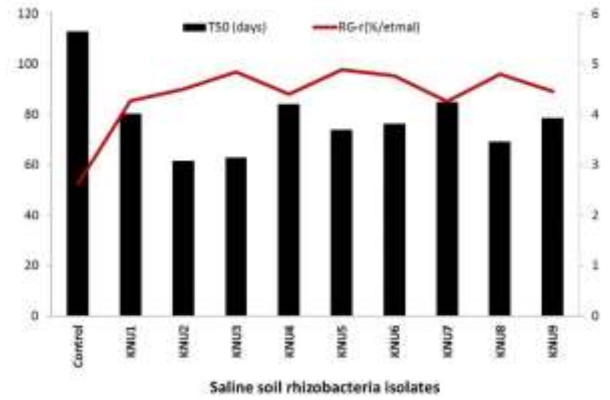

Fig. 2: The effect of rhizobacterial isolates from the rhizosphere of saline soil (explored from Lasolo sub-district) on T50 and RG-r of tomatoe seed. Note: The same letter notation on the bar chart with the same color shows no significant difference at the 5% significance level according to DMRT.

The parameter T50 shows how many days the seeds grow 50%, where the faster the time it takes for a seed to reach T50, it will indicate a seed has high vigor. The data in Figure 2 shows that when the seeds reach T50, each isolate shows a difference in the T50 value. This indicates that the isolated treatment of the T50 value has its role. From the table, it can be seen that in the control treatment, it took 120 hours, the highest time to reach 50% sprouts. In contrast, in the KNU3 isolate treatment, it was seen that there was a decrease in time to reach T50 but if you look at the T50 table all isolate treatments have the lowest T50 value when compared to the control. This shows that applying isolate treatment to seeds can lower T50 and speed up the time needed for seeds to germinate. The research before also found that the seed invigoration treatment integrated with rhizobacteria significantly reduced the time needed to achieve 50% germination of local upland rice seeds (T50) compared to other treatments and controls, [20]. The relative growth rate variable showed that all isolates could increase the growth speed relative to tomato seeds. The data in Figure 2 shows that the relative growth speed of the isolates inoculated seeds has a separate role where the inoculated seeds can significantly increase the relative growth speed. In the treatment isolates, KNU1, KNU4, and KNU7 showed relatively high growth rates when compared to other isolate treatments. The ability of these three isolates to increase the relative growth rate of tomato seeds inoculated with these isolates is related to the ability of these isolates to produce phytohormones that play a role in spurring plant growth, such as IAA. IAA hormones produced by bacteria exogenously can accelerate plant growth, [21].

3.2 Viability and Vigor Test of Tomato Seeds Inoculated with Saline Soil Rhizobacteria Isolates from Sawa Sub-District

Saline soil rhizobacteria isolates (explored from Sawa sub-district, North Konawe district) inoculated on tomato seeds showed the same and better performance in increasing MGP, GP and VI of tomato seeds compared to controls (Figure 2). The increase in MGP in seeds inoculated with saline soil rhizobacteria isolates reached the range of 48%-61%, while the increase in GP reached the range of 61%-80% and the increase in VI reached the range of 68%-87% compared to the control (Figure 3).

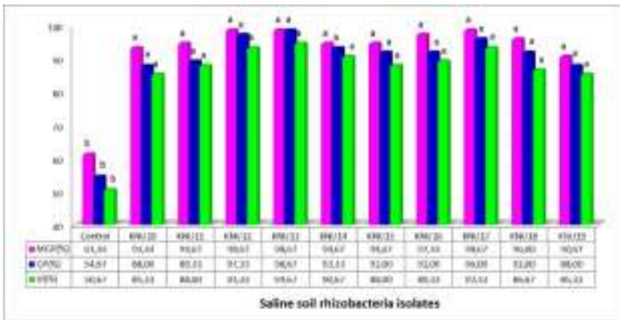

Fig. 3: The effect of rhizobacterial isolates from the rhizosphere of saline soil (explored from Sawa sub-district) on MGP, GP and VI of tomatoe seed. Note: The same letter notation on the bar chart with the same color shows no significant difference at the 5% significance level according to DMRT

Figure 3 showed that the highest maximum growth potential parameters of tomato plants inoculated with saline soil rhizobacteria from the Sawa sub-district were obtained in the isolated treatment KNU12, KNU13, and KNU17, significantly different from the control. This shows that the three isolates can potentially increase tomato plant growth in saline soil. The highest potential value for the maximum growth potential parameter in isolates obtained from saline soil rhizosphere in Sawa District. This situation is related to the isolate's ability to produce IAA to stimulate the germination of tomato seeds that IAA and GA-producing bacteria could stimulate seed germination and promote shoot emergence, [22]. In the germination percentage parameter, the isolates KNU12, KNU13, and KNU17 had the highest germination percentage and significantly differed from the control treatment. This indicated that the three isolates could increase the germination percentage.

The ability of the three isolates to increase the germination percentage of tomato seeds is inseparable from the ability of the isolates to produce the IAA phytohormone, which can accelerate the seed germination process. Rhizobacteria produced IAA in media by adding the amino acid tryptophan, [23]. The ability of rhizobacteria to promote plant growth could produce phytohormones such as IAA, gibberellins, cytokinin, and ethylene in the root environment. On the vigor index parameter, it can be seen that the isolated treatment can increase the vigor index value. In contrast, from the data in Figure 3, it can be seen that all isolate treatments have a significant effect on the vigor index when compared to the control. Figure 3 shows that the isolates KNU12, KNU13, and KNU17 had the highest vigor index values compared to the control treatment. This indicates that the three isolates can increase the growth vigor of the planted seeds. The seed vigor index is a value that can represent the speed of germination and categorize whether the seed is vigor or not [15].

The same was true for the relative growth rate variable (RG-r), all saline soil rhizobacteria isolates inoculated on tomato seeds gave the same and better effect in increasing the relative growth rate of tomato seeds compared to controls (Figure 4). The isolates explored from Sawa sub-district also showed different effects in lowering T50. Among the 10 isolates tested, there were only 3 isolates that were able to lower T50 better than the control and other isolates, namely KNU13, KNU15 and KNU17 isolates (Figure 4).

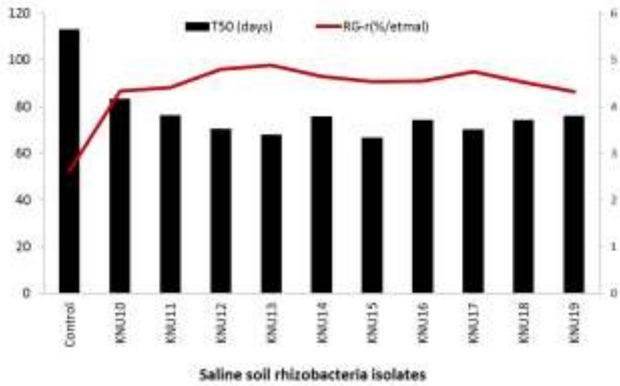

Fig. 4: The effect of rhizobacterial isolates from the rhizosphere of saline soil (explored from Sawa sub-district) on T50 and RG-r (relatif growth rate) of tomatoe seed. Note: The same letter notation on the bar chart with the same color shows no significant difference at the 5% significance level according to DMRT.

The variable T50 shows the number of days the seed grows 50%, where the faster it takes a seed to reach T50, it indicates that a seed has high vigor. The data in Figure 4 shows that when the seeds reach T50, there is a difference in the T50 value for each isolate, and the seeds inoculated with the isolates have the lowest T50 value compared to the control. This shows that the isolated treatment of the T50 value has its role. Figure 4 shows that in the control treatment, the time to reach T50 was 118. This time was the highest time to reach 50% germination.

In the treatment with KNU13 and KNU15 isolates, the time to reach T50 decreased. This shows that applying isolates to seeds reduces the T50 value so that seeds can germinate more quickly. This is in accordance with the result before that the seed invigoration treatment integrated with rhizobacteria significantly reduced the time to reach 50% germination of local upland rice seeds (T50) [20]. The relative growth rate variable showed that all isolates could increase the growth speed relative to tomato seeds. As shown in Figure 4, the relative growth speed of the seeds inoculated with the isolate increased the relative growth speed. The treatment with isolates KNU10 and KNU14 showed a relatively high growth rate compared to other isolates. The speed of growth is one indicator of a seed's vigor, where the high value of the growth speed indicates the high vigor of the seed [24]. The ability of these two isolates to increase the relative growth rate of tomato seeds inoculated with these isolates is related to the ability of these isolates to produce phytohormones that play a role in spurring plant growth, such as IAA. The IAA hormones produced by bacteria exogenously can accelerate plant growth [21].

The results showed that the rhizobacteria isolates explored from saline soil rhizosphere in North Konawe District, especially in Lasolo and Sawa sub-districts, were able to significantly increase the viability and vigor of tomato seeds compared to controls. The increase in viability and vigor of tomato seeds inoculated with rhizobacteria generally reached the range of 50%-89% compared to controls. This indicates that rhizobacteria from saline soil have a better ability to promote plant growth. In line with this study, reported that rhizobacteria isolated from saline soil could increase the growth of rice seeds as indicated by an increase in seedling height, root length and seedling dry weight compared to controls (without rhizobacteria), [25]. It was further reported that saline soil rhizobacteria were able to increase wheat yield by 24% compared to controls, [26].

Improvements that occurred in plants inoculated with saline soil rhizobacteria were reported to be related to the ability of rhizobacteria to synthesize growth hormones such as IAA, gibberellins and cytokinin, [11], [26]. In line with these results, reported that rhizobacteria isolated from saline soil were able to produce growth hormone IAA, fix biological nitrogen, dissolve soil phosphorus (P) and potassium, [27]. Further, reported that plants inoculated with saline soil rhizobacteria were able to synthesize IAA so that it could help improve seed germination and other growth parameters by increasing a number of physiological functions such as reducing osmotic stress, increasing K^+ absorption and decreasing Na^+ uptake, [28].

Salinity stress causes a decrease in IAA synthesis, thus disrupting the production of phytohormones, resulting in stunted plant growth. The role of auxin is very important, especially in influencing cell division and differentiation, seed germination, pigment formation and photosynthesis, [29], [30], [31], [32]. Furthermore, several research results show that the ability of plants to adapt to salinity stress depends on their interaction with microbes that have the ability to produce cytokinins, IAA, and or gibberellic acid, [33], [34], [35], [36], [37], [38]. Another study also found that inoculation of the beneficial rhizosphere microbiome significantly increased growth and yield of mungbean plants due to increased production of IAA through plant-microbial interactions under saline conditions. Microbial production of IAA not only enhances plant growth, but also provides protection from environmental stressors. It was further reported that 50% of the bacterial cells that did not produce IAA died under osmotic pressure, whereas the bacterial cells that produced IAA (70%) survived better than the control treatment, [39]. Phytohormone production is an important exploitable characteristic of PGPR. Therefore, it is necessary to continue to explore and isolate PGPR bacteria that are effective and can help reduce salinity stress in plants.

4 Conclusion

Seed treatment using rhizobacteria isolated from saline soil significantly increased the viability and vigor of tomato seeds, as could be seen in the variables of maximum growth potential, germination, vigor index, relative growth rate and reduced T50. From this study, 6 isolates were selected which have the potential to stimulate the growth of tomato seeds, namely KNU2, KNU3, KNU8, KNU13, KNU15 and KNU17. Further research is needed to test the effectiveness of this isolate in increasing the growth and yield of tomato plants under salinity stress conditions in the field.

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Conflicts of Interest

The authors have no conflicts of interest to declare that are relevant to the content of this article.